mation and repair constitute a relative orderly process, as in the rest of the body. The early changes are primarily exudative, with edema of the supporting stalk of the glomerulus and the pericapillary interstitial space. In severe injury a cellular exudate of mononuclear cells and polymorphonuclear neutrophils infiltrates the interstitial space from the blood stream. The pericapillary interstitial spaces may become so swollen from edema and invading cells that the basement membrane of an entire lobule is stripped from the capillary basement membrane and assumes a globular or club shape.

When injury is less severe or at a later stage of the disease, the number of polymorphonuclear neutrophils is reduced, and the predominating cell in the exudate is a mononuclear cell indistinguishable from monocytes or histocytes. Proliferative and reparative processes become evident. In the renal glomerulus the epithelial cells commonly multiply, and when the proliferation is pronounced, acute capsular glomerulonephritis is produced. Fibrils of basement membrane type are laid down either by epithelial cells or by accompanying connective tissue cells. With thickening of these fibrils, the familiar crescent scar is produced. Endothelial cells may multiply.

The interstitial cells of the glomerulus respond to inflammation with proliferation like any other connective tissue cell. In mild injury this proliferation may be the major finding, resulting in slowly progressive scarring. After injury of any extent, if scarring takes place, most of the connective tissue fibrils are laid down in the interstitial space of the stalk. The connective tissue laid down about the circumference of the capillaries in the tuft and between the capillary and epithelial basement membrane may obliterate the capillary lumen and forms the thickened basement membrane described in renal disease. Capillary obliteration and complete scarring give a characteristic picture of terminal chronic glomerulonephritis.

[This remarkable study may well force pathologists to revise many earlier concepts of glomerular lesions. It could explain the fact that in chronic glomerulonephritis there is no such uniformity of glomerular change as is noted in the acute diffuse disease. The fact that patients with chronic glomerulonephritis progress may be due to continuing functional disorder rather than to progressive chronic glomerulitis. Further equally good studies are indicated.—Ed.]

Virus Glomerulonephritis: Clinical and Experimental Studies. In the winter of 1948, L. Ristić, B. Jakšić and M. Jocković[7] (Belgrade) treated 45 young males for acute ne-

(7) Acta med. iugoslav. 4:250-267, 1950.

phritis which differed from the classic picture of acute glomerulonephritis. Onset was sudden. The patients were always febrile. Characteristic symptoms of general infection were absent in 31 per cent, symptoms such as extensive catarrh were present in 42 per cent and symptoms of gastrointestinal involvement were present in 27 per cent. Later, edema of varying localization developed. In the advanced stages there were bradycardia, systolic hypertension, diuresis, albuminuria, microscopic hematuria, chloride retention and/or nitrogen retention. The disease lasted three weeks, and no patient died.

Urine or blood from these patients was injected into guinea pigs. Later, suspensions of macerated kidney tissue from the animals were passed through other guinea pigs. At autopsy the animals showed only considerable edema of the kidneys. Microscopically, nephrons were involved but interstitial tissues spared. Glomeruli were often enlarged. There was a large quantity of serous cellular exudate in the glomerulus. The cellular elements consisted of lymphocytes and reticuloendothelial cells. Hemorrhagic areas were observed. The liver showed fatty dystrophy. No other organ showed changes.

The observations indicated that in man a virus may be the cause of acute glomerulonephritis.

[The upper respiratory infections which so commonly precede the onset of acute diffuse glomerulonephritis have been shown to be largely streptococcic, yet some may be viral. Even if that be so, it is a far cry to implicating a virus or viruses as a cause of renal disease. Note that Selye (p. 205) found inclusion bodies in the kidneys of his rats. Perhaps viral cause may be discovered, but it is not yet conclusively demonstrated.—Ed.]

Clinical Study of Hereditary Interstitial Pyelonephritis. Gerald T. Perkoff, F. E. Stephens, D. A. Dolowitz and Frank H. Tyler[8] (Univ. of Utah) studied 134 of 232 descendants of a single family extending through four generations. In 44, evidence of renal disease was demonstrated, but only 3 had any urinary symptoms; all others were apparently healthy. The urine of all 44 showed pus, red blood cells, albumin and casts. Urine culture from 37 patients revealed no growth in 20 and a variety of organisms in the rest. Study of two asymptomatic patients showed impaired renal function but normal blood chemistry. The two living symptomatic patients were men presenting clinical and pathologic evidence of severe progressive renal disease.

Two patients who died before the study was undertaken

(8) A.M.A. Arch. Int. Med. 88:191-200, August, 1951.

were examined post mortem. Findings in the kidneys were similar. Grossly the kidneys were small, scarred and contracted, without evidence of lower urinary tract obstruction. Microscopically there was diffuse infiltration of the cortex and medulla with lymphocytes and a few polymorphonuclear leukocytes. Except for secondary involvement within scarred areas the glomeruli were normal. The collecting tubules in scarred regions were dilated with flattened epithelial lining. Each lumen contained a precipitate of protein mixed with leukocytes, erythrocytes and granular debris. Elsewhere the tubules were thickened. The most striking change was the heavy infiltration of lymphocytes throughout the interstitial connective tissue. There were many small focal abscesses in the corticomedullary junction.

Of the relatives studied, 78 were women. Renal disease occurred in 29 women and 15 men. There were apparently no deaths due to pyelonephritis in any woman. Seven male descendants died. Transmission of the trait is probably incompletely sex linked.

The mechanism responsible for high incidence of pyelonephritis is not clear. It may be due to abnormal lymphatic connections between colon and kidney, which might be determined on a genetic basis, or to inherited susceptibility to kidney infection.

[The inference that the disorder is hereditary requires confirmation. The fact that any disease occurs in families may be due to environmental, hygienic and other factors.—Ed.]

Glomerular Lesions of Kidneys in Sarcoidosis (Boeck's Sarcoid). Gunnar Teilum[9] (Copenhagen) reports a case of generalized sarcoidosis in which the kidneys displayed glomerular lesions characterized by prehyalin or hyalin deposits in relation to the glomerular tufts. The lesions were essentially the same type as those in some other disorders of the mesenchymal tissue, which, during the active phase are associated with hyperglobulinemia.

Woman, 49, had progressive dyspnea, cough and edema. Sarcoidosis was diagnosed on the basis of roentgen examination of the lungs and lymph node biopsy. The blood count was normal. The urine contained hyaline casts but no albumin, blood or sugar. At autopsy, manifestations of cardiac insufficiency were present, with hypertrophy of the right ventricle, which was 8 mm. thick. Generalized sarcoidosis involved the spleen, liver, lymph nodes, adrenals, pituitary, bone marrow, lungs and kidney. The kidney measured

(9) Acta path. et microbiol. scandinav. 28:294-301. 1951.

3 × 6 × 11 cm. Scattered coarse retracted areas were evident on the cut surface. Microscopically, glomerular lesions were pronounced. Homogeneous prehyalin or hyalin deposits occurred in the glomerular tufts. The structural change was similar to the glomerular lesion in lupus erythematosus disseminatus. The material assumed a yellowish brown, partly red color with van Gieson's stain and violet blue with Mallory's stain but did not stain with methyl violet or Congo red. Almost all glomeruli were involved to some extent. Some showed only a portion involved by hyalinosis. The most pronounced form appeared as large confluent plaques and hyalin globules. Only a few entirely hyalin glomeruli were found. The capsular space was uninvolved and there was no proliferation of the capsular epithelium. In a few glomeruli, prehyalin precipitate simulated fibrinoid or hyalin thrombi. Hyalinosis of the reticular connective tissue and a number of hyalin deposits in the vascular walls were found. There were no granulomas and no hypertensive vascular changes.

[This case report is included because it may lead to further observations. Intercapillary glomerulosclerosis and amyloid are satisfactorily excluded, but without additional observations the lesion cannot be ascribed to sarcoidosis.—Ed.]

Liver as Factor in Experimental Renal Hypertension. Loyal Davis and Carlos A. Tanturi[1] (Northwestern Univ.) produced experimental renal hypertension in dogs by applying Goldblatt clamps to the renal arteries. Neither occlusion of the common hepatic artery nor section of the hepatic plexus decreased blood pressure levels. Ligation of the liver branches of the hepatic artery reduced the blood pressure to normal levels for transient periods. Occlusion of the portal vein produced a fall in the systemic blood pressure which persisted until the animal was killed, a period of one year in some cases. When both the common hepatic artery and the portal vein were occluded, blood pressure was decreased in every instance and the animals lived until they were killed.

After all these procedures the dogs showed no symptoms of illness. Following clamping of the portal vein, the intra-abdominal collateral circulation was well developed. To decrease the systemic blood pressure of these animals, reduction of the blood flow to the liver had to be sufficient to produce a fat infiltration of the liver cells without damaging their nuclei. This type of change was not always detectable by the presently accepted tests for liver function.

Results indicated that the liver plays a role in the humoral mechanism involved in experimental renal hypertension.

[It is a tribute to the surgical technic that the animals had no symp-

(1) A.M.A. Arch. Surg. 62:325-334, March, 1951.

toms of illness. However, there may be other aspects to the phenomena than alteration of hepatic circulation. Other articles on hypertension appear in the chapter on Cardiovascular System (pp. 134-138).—Ed.]

Urogenital Aspects of Coccidioidomycosis: Review of Literature and Report of Two Cases are made by James G. Rohn, Julio C. Davila and Thomas E. Gibson[2] (San Francisco).

Case 1.—Negro, 40, had swelling of the scrotum for six years. Examination showed a firm, nodular, enlarged epididymis and a slightly enlarged, smooth, rubbery testis. There was no tenderness. Epididymectomy was performed. Histologic examination showed many tubercle-like lesions which contained spherules of Coccidioides immitis. Recovery was uneventful.

Case 2.—Filipino, 38, had pain in both wrists and elbows for three years. Biopsy of the synovial membrane of the right wrist showed coccidioidomycosis. Several months later he complained of a mass in the right scrotum which had been enlarging gradually and was nontender. He had worked for varying periods in the San Joaquin Valley and in Arizona. Examination showed nodularity and induration of the lower pole of the right epididymis and questionable involvement of the right testis. Right orchiectomy and left partial vasectomy were performed. Histologic examination showed numerous tubercles in the epididymis which contained round to oval spherules. The other testis was not involved. Coccidioidomycosis of the epididymis was diagnosed.

Both patients belonged to the two racial groups in which coccidioidomycosis is most likely to undergo dissemination. Urogenital involvement is obviously a secondary manifestation of the disease, occurring only when there is dissemination. In most patients urologic manifestations of clinical significance are lacking, even though there may be urogenital involvement. Coccidioidomycosis mimics tuberculosis in almost all respects, with the notable exception of invasion of the kidney. Although there may be kidney lesions, they seldom progress to the point of clinical significance. Therefore, renal infection with coccidioides is of little importance, but the disease should be considered in the differential diagnosis of genital granulomas.

Glandular Tumors of Bladder. William M. Coppridge, Louis C. Roberts and David A. Culp[3] (Durham, N. C.) discuss a case of adenocarcinoma which arose from inclusions of urachal cell nests in the bladder dome. Of unusual interest was extensive involvement of the gastrointestinal tract by metastases from the primary tumor. There was no gross evidence of a break in the gastrointestinal mucosa and each tumor

(2) J. Urol. 65:660-667, April, 1951.
(3) Ibid., pp. 540-549.

mass microscopically showed an intact mucosa. A second case was an example of adenocarcinoma involving the base of the bladder, the origin of which was not clear.

Although most evidence points to the urachus as the origin of a majority of glandular bladder tumors, it is apparent that some arise in areas situated remotely from the bladder dome and are definitely not associated with urachal tissue. Various theories have been proposed to explain the origin of the latter. The anatomic theory is based on the supposition that the normal bladder contains glands. This question remains unanswered, but if glands are present in the normal bladder they could well be one site of origin of adenocarcinoma. The embryologic theory states that these tumors arise not only from urachus inclusions, which is well accepted, but from inclusions of cloacal cells. There is no definite proof that nests of cloacal cells can be found in the bladder mucosa. The metaplastic theory states that glandular elements arise in the bladder from transitional cell epithelium after long-continued irritation. The bulk of support for this theory is derived from cases of extrophy of the bladder, in which are invariably found mucus-producing glands that are similar to intestinal glands. Incidence of adenocarcinoma in extrophic bladders is much higher than that in normal bladders.

Presence of gelatinous or mucinous material in the urine in adenocarcinoma is actually the only symptom which tends to differentiate it from other types of bladder tumor. Structurally, glandular bladder tumors resemble adenocarcinomas of the gastrointestinal tract, especially of the rectum. Surgical removal is the preferred treatment whenever location and degree of extension of the tumor permit. Excision must be radical because, although distant metastases are rare, local recurrences at the site of operation are the rule.

Allergic Granulomas of Prostate Gland are reported for the first time by M. M. Melicow[4] (Columbia Univ.).

In man, 62, persistent asthma, weight loss, and eosinophilia developed. The prostate was normal on digital examination. One year later frequency, dysuria and nocturia had developed; the prostate was greatly enlarged. Urine contained albumin and red and white blood cells. Suprapubic prostatectomy was done because of retention. Total weight of surgical specimen was 14 Gm. Tissue was firm; on section, surface was a homogeneous yellow-white, studded with small, firm nodules. Microscopic examination revealed benign en-

(4) J. Urol. 65:288-296, February, 1951.

largement with scattered, irregular, deeply pink areas that were granulomatous in appearance. A few giant cells were seen but other stigmas of tuberculosis were absent. High power examination revealed the areas to be disintegrated connective tissue fibers surrounded by intense eosinophilic infiltration (Fig. 64). Round cells,

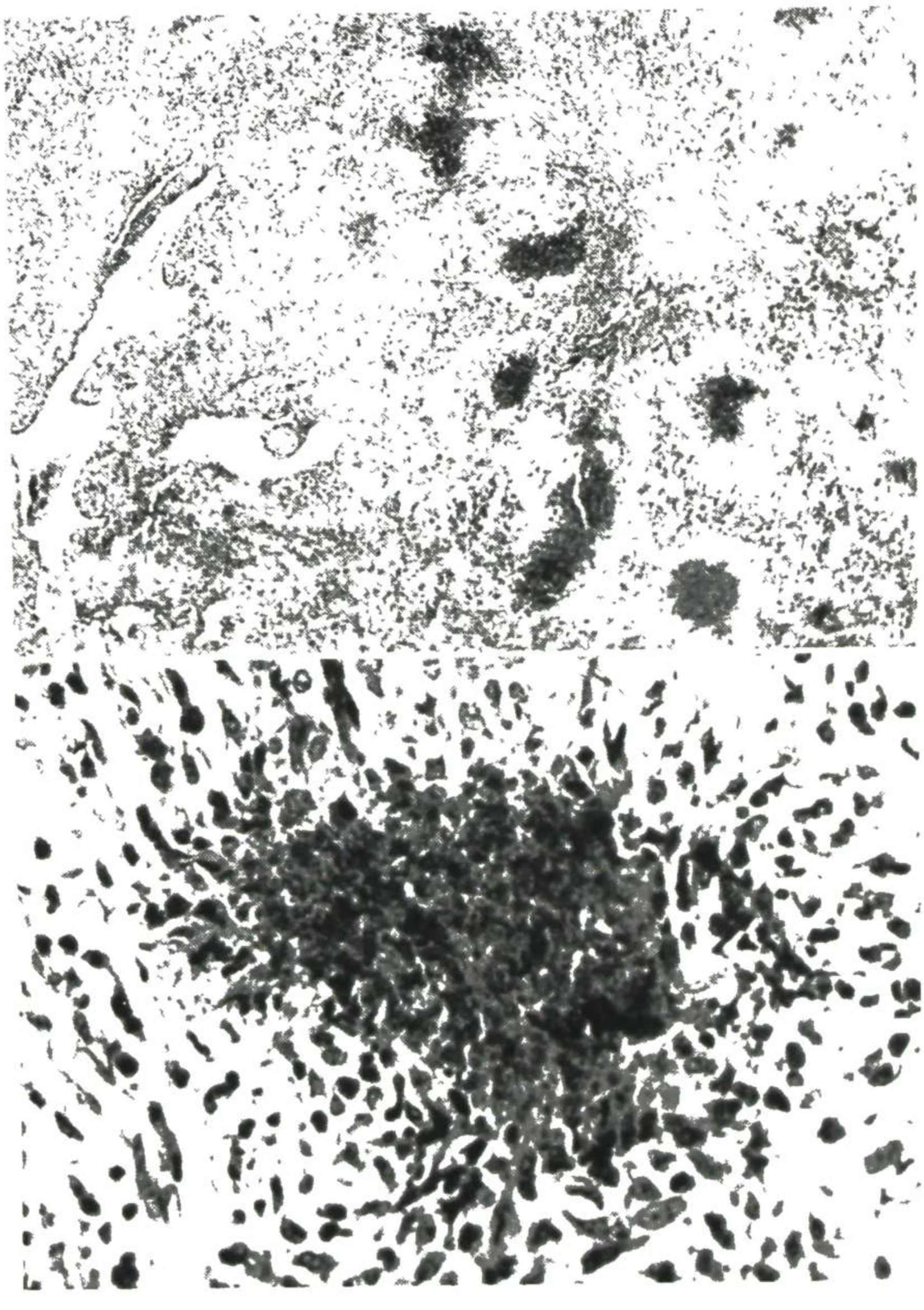


Fig. 63 (top).—Low power view of multiple granulomas; reduced from × 50. Several small acini are present nearby.

Fig. 64 (bottom).—High power view of granuloma; reduced from × 385. Note central connective tissue fibers and abundant cellular exudate predominantly of eosinophils. Hematoxylin-eosin.

(Courtesy of Melicow, M. M.: J. Urol. 65:288-296, February, 1951.)

plasma cells and lymphocytes were present. A small blood vessel with thickened intima and partially degenerated media, showing perivascular infiltration with many eosinophils, was seen.

The general condition did not improve. Transient areas of increased density were demonstrated in successive radiographs of the lungs. Weight loss and asthma progressed. Fever and bloody

diarrhea developed and thrombophlebitis appeared before death, seven months after operation. Postmortem examination revealed granulomatous lesions in the lungs, heart, liver, spleen, esophagus, rectum and seminal vesicles similar to those found in the bladder and prostate. There were also healed and healing periarteritis in the heart and muscle, widespread disseminated scars and interstitial nephritis.

The allergic granulomas of the prostate were local manifestations of a generalized vascular and connective tissue disturbance. The sudden involvement by the granulomas caused swelling of the prostate and rapid onset of symptoms of bladder neck obstruction. The evanescent pulmonary lesions were probably eosinophilic infiltrations similar to Löffler's syndrome.

[When Dr. Matthew J. Stewart, Emeritus Professor of Pathology, University of Leeds, lectured at the Armed Forces Institute of Pathology in the autumn of 1951, he described two similar cases which had not been reported in the literature. Whether the lesion is primarily allergic or represents a type of response in allergic men to other disorders in the prostate does not appear to be settled. It would be interesting to have studies of stained smears of the material obtained by prostatic massage from many patients.—Ed.]

FEMALE GENITALIA AND BREAST

Carcinoma of Mesonephric Duct: In Adults and Children. According to W. P. Plate[5] (Univ. of Utrecht), mesonephric remnants may be found in the cervix of the uterus and vaginal wall, occurring as tubules lined with a single layer of epithelium which does not show mucous secretion. In the cervix they may develop into adenomas and in the vaginal wall into cysts. If connection with the mesonephric duct can be proved, diagnosis is simplified. The tumors in the cervix usually originate in the lower part beneath the secretory epithelium and the stratified epithelium of the cervix and portio. This suggests a tumor which starts at the ampulla of the mesonephric duct. Adenocarcinoma may arise from these remnants and when the tumor breaks through to the outside, the carcinoma lies near the normal glands of the cervix. Cervical glands stain vividly with mucicarmine, whereas the glands of the mesonephric duct do not. The carcinoma often assumes a papillary form, the glandular tubules being filled with proliferating papillae. In other instances the tubules are filled with large, clear cells or the glandular character disappears and a solid mass is

(5) Gynaecologia 130:203-210, September, 1950.

found. Seven cases of carcinoma of the mesonephric duct in the cervix have been described previously and the author reports another. Close examination of patients with adenocarcinoma of the cervix may disclose others.

The primary adenocarcinoma of the vagina probably always starts from the mesonephric duct. Adenocarcinomas of the cervix and vagina in children probably all originate in mesonephric remnants. At least 11 definite cases of cervical carcinomas and 3 of adenocarcinomas of the vagina, including one described by Plate, have been reported in children.

Malignant Lesions of Uterus Associated with Estrogen-Producing Ovarian Tumors. Malcolm B. Dockerty and Elizabeth Mussey[6] (Mayo Clinic), in a study of 87 granulosa cell and theca cell tumors, found 15 associated uterine cancers. Three patients had mammary cancer. Association of uterine and ovarian lesions was 15 per cent in all patients and 27 per cent in women over 50. Carcinogenic influence of estrogen is suggested as a possible cause. Carcinoma-like lesions have been induced in cervices of mice receiving estrogens and metastasizing fundal carcinomas have been produced in old multiparous rabbits in which impaired livers prevented breakdown of circulating estrogens.

Uterine as well as mammary carcinomas are rarely observed clinically after oophorectomy; there is high incidence of fundal carcinoma in cirrhosis of the liver. Vaginal smear in a group of women with malignant uterine lesions showed sustained estrogen activity; patients with such lesions have a late menopause with mild symptoms. Development of uterine cancer has been reported in a woman who received 12,000,000 R.U. of estrogen for eight years.

Association with mammary cancer in three cases is further evidence for the role of estrogen in production of uterine tumors. Incidence of coexisting mammary and uterine cancer has been found to be 2.4 times as great as would be expected on the basis of chance alone.

Endometrial Carcinoma Associated with Feminizing Ovarian Tumors. James M. Ingram, Jr., and Emil Novak[7] (Johns Hopkins Univ.) review 50 cases from the literature and report 4. Of the 50 patients, 28 had thecoma and 22 granulosa cell tumor. Most were over 50, and the presenting

(6) Am. J. Obst. & Gynec. 61:147-153, January, 1951.
(7) Ibid., pp. 774-789, April, 1951.

symptom of 85 per cent was postmenopausal bleeding. The four new cases were found among 66 consecutive cases of feminizing ovarian tumor. The four comprised 12 per cent of the postmenopausal group.

Endometrial hyperplasia accompanies most estrogen-producing tumors. It is suspected that many of these patients bleed for years from a hyperplasia which finally gives rise to carcinoma. In the presence of estrogen-producing tumors in postmenopausal women, endometrial carcinoma develops quite often, whereas combined carcinoma of the cervix and breast develops rarely. Apparently, degree of carcinogenic response of these tissues to estrogens in postmenopausal life parallels their degree of physiologic response to estrogen during menstrual life. Hyperestrogenism may be the added factor which initiates carcinogenesis in women already predisposed to cancer.

Since in this series thecoma occurred more often in combination with uterine carcinoma than did granulosa cell tumor, despite the much greater general incidence of granulosa cell tumor, it is suggested that the thecoma, by means of greater estrogen production, has the greater carcinogenic effect. This observation supports the concept that the thecal and not the granulosa cells are the sole or chief source of estrogen.

[This and the preceding paper give concrete evidence of the influence of tumors which undoubtedly produce estrogenic substance. Individual case reports have appeared, but they could not be interpreted conclusively. The view that theca cell tumors of the ovary produce more estrogens than the granulosa cell tumors requires confirmation.—Ed.]

Study on Biologic Activity of Transplanted Granulosa Cell Tumor in Castrate C57 Mice. E. S. Crelin and J. T. Wolstenholme[8] (Yale Univ.) castrated 18 female and 35 male C57 black strain mice. The 7 females and 14 males used as controls were killed after 60 days. A small fragment of granulosa cell tumor was transplanted to the other mice, which were killed 62-100 days later.

By the end of 52 days, "takes" could be palpated in all the animals with transplants. At autopsy, they had dilated and slightly heavier hearts than did the controls. Their livers were enlarged and hyperemic. There was no significant weight change in the kidneys of tumor-bearing animals as compared with those of controls. Microscopically the kidneys of the castrated male controls showed cuboidal cells in the parietal

(8) Cancer Research 11:212-215, March, 1951.

epithelium of Bowman's capsules, which indicated that they were of the male type. In some female controls squamous cells were predominant, whereas in others there was about an equal number of both cell types. In tumor-bearing animals the parietal epithelium was predominantly of squamous cell or female type. Control animals and those with small tumors had columnar cells lining the terminal tubules of the submaxillary gland. All animals with tumors weighing more than 3.95 Gm. had cuboidal cells lining these tubules which were characteristic of the estrogen-stimulated female type. There were no changes in the adrenal or pituitary glands.

Weight of the uteri of the female animals with tumor increased progressively with weight of the tumor. Histologically, uteri of control females were of the castrate type, whereas those of all tumor-bearing females showed varying degrees of estrogenic stimulation. Animals with the largest tumors showed the most pronounced stimulation. Changes in the mammary glands correlated similarly with tumor weight.

It was concluded that weight of the granulosa cell tumors correlated closely with the amount of estrogenic substance secreted and with the severity of hypervolemic changes.

Arrhenoblastoma: Incidence of Malignancy and Relation to Pregnancy, to Sterility and to Treatment. Carl T. Javert and William F. Finn[9] (Cornell Univ.) analyzed reports in the literature and obtained follow-up data from the authors by circulation of a questionnaire. Among the 122 reported cases, 27 are examples of malignant arrhenoblastoma. If histologic carcinoma is accepted as evidence of malignancy, five more cases can be added to this group. However, it should be pointed out that in these five patients the subsequent course has been uneventful. Malignant arrhenoblastomas usually manifest their true nature either before or at operation, so that there is seldom any question as to whether the tumor is benign or malignant at the time of operation. No patient has been known to survive or to be free from recurrence. In several, recurrence after apparently benign tubular adenomas has been reported.

Simultaneous appearance of arrhenoblastoma and pregnancy has been reported only five times. Pregnancy before appearance of the tumor has been reported at least 100 times in 38 patients. Pregnancy after surgical removal is known to

(9) Cancer 4:60-77, January, 1951.

have occurred at least 30 times in 25 patients. Simultaneous existence of arrhenoblastoma and amenorrhea may account for some of the relative sterility and infertility. At least 6 of the 51 patients who had never been pregnant had associated congenital anomalies which made conception impossible. History of the tumor in parents or siblings is unusual. A case reported by the authors is unusual in this respect, since the patient's mother had such a lesion removed eight years before the birth of her daughter.

Bilateral salpingo-oophorectomy and total hysterectomy are mandatory in patients with weight loss, ascites, hydrothorax, rapid growth and invasion. Genital anomalies, postmenopausal age, adhesions and bilateral tumors suggest the need for complete extirpation of pelvic organs even when the tumor appears benign. Conservative surgery such as unilateral oophorectomy should be reserved for the young woman with an apparently benign unilateral tumor, if the uterus and contralateral tube and ovary are normal. Metastases occur chiefly in the intermediate and undifferentiated sarcomatous type, rarely in the tubular adenomatous. If microscopic examination reveals malignancy when unilateral oophorectomy alone has been performed, removal of the remaining pelvic organs should be considered. Incidence of malignancy in such patients is about 25 per cent and they should be followed carefully when pelvic organs remain. The return of virilization is one of the early manifestations of recurrence. Development of a pelvic mass or ascites indicates recurrence. A rising ketosteroid titer may indicate recurrence.

[The questionnaire was concerned almost solely with the clinical aspects but added a question about autopsy findings. There is no indication that there were assays of steroids in the cases included in the answers. —Ed.]

Mesothelioma of Female Genital Tract: Review of Literature and Report of Five Cases Involving Uterus are presented by Robert C. Horn, Jr., and George C. Lewis, Jr.[1] (Univ. of Pennsylvania). Twenty-four cases in the literature are accompanied by histologic descriptions and photomicrographs which leave little doubt as to the correctness of the diagnosis.

Most mesotheliomas were found incidentally at surgery or in autopsy specimens. There were no characteristic symptoms. Most patients were aged 30-50. Size of the tumor varied considerably. Some authors describe circumscribed tumors or

(1) Am. J. Clin. Path. 21:251-259, March, 1951.

indistinct capsules, but in the present cases there was the same circumscription as with uterine leiomyomas. Color varied from pink to red.

Microscopically the tumors described in the literature varied from a netlike structure which displaced normal tissues to single or grouped cells scattered through a fibromuscular background. The cells varied from flattened, endothelium-like elements to cuboidal or low columnar cells. The gland or vessel-like spaces they enclosed were unequal in size and

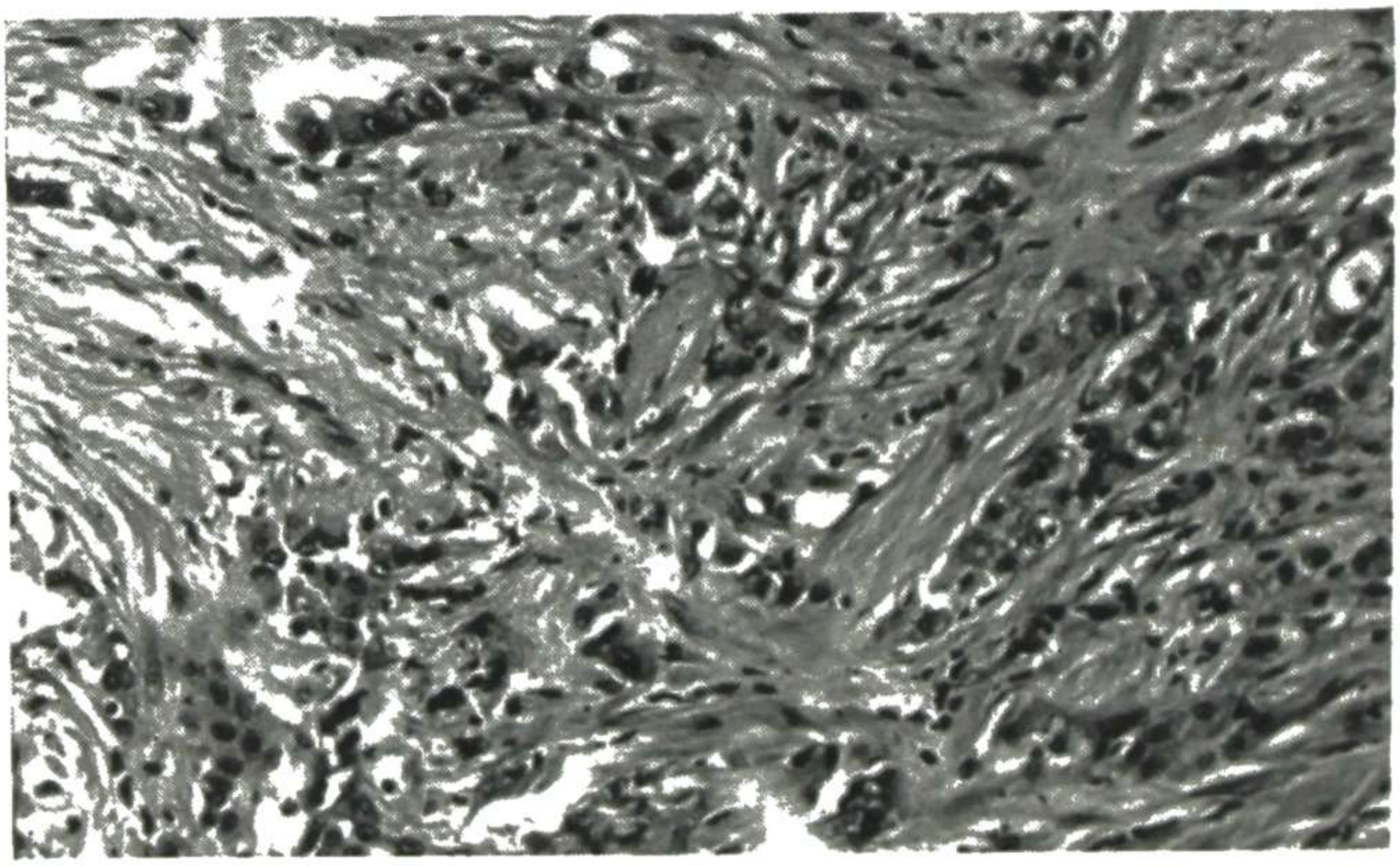


Fig. 65.—Uterine mesothelioma showing distribution of neoplastic cells, largely in solid cords without lumens in dense stroma. Hematoxylin-eosin; reduced from × 175. (Courtesy of Horn, R. C., Jr., and Lewis, G. C., Jr.: Am. J. Clin. Path. 21:251-259, March, 1951.)

shape. The cells were often in solid cords without lumens (Fig. 65). Frequently the cells were vacuolated, with material resembling mucin in the vacuoles. In some cases lymphocytic aggregates in the stroma were striking. In the present series the histologic picture was similar except that lymphocytic infiltration was not significant.

These tumors are the counterpart of similar lesions in the tunica vaginalis testis in the male. This group of tumors probably originates from the mesothelial lining cells of the peritoneum. With rare exceptions, most mesotheliomas of the female genital tract behave like benign tumors.

Demonstration of Chorionotrophic Virus in Four Cases of Hydatid Mole and One Case of Pulmonary Metastases of Chorioepitheliomas. Using a technic reported previously,

Roland de Ruyck[2] inoculated filtrates into the amniotic cavity and on the chorioallantoic membrane of chick embryos, then made successive passages in chick embryos.

Though there was some variation in the appearance of inclusion bodies and ectodermic reactions, the results were fairly constant and similar to those of previous experiments. Ectodermic lesions resembled those found with other viruses: hyperplasia, metaplasia and formation of inclusion bodies. In one case the ectodermal reaction appeared anarchic and invaded the mesoderm. Entodermal lesions always presented tumorous proliferations which were sometimes extensive and in which inclusion bodies could be demonstrated. The amniotic membrane formed numerous vesicles suggestive of hydatidiform mole. In the liver the reticuloendothelial system produced round cell accumulations of macrophagic appearance which seemed to be the source of profuse mucous secretion. Finally, examinations with the electron microscope of centrifugates of the four moles and of inoculated amniotic membranes showed many elementary bodies of the same size and appearance as those previously described.

In view of the complexity and polymorphism of these lesions, the chorionotrophic virus seems definitely distinguishable from other known viruses. Though hydatidiform and choriocarcinomatous transformations have not been produced experimentally in mammals, the continued finding of this virus implicates it more and more in such changes.

[The fact that some sort of filtrable agent was recovered seems to be established, but there is much more to be done before it can be accepted as the inciting agent in choriocarcinoma.—Ed.]

Papillary Neoplasms of Breast: Benign Intraductal Papilloma. C. D. Haagensen, Arthur Purdy Stout and Jim S. Phillips[3] (Columbia Univ.) reviewed 353 cases diagnosed as papilloma of the female mammary gland. Of these, 108 benign intraductal papillomas were evident on gross inspection. They occurred within a relatively localized, dilated portion of a duct or in several adjacent ducts. A serous or bloody discharge was almost always the symptom which attracted the patient's attention. In many the papilloma could not be palpated but in others it was suspected or felt as a radial linear thickening beneath or adjacent to the areola. Most lesions are situated

(2) Bull. Assoc. franç. étude cancer 38:252-268, 1951.
(3) Ann. Surg. 133:18-36, January, 1951.

in the central area of the breast in the larger ducts near their termination in the nipple.

There were 243 microscopically benign intraductal papillomas. These lesions are one of the manifestations of the protean lesion, cystic mastitis. They appeared as small multiple papillary projections, with or without fibrous cores, which projected into the ducts and cysts of chronic cystic mastitis. Always multiple, they often involved ducts in many areas of the breast and had no predilection for any special part of it. They rarely, if ever, produced nipple discharge.

The grossly visible intraductal papillary tumors are proliferations of duct epithelium which project outward into a dilated lumen from one or more focal points. The proliferative epithelial cells are supported on vascular stalks. They may be obviously papillary or so completely anastomosing as to seem to form glandlike spaces. If they are subjected to trauma, cicatrices can form. Such scars may include glandlike tubes which create a false impression of infiltrative growth.

The criteria permitting differentiation between benign and malignant growths are found in the cells. In benign growths the cells resemble normal duct epithelium. It must be remembered that normal duct cells vary considerably, enlarging during secretory activity, acting as phagocytes and undergoing degenerative changes. In doubtful cases the decision may rest on the nucleus; if this has the appearance of anaplasia with hyperchromatism, accentuation of the chromatin network or large nucleoli, and particularly if there are more than rare, widely separated mitoses, the growth can safely be considered cancer. Recognizable solitary nodules of papillary carcinoma almost never show traces of benign intraductal proliferation. In this series there was no proof that benign papillary tumors undergo malignant change. The rare papillary carcinomas begin as such and maintain their characteristic low grade of malignancy throughout. They can be differentiated microscopically from intraductal papillomas.

Local excision done meticulously according to a plan makes possible the identification of the duct or ducts containing the papilloma and removal with a reasonable margin of surrounding breast tissue suffices for cure. There is no justification for simple mastectomy in treatment of intraductal papilloma. If features suggesting malignancy are found,

biopsy only should be done and the wound closed. After examination of paraffin sections the lesion is locally excised, if benign; radical mastectomy is performed if it is malignant.

GLANDS OF INTERNAL SECRETION

Colloidophagy in Human Thyroid Gland is discussed by C. Alexander Hellwig[4] (Halstead, Kan.). This phenomenon of invasion and ingestion of colloid by macrophages in the thyroid has been observed in animals, particularly in response to administration of pituitary extract or thyrotrophic hormone. In man, cells within the follicles have been described,

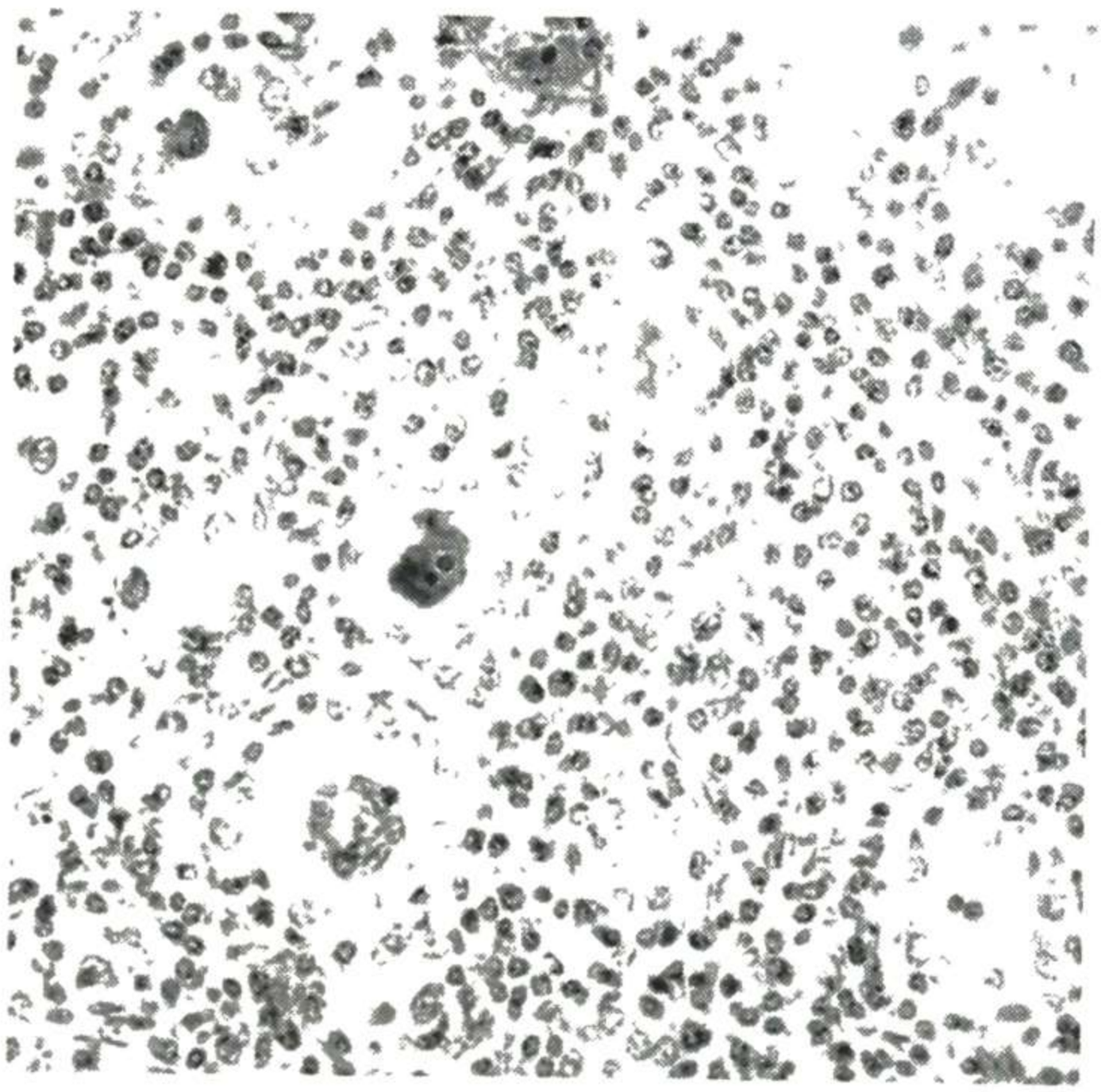


Fig. 66.—Normal thyroid tissue with macrophages in follicles. Colloidophages; reduced from × 400. (Courtesy of Hellwig, C. A.: Science 113:725-726, June 22, 1951.)

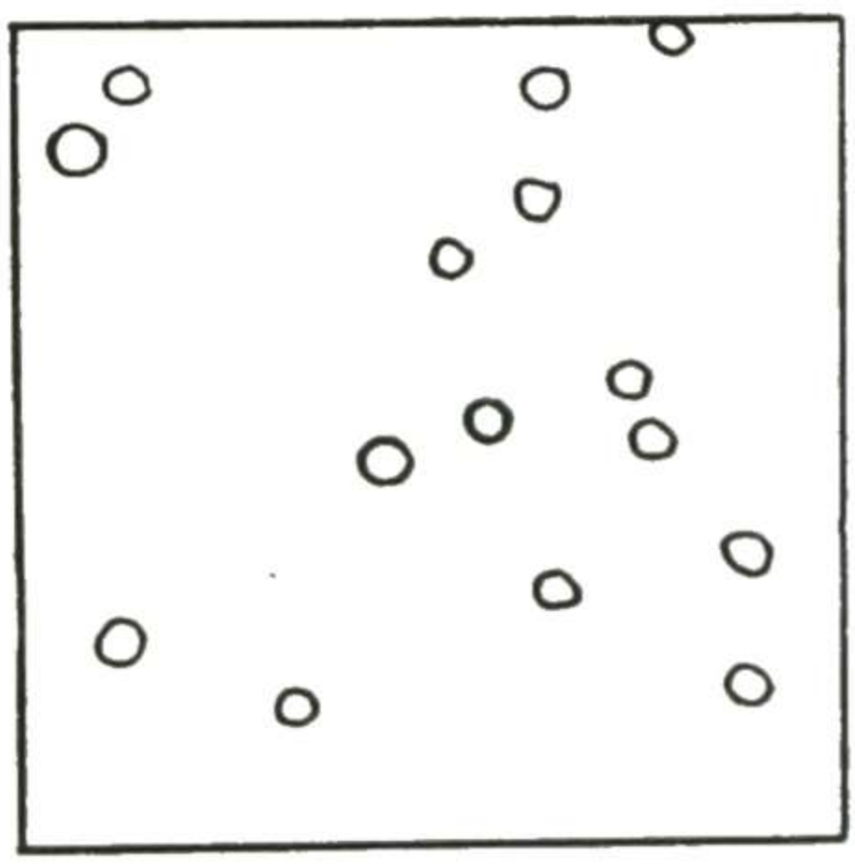
and have usually been regarded as desquamated cells of the follicle wall.

Of 435 patients who underwent thyroidectomy for goiter, 63.9 per cent had such cells in the colloid material. They were present in 16.2 per cent of 619 normal thyroid glands obtained by autopsy. There were 1-20 cells in one lumen. The cells were large, with eosinophilic cytoplasm and an oval or kidney-

(4) Science 113:725-726, June 22, 1951.

shaped nucleus. No evidence could be found to suggest origin from follicular epithelium. When fresh goiter material was teased and stained supravitally with neutral red, 11 of 23 specimens showed typical macrophages filled with large salmon-red granules in the follicles. They were also present in the interfollicular tissue. The epithelium was unstained.

Normally, macrophages containing colloid re-enter the blood vessels. In exophthalmic and lymphadenoid goiter, they fuse together in the follicular lumen, forming large multinuclear syncytial masses (Fig. 66). The colloid-containing macrophages may not reach the blood stream but degenerate



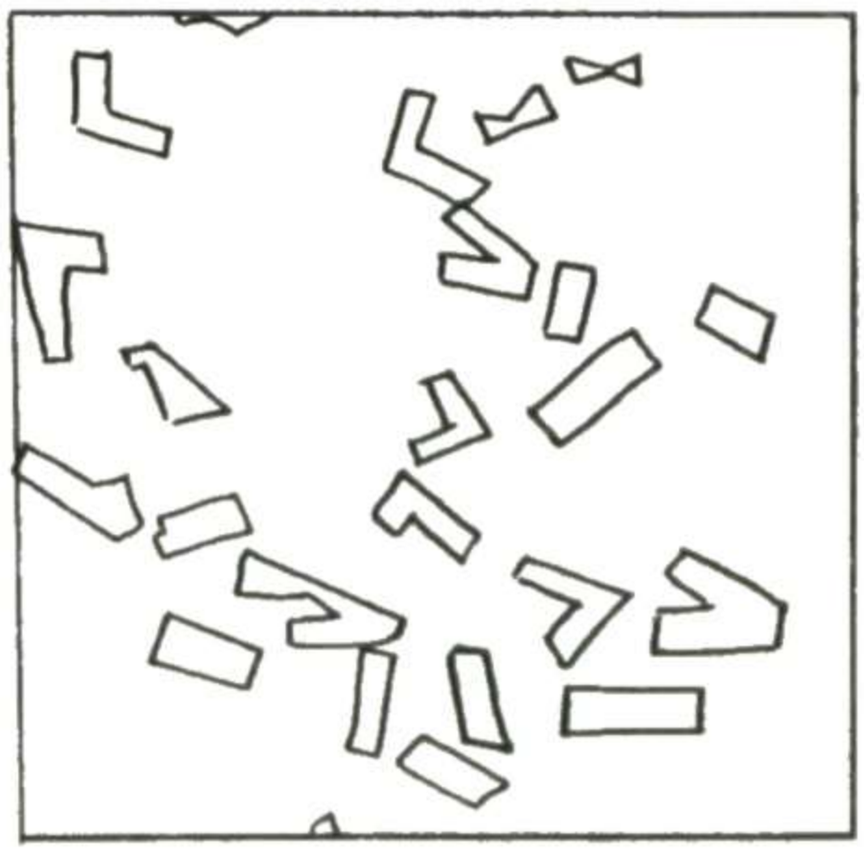


Fig. 67 (left).—Colloid of normal thyroid tissue, without colloidophagy. Camera lucida drawing of original electronmicrograph.
Fig. 68 (right).—Colloid of lymphadenoid goiter with colloidophagy. Camera lucida drawing of original electronmicrograph.
(Courtesy of Hellwig, C. A.: Science 113:725-726, June 22, 1951.)

in the interfollicular tissue. The liberated colloid attracts lymphocytes, and masses of red-stained colloid material remain in the center of lymph follicles.

Colloidophagy is apparently the underlying cause of so-called chronic thyroiditis. Overstimulation of the thyroid by thyrotrophic hormone may change the properties of the colloid in such a way that it attracts macrophages. In the electron microscope, normal colloid appears to be composed of globules about 25 mμ in diameter (Fig. 67). The colloid of lymphadenoid goiter in man (Fig. 68) and of rabbit thyroids stimulated by thyrotrophic hormone shows angular, wedge-shaped and sharp-edged particles about 160 mμ long.

[After publication of the paper, Dr. Hellwig received a letter from Dr. Washington Buño of Montevideo, Uruguay, who wrote that he had described the process in 1943 and had related it not only to chronic thy-

roiditis but also to so-called Hashimoto's struma. Dr. Hellwig reviewed a translation made for him from the Spanish and points out that Buño believes that monocytes attack the colloid only after inflammatory destruction of the follicular wall. Hellwig looks on the inflammation as of chemical origin and believes that the migration of the phagocytes is, in a sense, chemotactic, perhaps because of a change in the colloid due to a mixture of phospholipid. We have no desire to assess priority but, in any event, Hellwig's independent observations first drew attention to the matter in American literature.—Ed.]

Chronic Thyroiditis: Supravital Studies of Surgical Goiter Specimens. V. E. Chesky, W. C. Dreese and C. A. Hellwig[5] (Halstead, Kan.) report observations which support the concept that chronic thyroiditis is a chemical inflammation. In most of 54 goiters examined after supravital staining with neutral red, large cells with salmon-red granules in the cytoplasm were found within the follicles or interfollicular tissue. In paraffin sections of thyroid gland, aggregations of lymphocytes associated with follicles containing these large macrophages were often found. The cells were large with abundant eosinophilic cytoplasm and oval nuclei. The number varied from 1 to 20 in one follicle.

Invasion of intrafollicular colloid by these wandering cells, known as colloidophagy, is an outstanding characteristic of chronic thyroiditis, particularly lymphadenoid goiter. Development of chronic thyroiditis can be followed by the morphologic changes of the gland. In the early stage, one or several macrophages enter the colloid, which appears unchanged by histologic staining methods. The colloid of the involved follicle begins to disappear, and the macrophages increase in size and stain more intensely (Fig. 69). Later the follicles collapse and the lining epithelial cells are higher than the cells of the noninvolved follicles. Within the diminished lumen a syncytial mass of cells is formed by fusion of the macrophages, which have pyknotic nuclei. Some of the colloid-filled macrophages re-enter the interfollicular stroma but do not reach the circulation. They degenerate in the stroma, liberating colloid which attracts lymphocytes (Fig. 70). The lymphocytes accumulate around the colloid plaques. If the lymphocytic infiltration progresses, the follicles within the area are so compressed and isolated that they undergo atrophy and degeneration. Terminal fibrosis follows destruction of the thyroid epithelium. An excess of thyrotrophic hormone or increased sensitivity of the

(5) Surg., Gynec. & Obst. 93:575-580, November, 1951.

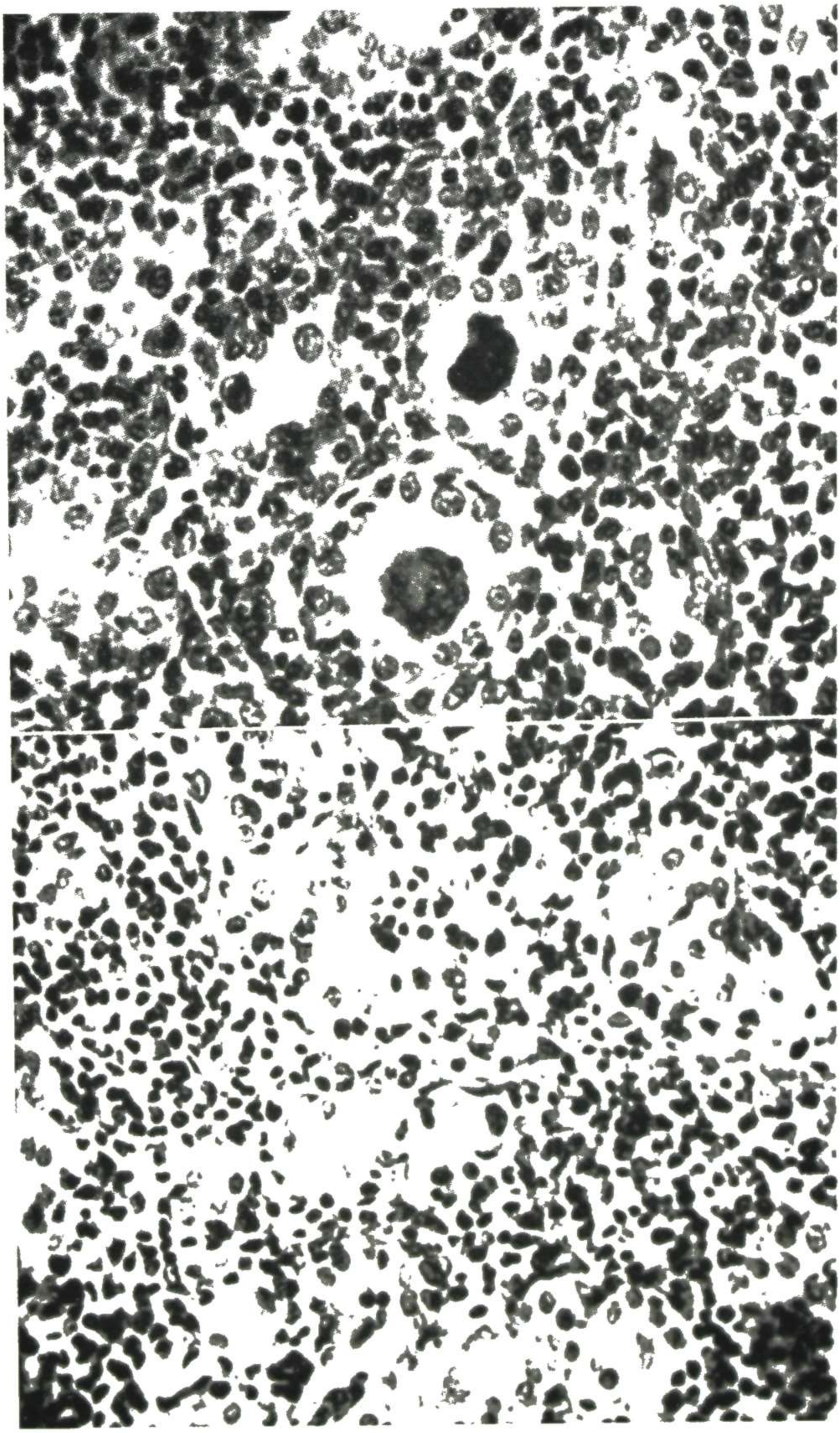


Fig. 69 (top).—Resorption of colloid by monocytes. (Second stage of colloidophagy.)

Fig. 70 (bottom).—Colloid remnants in interstitial tissue attracting lymphocytes. (Courtesy of Chesky, V. E., *et al.*: Surg., Gynec. & Obst. 93:575-580, November, 1951.)

thyroid cell to the hormone probably changes the properties of the colloid so that it attracts these mononuclear cells by chemotaxis.

Nodular Goiter and Carcinoma of Thyroid. Edwin R. Fisher and Bernard Fisher[6] (Univ. of Pittsburgh) analyzed 38 cases of thyroid carcinoma. Incidence of carcinoma in all thyroidectomies was 1.6 per cent. Carcinoma was present in 3.7 per cent of patients with nontoxic nodular goiter. Average age of patients was 49; two thirds were women. In addition to enlargement, which was of firm consistency in only 60 per cent of the patients, most frequent symptoms were local manifestations of pressure. Enlargement was rapid in 13 patients, and tumefactions were of long standing in 13. A solitary nodule palpable before operation, present in 33 patients, was unassociated with other nodules at surgery in 21 but was found to be one of several nodules in 12. Diagnosis of carcinoma was made in only 10 patients before surgery. Carcinoma was recognized or suspected in only 65 per cent of patients by the time of gross examination of the specimen, diagnosis depending on microscopic study in the rest.

The criterion for microscopic diagnosis was the demonstration of capsular, lymphatic or venous invasion by the tumor associated with some degree of cytologic change. Papillary adenocarcinoma was present in 15 thyroids. Lymphatic vessel invasion was frequent. Lateral aberrant thyroid tumors were the presenting symptom of three patients. Survival time was longest in patients with papillary adenocarcinoma. Of 12 patients followed only 4 died of the disease; the rest had apparent freedom from recurrence up to 16 years. Of the tumors removed from these long survivors, lymphatic vessel invasion was present in all and vascular invasion in one. In all but one of nine patients with alveolar adenocarcinoma, nodular goiter had been present for years and vascular invasion was common. Only two of the nine lived as long as one to two years after operation. Death of the others was usually due to metastases. Small cell type carcinoma was found in 12 patients. These tumors were composed of solid masses of polyhedral cells with pale cytoplasm and hyperchromic nuclei. Invasion of vascular and lymphatic vessels as well as capsule and skeletal muscle was usual. Of nine patients followed, five died less than 18 months postoperatively,

(6) Am. J. Surg. 82:202-208, August, 1951.

three lived 1 year after surgery and one had recurrence after 2 years. Of the 38 patients, 1 had epidermoid carcinoma and 1 Hürthle cell tumor.

Thyroid neoplasms probably arise from the parenchyma of the gland itself rather than from a pre-existing adenoma. Because of the difficulty in recognition of malignant nodules, nodular goiter is primarily a surgical problem. Patients with thyroid carcinomas not suspected before operation have the longest survival period. Death within one year of confirmed diagnosis of the clinically obvious cases of all histologic types is common. The survival prognosis in carcinoma of the thyroid is not significantly related to the microscopic presence or absence of vascular, lymphatic or capsular invasion but seems associated with the histologic type of tumor and degree of differentiation.

Nodular Goiter and Malignant Lesions of Thyroid Gland. Oliver H. Beahrs, John DeJ. Pemberton and B. Marden Black[7] reviewed data on 360 cases of malignant lesion of the thyroid seen at Mayo Clinic during 1938-47. The findings support the belief that clinically unrecognized carcinoma occurs often enough in nodular goiter to warrant thyroidectomy in most cases. In 284 cases, diagnosis was verified histologically. In 76, the lesion was considered inoperable or diagnosis was established from tissue removed elsewhere and no further treatment was thought indicated. The lesions were classified as: papillary carcinoma, 174 cases; carcinoma in an adenoma, 52; diffuse adenocarcinoma, 50; epithelioma, 3, and sarcoma, 5. Incidence of tumor type in this series differed from that in a series seen in 1907-37. In the present series papillary lesions formed 61.3 per cent of the total cases, whereas in the 1907-37 series they had made up only 30 per cent. Incidence of carcinoma in adenoma and diffuse adenocarcinoma decreased in the present series.

Carcinoma of the thyroid may occur in young persons. About 15 per cent of the papillary adenocarcinomas were found in patients under age 20 and over 30 per cent in patients under age 30. Such lesions are not limited to the younger age groups, however; 36 per cent of the patients were age 50 or older. Incidence of males to females in the entire series was 1:2.

No significant relation could be demonstrated between

(7) J. Clin. Endocrinol. 11:1157-1165, October, 1951.

incidence of carcinoma and presence of a single nodule palpable in the thyroid. Carcinoma was found in 4.8 per cent of all cases of nodular goiter (5,679), a term designating all thyroid lesions which were nodular on clinical examination. It was present in only 0.5 per cent of 3,029 cases of exophthalmic goiter. Carcinoma was found in 1.2 per cent of 2,229 cases of adenomatous goiter with hyperthyroidism and in 7.5 per cent of 3,247 cases without hyperthyroidism. There was no clearcut correlation between duration of goiter and presence of carcinoma. In only 27 per cent of the cases was there any evidence in the clinical record that the goiter had recently changed in any way. There was no relation between size of the goiter when the specimen was weighed and type of carcinoma.

In 44.5 per cent of the cases of carcinoma, the clinical diagnosis was benign goiter. About half of the papillary lesions, two thirds of the malignant adenomas and one fifth of the diffuse adenocarcinomas were not suspected on clinical examination.

[This and the preceding article emphasize the view that nodular goiter is potentially dangerous and also the fact that, in many instances, carcinoma is not suspected clinically. Perhaps methods for clinical diagnosis will be developed. Of importance also is the disclosure that a supposed solitary nodule is proved on pathologic examination to be one of several. This renders difficult the acceptance of the statement frequently made that in about one fifth of the cases a solitary nodule is cancerous. The survival of so many patients with thyroid carcinoma means that this disease behaves differently from other glandular carcinomas, that the pathologic criteria need revision or that surgical excision is especially complete in this organ. Pathologists should carefully evaluate the natural history of the neoplasm. In the near future, S. Warren and W. A. Meissner's fascicle on "Tumors of the Thyroid" of the *Atlas of Tumor Pathology* will appear. —Ed.]

Myxedema: Autopsy Report with Histochemical Observations on Nature of Mucoid Infiltrations is presented by Douglas B. Brewer[8] (Univ. of Birmingham).

Man, 56, had a clinical picture of advanced myxedema. At autopsy, patches of fibrosis were scattered through the myocardium. In a few fibers in the heart muscle the central portion of sarcoplasm was replaced by amorphous, faintly basophilic material. The epithelium of the tongue was slightly thickened, and there was mild lymphocytic infiltration of the supporting tissue. Between this zone and the deeper muscle there was a broad band of mucoid infiltration lying parallel to the surface. The epidermis was atrophic, and the superficial dermis showed a slight diffuse infiltration with a basophilic mucoid substance. The thyroid was replaced by dense fibrous

(8) J. Path. & Bact. 63:503-512, July, 1951.

tissue containing a few islands of densely packed lymphocytes. The adrenal cortex was thinned, and the zona glomerulosa not sharply defined.

The infiltration in the tongue and myocardium stained positively by the periodic acid-Schiff (P.A.S.) method, as did the mucins in control material — sections of umbilical cord, bronchus, submaxillary gland and cornea. The infiltration in the tongue was strongly metachromatic with toluidine blue. When the section of tongue was exposed to hyaluronidase be-

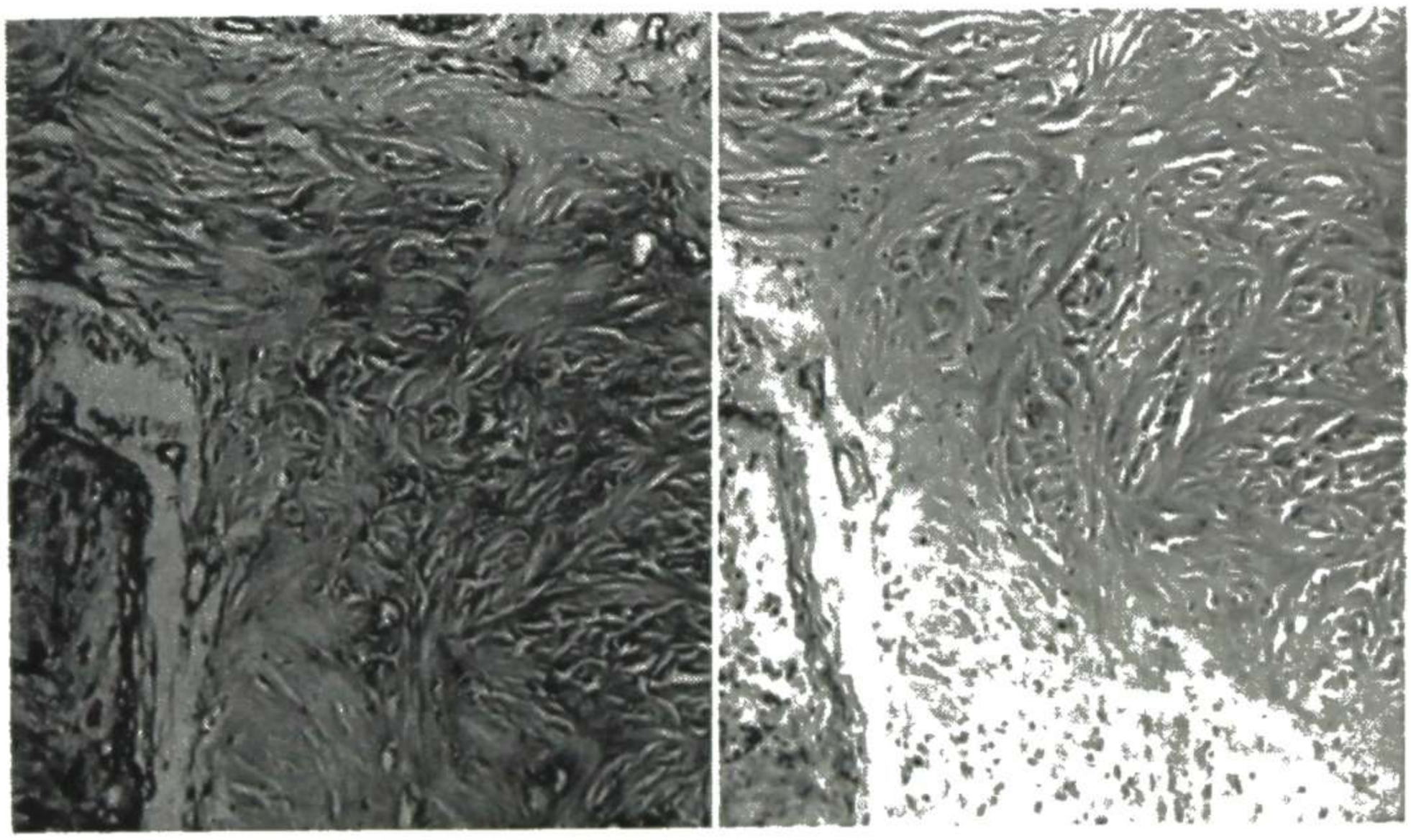


Fig. 71 (left).—Periphery of area of mucoid infiltration in tongue. Control section incubated in saline for 24 hours. Toluidine blue; green filter; reduced from × 100.

Fig. 72 (right).—Adjacent section to that in Figure 71. Incubated with hyaluronidase for 24 hours. Toluidine blue; green filter; reduced from × 100.

(Courtesy of Brewer, D. B.: J. Path. & Bact. 63:503-512, July, 1951.)

fore staining with toluidine blue, the metachromatic reaction at the periphery of the area was slightly reduced, whereas that at the central portion was unaffected by hyaluronidase (Figs. 71 and 72). The metachromasia exhibited by the interstitial substance of the umbilical cord and of the substantia propria of the cornea was almost completely abolished by hyaluronidase. Bronchial cartilage retained metachromatic staining after exposure to hyaluronidase. Staining of the myocardial infiltration by the P.A.S. method was not affected by hyaluronidase. Exposure to a diastase solution did not change the staining reaction of the material in the heart and tongue. Diastase destroyed the metachromatic staining of bronchial cartilage.

The isoelectric point of the infiltration was investigated by

staining with methylene blue in buffer solutions of different pH. Infiltration in the tongue took up the stain at pH 2.5, but the material in the heart required a pH 6. Mucins in the bronchial cartilage took up the dye at a pH 2.5 and mucins of the umbilical cord, submaxillary gland and cornea stained at pH 5. Infiltration in the skin and tongue but not the heart stained with Southgate's mucicarmine.

Brewer concluded that the infiltrate in the tongue was probably a mixture of mucoproteins containing hyaluronic and chondroitin sulfuric acid but that the material in the myocardium was a histochemically distinct mucoprotein. It is suggested that the increased interstitial fluid represents a binding of water by mucoprotein in the form of a hydrophilic suspensoid.

[This is another example of excellent work in histochemistry. When the methods become quantitative, results will improve. This would be valuable in examinations of skin and subcutaneous tissues because hyaluronic acid is a normal constituent. In Brewer's case, the amount of skin and the limited myxedema permitted only the observation that the interstitial material is positive by the P.A.S. method and is metachromatic with toluidine blue.—Ed.]

Suprarenal Hemorrhage and Necrosis in Pregnancy. Margaret D. Crawford[9] (St. George's Hosp., London) reports observations on 14 pregnant or puerperal patients in whom adrenal hemorrhage with or without necrosis of the cortex was found at autopsy. The patients had a variety of the more severe complications of pregnancy with accompanying metabolic disturbances. The condition did not seem to be related to labor, but toxemia of late pregnancy appeared to be a predisposing factor.

In over half of cases both adrenals were equally involved. In the others one gland was more affected than the other, and in one case a unilateral lesion only was found. The change was always confined to the cortex. Early in severe cases the glands were swollen and plum colored; later they tended to be more brick red. Often an associated retroperitoneal edema was present, sometimes blood stained.

Microscopically, the different lesions in any particular case appeared to be of about the same duration. Two main histologic types of lesions were seen: multiple hemorrhagic and necrotic. Hemorrhages ranged from small patches associated with hyperemia of the zona fasciculata to complete disruption of the cortex. In mild cases the essential change

(9) J. Path. & Bact. 63:365-376, July, 1951.

was hemorrhage, often related to masses of swollen vesicular cortical cells, some of which showed signs of disintegration. In severe cases hemorrhages became confluent, sometimes involving the entire cortex. When patients had survived for 24 hours, the cortical cells showed signs of degeneration near the center of the lesion. Yellow pigment was deposited around the lesion, and polymorphonuclear leukocytic infiltration occurred at the periphery. At a later stage necrosis of the cortical cells was the outstanding feature. In the necrotic type the cortex showed massive degeneration. The entire cortex was sometimes necrotic, except for a few groups of cells in the zona glomerulosa. In the oldest lesions nuclear staining was largely absent, the cortical cells remaining as ghosts, outlined by the columnar fibrous structure which was not disrupted. Endothelial and fibroblastic proliferation and polymorphonuclear infiltration of varying extent was manifested in different cases. Arterial thrombi were not found in any case. Venous thrombosis was found in three. In one instance thrombosis of a central vein was associated with cortical hemorrhage, but there was also hemorrhage in the opposite gland without venous thrombosis.

No obvious local primary lesion was found to account for the adrenal hemorrhages and necrosis. Patients may recover from quite extensive lesions of the adrenals and later present manifestations of Addison's disease with severely scarred or almost nonexistent cortices.

[It might be supposed that in some cases of this sort, acute adrenal cortical insufficiency would be apparent clinically. Although the matter is discussed in the paper, no clearcut evidence is adduced. Further study at St. George's and other clinics may provide more satisfactory clinicopathologic correlation.—Ed.]

Human Adrenal Cortex after Administration of ACTH and Cortisone: Morphologic Changes which might serve to indicate the functional state of the cortex are described by Ward M. O'Donnell, Stefan S. Fajans and Jack G. Weinbaum[1] (Univ. of Michigan). Concentration of sudan-stained lipids in cortical cells was used as an indication of storage and secretion of adrenocortical hormones or their precursors.

The adrenals of nine patients who died of various diseases up to 23 days after receiving ACTH were examined. Total dosage was 40-3,785 mg. Reduction of lipids and hypertrophy of cells in the outer zona fasciculata were early changes. In the

(1) A.M.A. Arch. Int. Med. 88:28-35, July, 1951.

patient who received the largest dose, loss of sudanophilic material was apparent in all layers of the cortex. In some patients cells of the zona fasciculata and reticularis were hypertrophic. The zona glomerulosa appeared narrow, ill defined

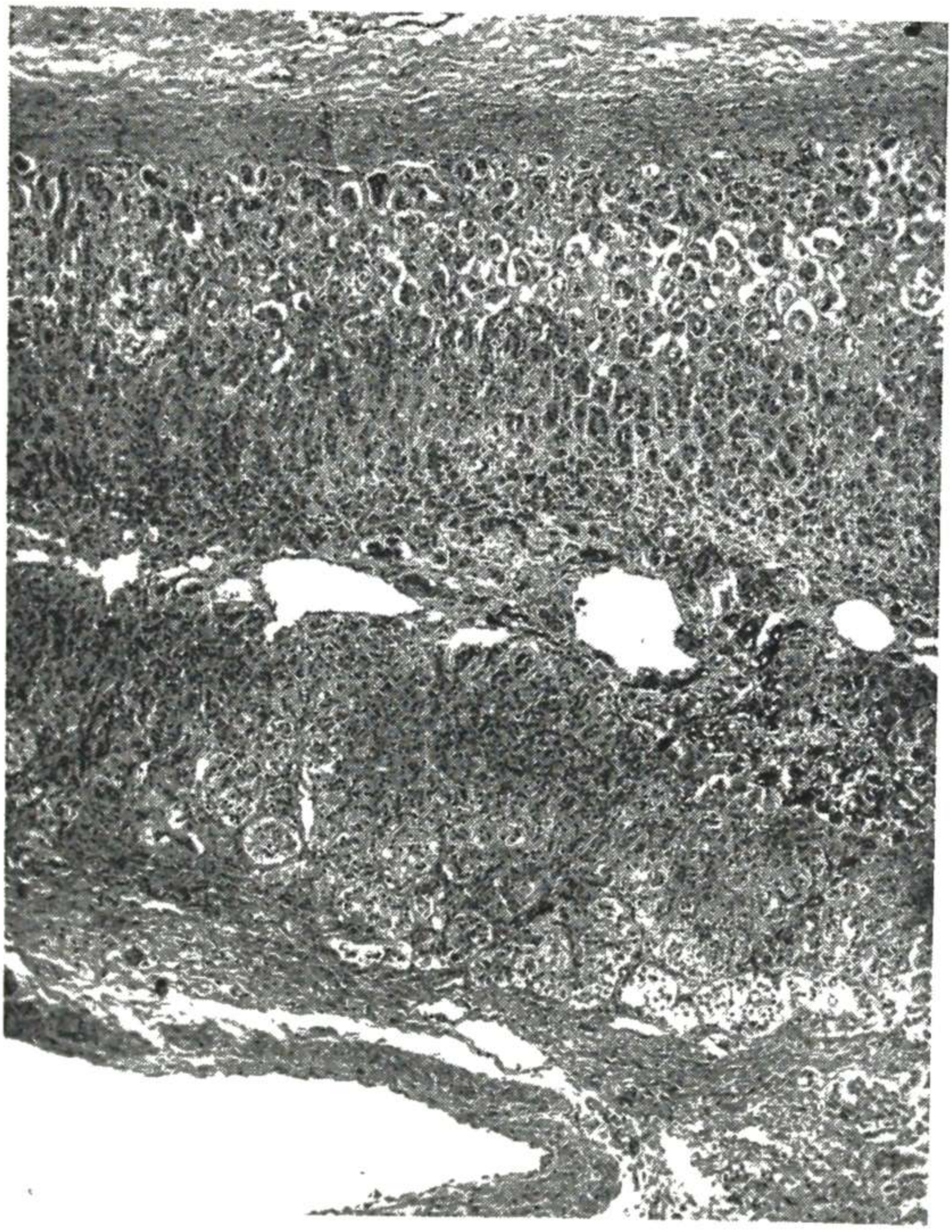


Fig. 73.—Adrenal gland after administration of cortisone. Hematoxylin-eosin; reduced from × 56. (Courtesy of O'Donnell, W. M., *et al.*: A.M.A. Arch. Int. Med. 88:28-35, July, 1951.)

and less prominent than in a normal gland. Some of its cells were normal in size, others hypertrophic.

The adrenals of five patients who died of different diseases and who had received 1-6,400 mg. cortisone were studied. Cells of the zona fasciculata and reticularis were atrophic (Fig. 73). Abundant brown pigment was seen in the cells of the reticular layer. The zona glomerulosa was broadened; its cells were normal in size. Sudanophilic substance, although reduced in concentration, was seen in the cells as intracellular globules or fine granules. In one case adrenals were examined 51 days

after cortisone had been discontinued; the zona glomerulosa was of normal width and the lipids showed a normal pattern. When stress is a factor, evidence of hyperfunction follows the return to normal of the adrenal when cortisone is discontinued. The cells of the fascicular and reticular layers undergo hypertrophy and lipid content of the entire cortex is reduced.

Cortisone may induce adrenal atrophy by suppressing secretion of pituitary adrenocorticotrophic hormone. The morphologic return to normal after it is discontinued is evidence that the effect of cortisone on the adrenal is reversible. After return to normal the organ may even respond to stress. The alteration of the adrenal cortex after administration of cortisone is similar to that seen in patients with clinical and autopsy evidence of hypopituitarism. The accumulation of lipid material associated with cell atrophy of the inner cortex is regarded as evidence of storage of adrenal steroids or their precursors. Lipid depletion and cortical cell hypertrophy are interpreted as indications of increased secretory activity of the adrenal cortex. Abundant cortical lipid associated with pronounced cortical hypertrophy is thought due to gradual and progressive stimulation. The observations indicate that the zona glomerulosa is stimulated by ACTH. Morphologic changes due to ACTH resemble those that result from certain types of stress. The morphologic pattern of the adrenal cortex after administration of ACTH or cortisone depends on the preceding state of the cortex, dosage, preparation used and interval between cessation of administration and death.

Feminizing Adrenal Tumor in an Adult Male. Henry Mortensen and Leonard Murphy[2] (Melbourne, Australia) report the 14th case.

Man, 38, had gynecomastia in 1949. The breasts had been enlarging for 18 months. Loss of libido had been present for a year. Physical examination showed a normal masculine physique without adiposity. The breasts and nipples were well developed; genital organs, small and atrophic. Serum sodium value was 288-320 mg./100 cc.; chloride, 468-480 mg./100 cc. Result of an Aschheim-Zondek test was negative, that of a glucose tolerance test was not remarkable. Urinary excretion of 17-ketosteroids was 7.6 mg./24 hours. X-rays of the pituitary fossa and chest were negative. Perirenal insufflation of air showed a normal left adrenal gland and a right adrenal which was oval and of a density suggesting a rounded tumor. Through a transthoracic approach a tumor of the right adrenal, slightly smaller than a golf ball, was removed. Macroscopically the cut surface was

(2) J. Urol. 65:709-714, May, 1951.

yellow and homogeneous; the tumor was surrounded by a well defined capsule. Microscopically the cells varied in size and appearance, some resembling cortical cells whereas others showed cytoplasm which stained more deeply with eosin. In some cells there were two or three nuclei. Size of the breasts began to decrease within 10 days postoperatively. Libido returned. When seen five months after operation, he was symptom free and there was no evidence of metastases.

This is the fourth patient reported to have survived excision of such a tumor and to have been free from metastases at the time of report.

Vasopressor Components of Pheochromocytomas. Analysis of pressor amine content of two pheochromocytomas and study of the metabolism of the hypertensive agents in these and a third tumor are reported by Karl H. Beyer, Charles A. Ross, Virgil D. Wiebelhaus, William S. Waller and Grace S. Schuchardt[3] (Glenolden, Pa.).

The thoracolumbar division of the autonomic nervous system activates organs by humoral transmitters. Epinephrine is one of these chemical mediators. Nor-epinephrine may also arise from sympathetic stimulation. It is likely that adrenergic nerves are capable of transmethylating l-arterenol more or less quantitatively, and the amount of l-epinephrine appearing after stimulation of such nerves depends on the rate of synthesis. Recent evidence indicates that transmethylation of l-arterenol in the adrenal medulla may be impaired in pheochromocytomas, leading to an accumulation of nor-epinephrine. Another vasopressor component of pheochromocytomas has been described as differing from l-arterenol and epinephrine. It was thought to have the nature of hypertensin principally because its pressor effect was not abolished by previous administration of an adrenolytic agent, dibenamine.®

History and physical findings of the three cases were consistent with diagnosis of pheochromocytomas. Before surgical removal of the tumor in one, up to 1.5 cc. of 1:1,000 epinephrine solution subcutaneously had no effect on the blood pressure but 2-2.5 cc. elevated blood pressure, whereas in another, 0.5 cc. had no effect but 1 cc. caused a rise from 130/100 to 210/140 in five minutes. In the former, injection of 10 mg. acetyl-beta-methylcholine subcutaneously produced an alarming rise in blood pressure, and amyl nitrite had its usual vasodilator effect. In this case, postoperative injection of as little

(3) Ann. Int. Med. 35:117-133, July, 1951.

as 0.25 cc. epinephrine solution caused a pronounced rise in the blood pressure.

Portions of the three tumors were frozen and homogenized extracts kept stable under carefully controlled conditions. Chemical analysis was done by the idoadrenochrom method and relative concentration of the substance determined. This method does not distinguish between beta-(3,4-dihydroxyphenyl) ethylamines such as l-arterenol and epinephrine, but excludes all unsubstituted phenyl and monophenolic analogs. Inactivation of the adrenal extract by amine oxidase and phenol oxidase was studied. Pressor assays were performed on dogs. All chemically determinable content of the tumor homogenate was in a freely diffusible form, unbound to any protein complex.

Vasopressor effects demonstrated in the extracts of two tumors could be accounted for entirely on the basis of presence of l-epinephrine, l-arterenol or their precursors. This was demonstrated by abolition of all pressor effects when extracts were incubated with amine oxidase and phenol oxidase. Destruction of all pressor activity by both enzymes indicates that the active principle of the tumors was catechol alkylamines such as l-epinephrine or l-arterenol. Hypertensin is not inactivated by either phenol oxidase or amine oxidase.

The most reliable clinical diagnostic test for pheochromocytoma appears to be intravenous injection of a small amount of histamine. If nonpsychogenic hypertension and glycosuria follow, it offers strong presumptive evidence of presence of pheochromocytoma. However, the sudden secondary hypertension makes the test hazardous. The decreased sensitivity to injected epinephrine supposed to exist in the presence of tumor may not be entirely reliable because both l-epinephrine and l-arterenol may be produced by the tumor. Response to injection of epinephrine can be reversed by doses of sympatholytic agents that have little effect on the precursor action of l-arterenol or hepatic nerve stimulation. When a pheochromocytoma contains a preponderance of l-arterenol, use of adrenolytic agents such as benzodioxane® or dibenamine® might not cause a fall in blood pressure.

Caution is voiced regarding type of anesthesia for operation when pheochromocytoma is suspected. Because cyclopropane enhances development of cardiac arrhythmias follow-

ing injection of epinephrine and related compounds, ethyl ether should be a more suitable anesthetic agent.

Glomus Tympanicum: Its Occurrence in Man and Its Relation to Middle Ear Tumors of Carotid Body Type. L. Zettergren and J. Lindström[4] (Upsala) studied sections prepared from a small block of bone sawed from one temporal bone from each of 10 adults. The block included the middle ear, the

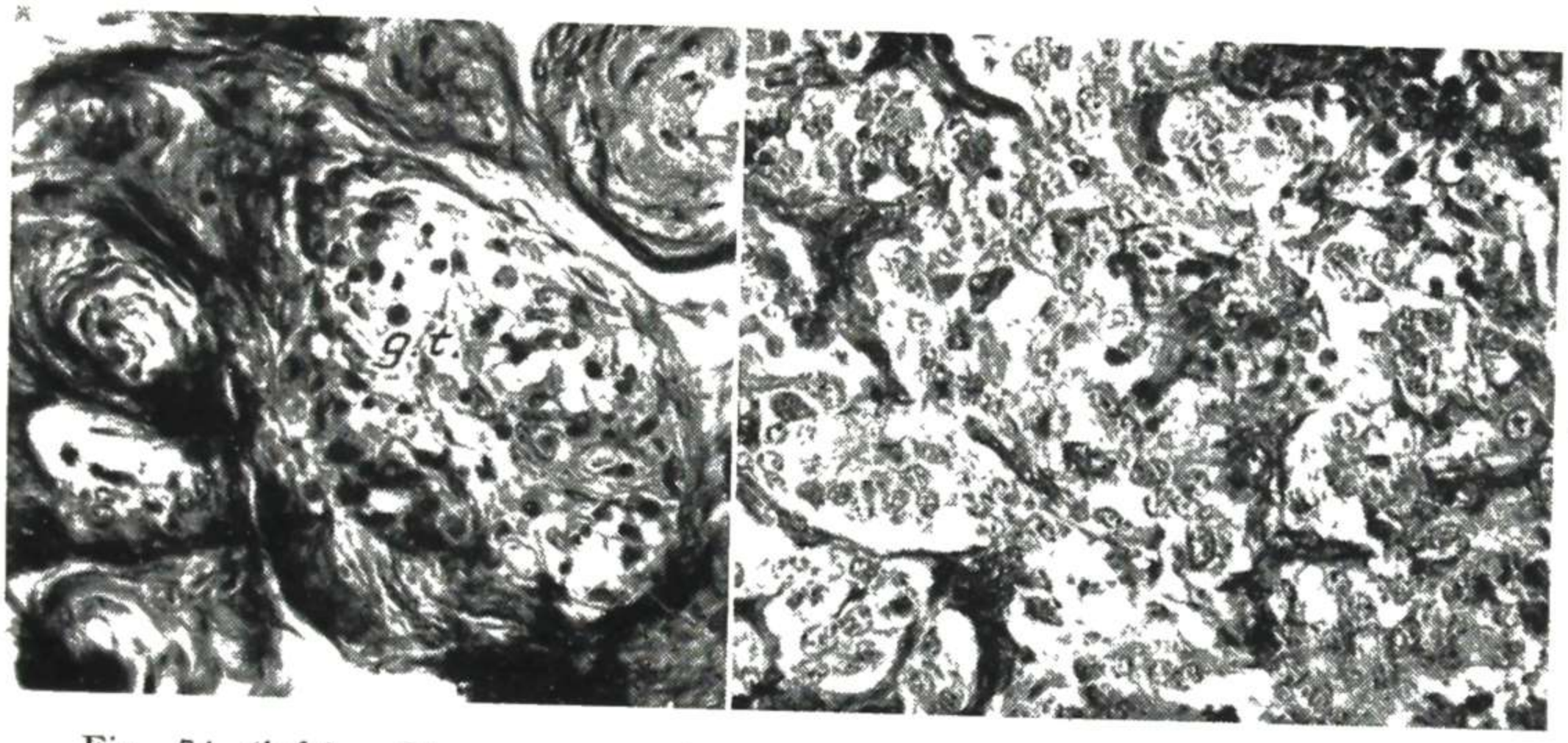


Fig. 74 (left).—Glomus tympanicum situated in middle section of canaliculus tympanicus.

Fig. 75 (right).—Section from tumor of glomus tympanicum showing striking structural similarity to matrix tissue.

(Courtesy of Zettergren, L., and Lindström, J.: Acta path. et microbiol. scandinav. 28:157-164, 1951.)

cell system of its floor, the bulb of the jugular vein and part of the glossopharyngeal nerve with its petrous ganglion. In 9 of the 10 bone preparations an organ having a histologic structure like that of the glomus caroticum was found.

The glomus tympanicum usually took the form of a single round or ovoid mass of tissue with diameters of 0.28-0.38 and 0.15-0.18 mm. and a thickness of about 0.20 mm. Occasionally the glomus was divided into two portions. In all cases it was situated near the tympanic artery and nerve. The structure resembled a ball of winding capillary and precapillary vessels separated by delicate collagenous connective tissue stroma containing numerous epithelioid cells rich in cytoplasm with round or oval nuclei fairly uniform in size (Fig. 74). The chromatin pattern of the nuclei was extremely loose and delicate.

The glomus tympanicum is probably a rudimentary organ

(4) Acta path. et microbiol. scandinav. 28:157-164, 1951.

without any function; its chief significance may be as a site of origin of a middle ear tumor of carotid body type (Fig. 75).

[This and the succeeding articles in this chapter are placed here for convenience rather than because they deal with endocrine glands. A survey of the group of tumors is found in P. M. LeCompte's fascicle on "Tumors of the Carotid Body and Related Structures (Chemoreceptor System)" of the *Atlas of Tumor Pathology*.—Ed.]

Tumor of Carotid Body Type Presumably Arising from Glomus Jugularis. James L. Poppen (Lahey Clinic) and P. A. Riemenschneider[5] (New England Deaconess Hosp.) report two cases.

Case 1.—Woman, 26, had tinnitus, deafness on the right,

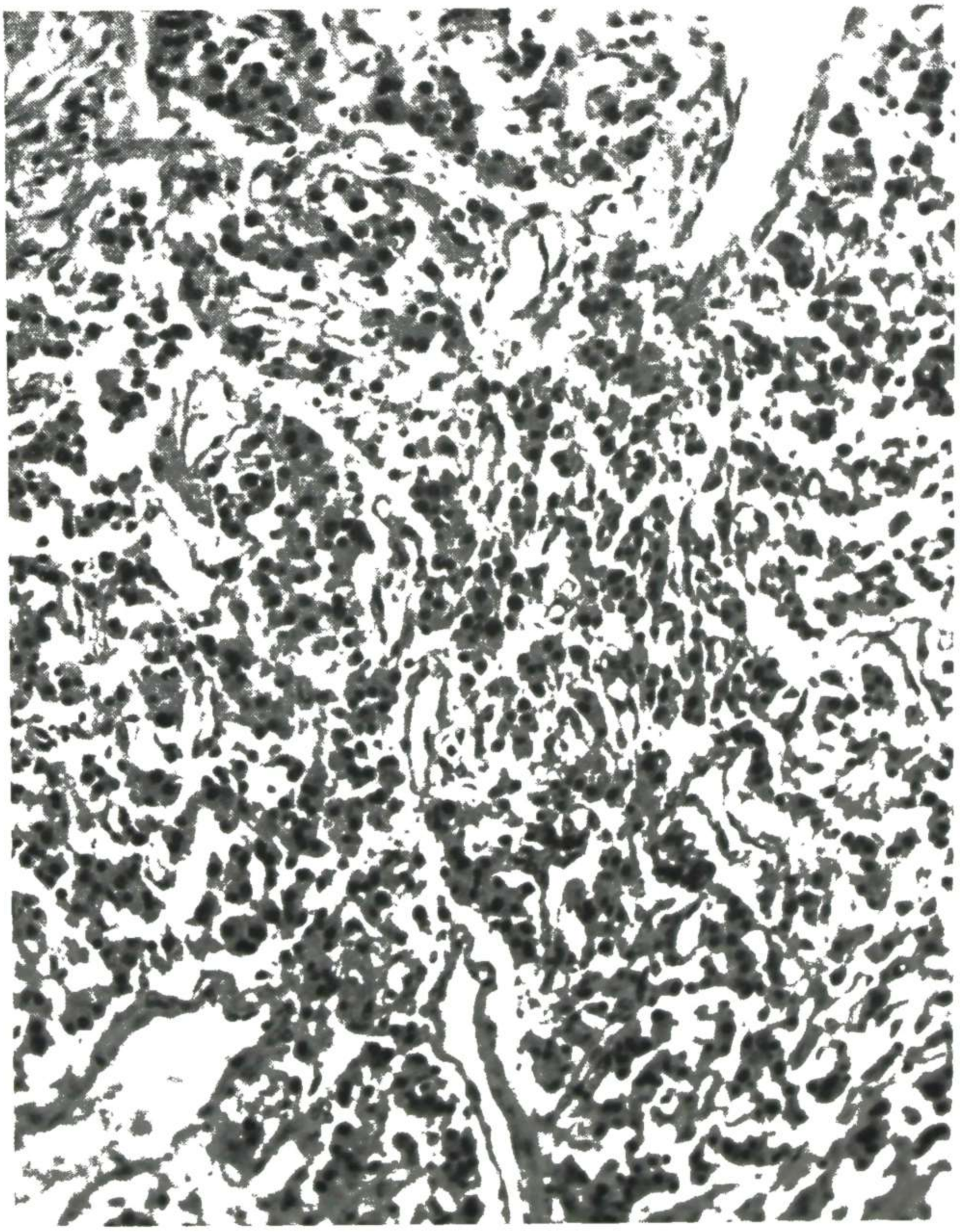


Fig. 76.—Carotid body type tumor; reduced from × 200. (Courtesy of Poppen, J. L., and Riemenschneider, P. A.: A.M.A. Arch. Otolaryng. 53:453-458, April, 1951.)

(5) A.M.A. Arch. Otolaryng. 53:453-458, April, 1951.

diplopia, vertigo and vomiting 4½ years before death. A brain abscess was allegedly drained through burr holes without improvement. Exploration four years before death revealed an inoperable vascular mass in the right cerebropontile angle. It was thought to be an angioma. Deep x-ray therapy was given postoperatively, and she improved slightly. The right vertebral artery was ligated. This was followed by two years without symptoms. Eighteen months before death the symptoms recurred, accompanied by a polypoid growth in the external ear.

Autopsy revealed a friable mass adherent to the dura, which displaced the cerebellum and medulla to the left, with involvement of the eighth to the twelfth right cranial nerves. The petrous ridge was destroyed, with extension of the tumor to the middle ear and mastoid cells. Tumor extended down as far as the right side of the pharynx. Microscopically the growth was composed of pseudosyncytial epithelioid cells which were separated into groups by a fine reticular framework, stained with silver (Fig. 76). The clear eosinophilic cytoplasm contained a few brown granules. The nuclei were pleomorphic and occasional multinucleated cells were observed. The growth was extremely vascular.

Case 2.—Woman, 63, had a 4½ year history of a pulsating tumor beneath the right mastoid. The right common carotid artery was ligated without improvement. An x-ray of the skull revealed invasion of the temporal bone and mastoid cells, with extension into the posterior pharynx. The tumor was removed together with the right internal and external carotid arteries and jugular vessels. Microscopically, it was of a carotid body type. Metastases were present in five cervical lymph nodes removed at operation. She did well after discharge, but was reported to have died a year later of a cerebrovascular accident.

Nonchromaffin Paraganglioma of Middle Ear (Glomus Jugulare Tumor). Two cases are reported by J. S. Lewis and R. N. Grant[6] (Memorial Hosp., New York City).

Case 1.—Woman, 63, had a pink-gray granular tumor filling the left external auditory canal. No involvement of the mastoid was apparent on x-rays. By a left tympanomastoidectomy by endaural approach the richly vascular growth filling the tympanic cavity was removed. Glomus jugulare tumor was diagnosed. Postoperative course was uneventful.

Case 2.—Woman, 80, had a tumor occluding the right external auditory meatus. Previously hemangioendothelioma had been diagnosed on biopsy. No bone involvement was evident. Biopsy was followed by severe hemorrhage. Histologic diagnosis was glomus jugulare tumor. The patient refused operation. Low voltage roentgen therapy, total of 1,200 r, was given without visible effect on the tumor. After 40 months, no signs of tumor extension were noted.

Early removal of such tumors is advised to prevent local bony extension. Because of the rich vascularity and origin

(6) A.M.A. Arch. Otolaryng. 53:406-410, April, 1951.

from the wall of the jugular bulb, considerable hemorrhage occurs on manipulation. This necessitates adequate exposure. Endaural approach facilitates removal by tympanomastoidectomy.

Carotid Body-Like Tumors of Temporal Bone: With Particular Reference to Glomus Jugulare Tumors. Marvin J. Tamari, Robert J. McMahon and Emil H. Bergendahl[7] (Chicago) review the literature and report two cases.

Case 1.—Woman, 73, had facial paralysis with bleeding from the right ear and loss of hearing for three months. X-ray showed mastoid destruction and temporal bone involvement. Biopsy of a hemorrhagic mass in the external auditory canal revealed a malignant neoplasm of undetermined type. At operation a vascular, friable granular mass filled the mastoid area and extended into the labyrinthine bone. Invasion was so widespread that the entire tumor could not be removed. Microscopically the surgical specimen, removed by postauricular approach, was made up of whorls and clusters of spindle-shaped, fusiform or polygonal cells, closely spaced in irregular groups. Silver stain preparations exhibited reticular fibers in the honeycomb network between cell clusters. Vascular channels of different sizes were frequently lined by tumor cells. Sequestrum formation and rarefaction of the temporal bone were present. Deep in the petrous portion tumor clusters filled vascular channels. There was little tissue reaction to tumor growth. Osteocytes were absent and no new bone formation existed in areas of destruction. The lacunae were empty; frequently haversian and Volkmann's canals were partly filled with tumor cells. Tumor infiltration had reached the promontory wall of the labyrinth just short of the eustachian tube. There was degeneration of the nerve fibers of the modiolus and ganglion of Rosenthal. Disintegration of ganglion cells was accompanied by deposition of brown pigment. Signs of meningitis developed and the patient died on the ninth postoperative day. Metastases to the liver and lungs were demonstrated. These areas were seen microscopically as small focal seedings of endothelial cells.

Case 2.—Woman, 56, had hemorrhage from the right ear for 10 years. In a few instances bleeding occurred from the nose and mouth as well. At operation the right mastoid was filled with a friable necrotic mass which extended into adjacent parts of the temporal bone. Hemorrhage prevented complete removal, and prolonged convalescence. Microscopically there was degeneration of the mastoid bone. Fibrosis was prominent. The typical picture of an endothelial glomus tumor was represented by isolated clusters of ovoid cells surrounded by argentophil fibers.

Chemodectoma ("Nonchromaffin Paraganglioma") of Mediastinum: Report of Two Cases is made by Douglas K. Duncan and John R. McDonald[8] (Mayo Clinic and Found.).

(7) Ann. Otol., Rhin. & Laryng. 60:350-364, June, 1951.
(8) Am. J. Clin. Path. 21:515-520, June, 1951.

Chemodectoma is a term used to designate a tumor composed of chemoreceptor cells which are associated with the distribution of parasympathetic nerves and structures connected with afferent nerve fibers. They may also originate in the adventitia of blood vessels connected with afferent nerve fibers, or occur along branches or in ganglions of the glossopharyngeal and vagus nerves. Only three cases of mediastinal chemodectoma have been reported previously.

Mediastinal chemoreceptor bodies are usually found near the bifurcation of the innominate artery; on the anterolateral aspect of the descending aortic arch near the left subclavian artery; near the pulmonary end of the ductus arteriosus, and on the right side and posterior surface of the pulmonary trunk (the last is a variable, poorly localized body).

Case 1.—In a youth, 18, tumor of the right thorax was discovered on routine x-ray examination. The only symptom was pain in the right upper quadrant of the abdomen. There was a rounded mass in the right posterior portion of the thorax adjacent to the ninth and tenth thoracic vertebrae, with erosion of the tenth rib. The extrapleural tumor was removed surgically. Grossly, it was round and smoothly lobulated, measuring $7 \times 5 \times 4$ cm. and weighing 50 Gm. Histologic study showed a vascular tumor composed of small and large nests of cells separated by connective tissue septa. The cells had round, vesicular, deeply stained nuclei and finely granular, eosinophilic cytoplasm. No mitotic figures were seen. Silver staining showed reticulum fibers in the septa but did not reveal any in the cells. There were scattered areas of necrosis throughout the tumor. He was living and well six years after operation.

Case 2.—In a woman, 33, routine x-ray examination disclosed a mass in the right posterior thorax adjacent to the spinal column at the level of the sixth to eighth rib. The extrapleural tumor was excised surgically. Histologically, it was identical with the tumor in Case 1. The postoperative course was uneventful and recovery satisfactory. Fourteen years later, neurologic manifestations developed which were due to destruction of the seventh thoracic vertebra. A large, ovoid paravertebral mass was seen by x-ray. Biopsy was not done.

BONES AND MUSCLES

Experimental Infarction of Bone and Bone Marrow: Sequelae of Severance of Nutrient Artery and Stripping of Periosteum from the femurs of rabbits of varying ages are reported by Lee N. Foster, Robert P. Kelly, Jr., and Walter M. Watts[9] (Lawson Veterans' Admin. Hosp., Chamblee, Ga.).

(9) J. Bone & Joint Surg. 33-A:396-406, April, 1951.

In young, rapidly growing rabbits which showed extensive infarction of bone and marrow, thickening of the cortical bone was the most noticeable of gross changes. Extensive infarction was seen throughout the middle third of the bone and marrow of the femoral shaft of all animals killed less than two months after operation. After two months, regeneration of marrow and reorganization of bone made histologic recognition of the degree of initial injury difficult, but x-ray studies confirmed presence of the lesion for as long as four months after complete or almost complete replacement of infarcted bone with viable tissue. After operation, most sinusoids in the devascularized tissue collapsed. Larger vessels contained blood but occasionally were filled with fibrin networks, and proliferating fibroblasts occluded the lumen of some smaller vessels by the fifth postoperative day. A partial blood supply could be recognized in all animals within two months after operation. In the infarcted areas myeloid elements ceased to mature, there was coagulation necrosis of endosteal osteoblasts and osteoclasts, and erythrocytes lost their usual brilliant eosinophilic character but megakaryocytes remained the most resistant of all cells to this kind of decomposition.

In these young animals, striking alteration in the normal pattern of enchondral bone formation followed the operation. Within 24 hours a well defined infarct, its base formed by the epiphyseal cartilage, could be recognized. The area of the infarct varied in size but its wedge shape was demarcated by heavy lines of hemorrhagic extravasation. Cells in the central portion of the wedge underwent coagulation necrosis. Proliferation of cartilage progressed without interruption as occluded vessels failed to resorb the growing columns of cells. By the tenth day this cartilage growth was invaded by new vessels from the periphery and within 21 days it was almost completely gone. The only remnant of the previous disturbance was a small island of unrecognized infarcted enchondral bone. Infarction of cortical bone was recognizable in the first 24 hours by fading of the nuclei of osteocytes and plugging of haversian vessels by neutrophils. Circumferential bone growth was demonstrated microscopically 72 hours after operation.

In mature animals the operation had varied effects. Persistence of marrow infarcts for five or more months accom-

panied ingrowths of fibrous tissue and occasionally stimulated production of bone in the marrow compartment. Cortical infarcts in the bones of these animals were repaired so slowly that large amounts of dead bone were present for as long as nine months after the initial injury.

[Studies such as this may give a clue to interpretation of some of the lesions of human bone which are still puzzling.—Ed.]

Generalized Hypertrophic Osteoarthropathy: Pathologic Study of Seven Cases in which all the well known clinical and x-ray manifestations were represented is reported by Edward A. Gall, Granville A. Bennett and Walter Bauer[1] (Harvard Univ.).

In the early stages the periosteum thickens and divides into two fairly distinct layers. In the outer, more densely fibrous layer, inflammatory cells accumulate in large numbers. The inner layer initially is fibrillary, loosely textured and edematous. The fibrillar material is soon replaced by deposition of osteoid between the original bone and the inner layer of periosteum (Fig. 77) with resultant displacement of periosteum from the compact bone of the cortex. As growth proceeds the osteoid matrix assumes a palisade-like structure with poorly defined homogeneous columns perpendicular to the bone's surface (Fig. 78). With continued growth the periosteum displaces itself centrifugally and the columns are further elongated. The new subperiosteal bone layer is fused with the original cortical surface and as mineralization progresses the palisaded columnar appearance is diminished or lost. Formation of a pseudocortex results. Fluctuations in periosteal function depend both on activity of the basic disease in the thorax and on local factors. Various phases of the skeletal lesions may be observed simultaneously in different bones in the same patient.

In long-standing lesions it may be impossible definitely to establish the limits of the original cortex. Generally, staining power of the two parts differs slightly and single or multiple cement lines persist at the points of fusion (Fig. 78). In the involved extremities articulations show mild to moderately severe chronic synovitis associated with nonspecific degenerative changes in the articular cartilage. All patients in this series exhibited digital clubbing. This appeared to result from

(1) Am. J. Path. 27:349-381, May-June, 1951.

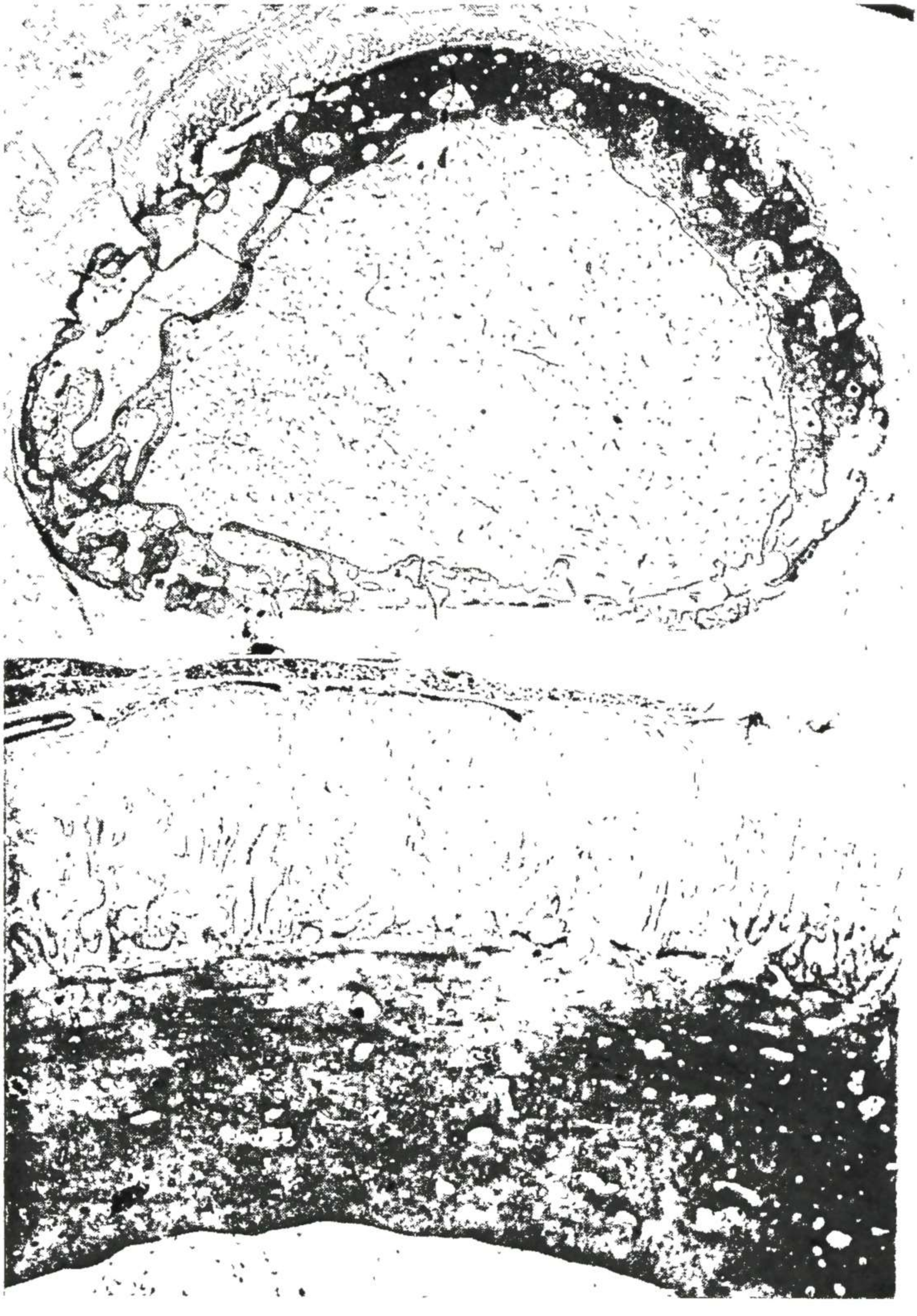


Fig. 77 (top).—Cross section through a metatarsal. Irregular deposit of new bone on the cortex shows considerable osteoporosis; reduced from × 11.

Fig. 78 (bottom).—Cross section through wall of femur. Ossification is more advanced in the deeper portions. Point of fusion with the cortex is shown by a densely staining but broken cement line. Blood vessels entering from the periosteal layer are perpendicular and form a series of arcades; reduced from × 11.

(Courtesy of Gall, E. A., *et al.*: Am. J. Path. 27:349-381, May-June, 1951.)

an increase of soft tissue bulk and was associated with few or no morphologic changes in the terminal phalanges or their periosteum.

[In addition to the detailed information given, the reports on the terminal phalanges are convincing.—Ed.]

Osseous Changes in Congenital Biliary Stenosis. Robert C. Hendrix[2] (Univ. of Michigan) reviewed the records of 12 patients, aged 6½ hours to 8 months at death. Clinical diagnosis of rickets was made in five by observation of enlarged

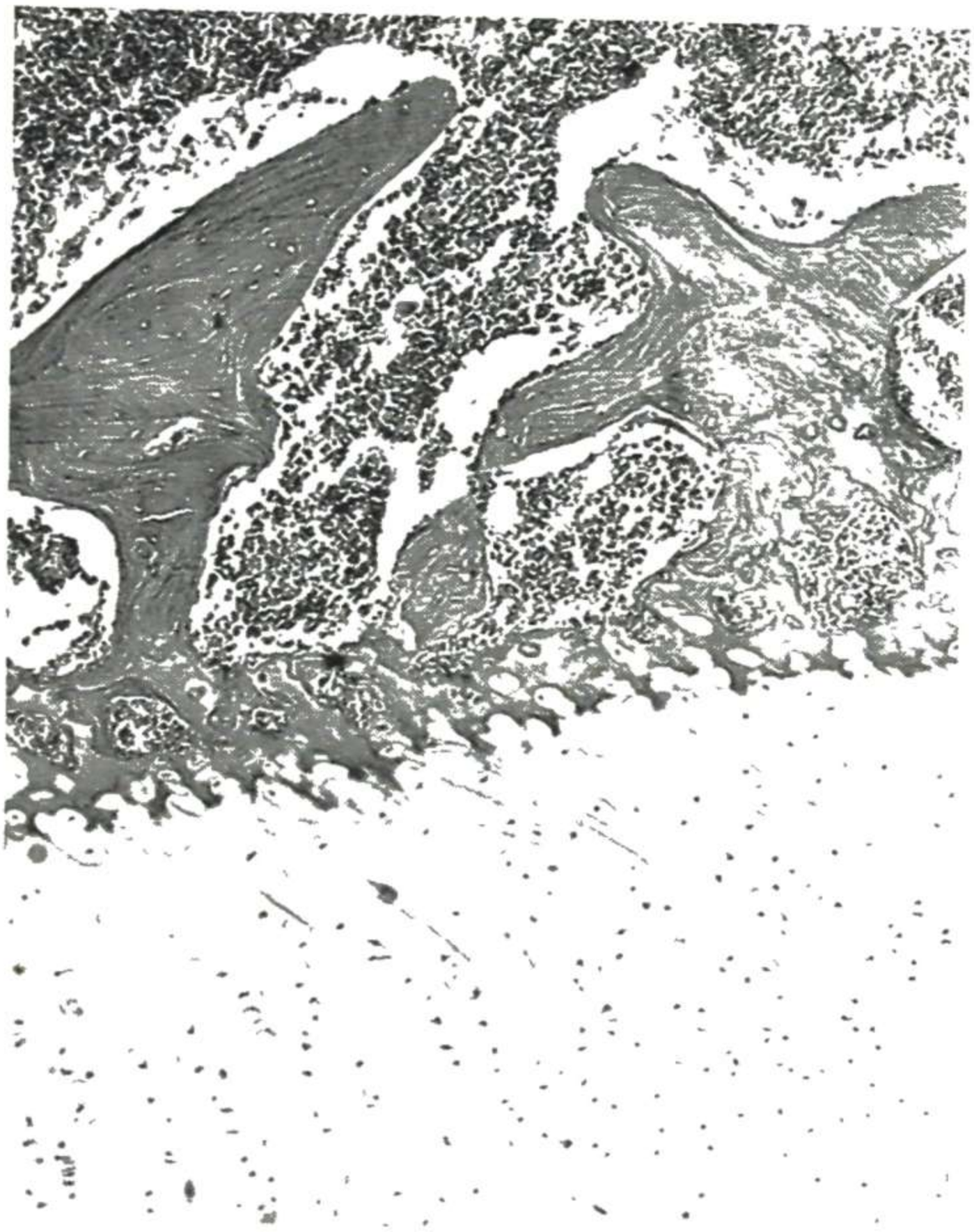


Fig. 79.—Cartilage plate of vertebral body showing long cords of large cartilage cells. Hematoxylin-eosin; reduced from × 150. (Courtesy of Hendrix, R. C.: A.M.A. Arch. Path. 51:518-526, May, 1951.)

costochondral junctions, by x-rays of the long bones or by unusual softness of the occipital bone. Rickets was not apparent clinically in the other seven, probably because of the short survival period, since six months was the earliest age at which rickets was detected. All patients but one had frank icterus at death and obstructive biliary cirrhosis of varying degree. The bile ducts were completely occluded somewhere along their course.

(2) A.M.A. Arch. Path. 51:518-526, May, 1951.

The earliest skeletal changes were found in two infants who died at 6 months. Sections of costochondral junctions showed striking irregularity of the cartilage cords, decreased calcification of the cartilaginous matrix, irregular destruction of cartilage cells, capillary buds growing into the cartilage plate and large amounts of osteoid tissue about the trabeculae. Vertebral trabeculae were covered with abundant

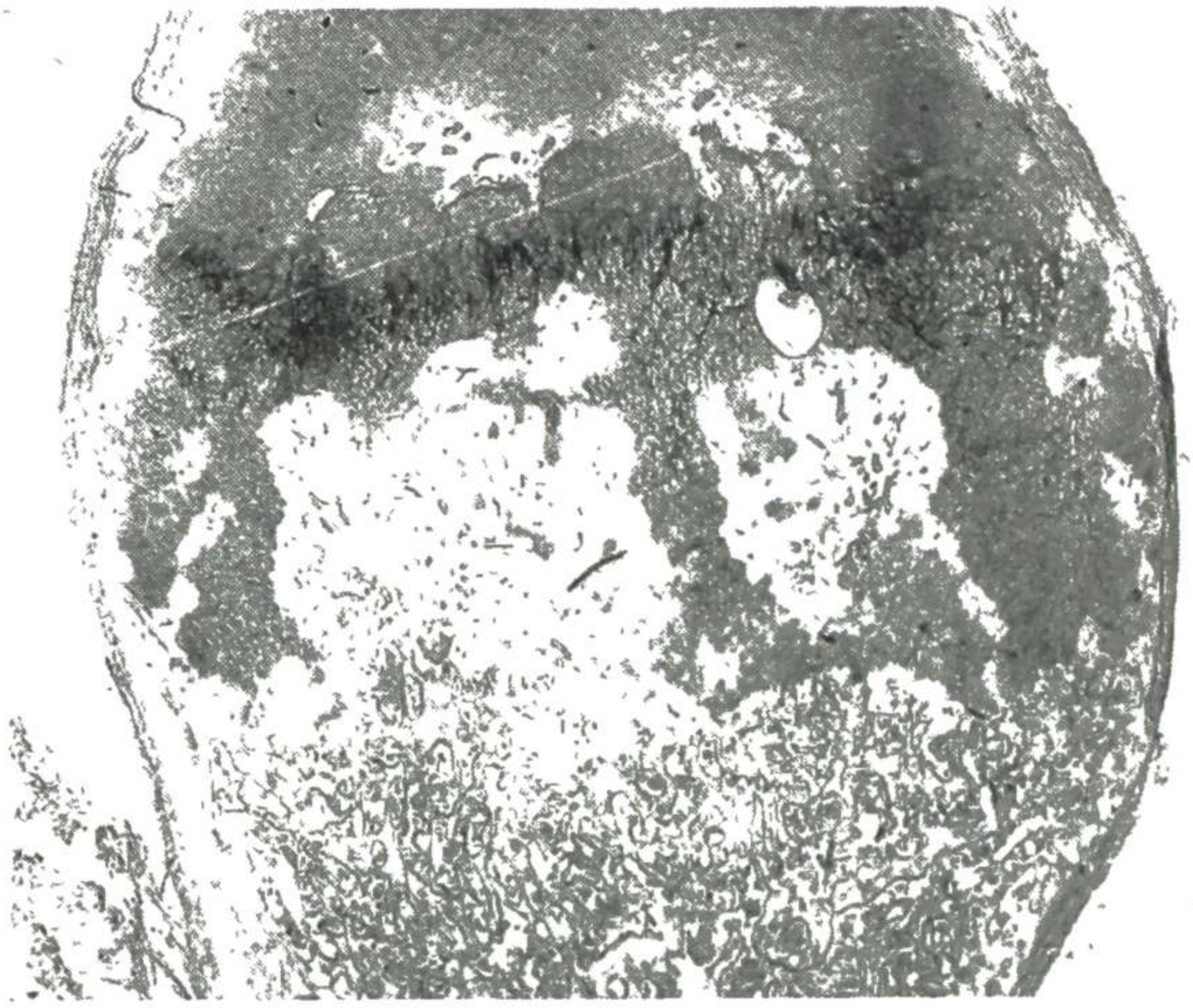


Fig. 80.—Striking rachitic changes of costochondral junction in infant, aged 8 months. Hematoxylin-eosin; reduced from × 10. (Courtesy of Hendrix, R. C.: A.M.A. Arch. Path. 51:518-526, May, 1951.)

osteoid tissue and the proliferating cartilage cells were large, arranged in long cords and only slightly calcified (Fig. 79). The most advanced change was found in infants who survived 8 months. In the costochondral junctions the proliferating cells were large and had no semblance of orderly arrangement. Calcification was slight, destruction of cartilage cells irregular and numerous large capillary bushes penetrated the cartilage plate. Osteoid tissue was abundant about the trabeculae (Fig. 80). In the seven youngest patients no skeletal changes were found.

The cause of rickets is known to be a vitamin D deficiency. As a fat-soluble material this vitamin is not absorbed in the absence of bile salts. Hepatic damage may increase the adverse effect on utilization of vitamin D even if intestinal absorption of fats is not greatly impaired.

X-ray Diffraction Studies on Bone Tissue during Hyperparathyroidism. A. Engström and B. Engfeldt[3] (Karolinska Inst.) used an x-ray microdiffraction technic to study the diffraction patterns of areas as small as part of a haversian system in dogs given large doses of parathyroid hormone. No appreciable changes in the molecular structure were noted during decalcification caused by the injection. The x-ray diffraction pattern was the same as that of bones from normal dogs. Apparently, spacing of structures in bones subjected to heavy decalcification by parathyroid hormone does not deviate from that in normal bones.

Muscle, Nerve and Synovial Changes in Lupus Erythematosus. Edward W. Lowman[4] (Rochester, Minn.) studied muscle specimens from 15 cases of lupus erythematosus, peripheral nerves from 15 and synovia from 5.

Muscle degeneration was seen much more often in cases of lupus erythematosus than in controls. Focal muscle degeneration could not be correlated with focal vascular changes although they were never completely dissociated. Muscle degeneration varied from early loss of striation to old falling out of muscle tissue with fibrotic replacement.

Vascular involvement in muscle and nerve tissue was widespread and characteristic. It was typically venous in location, with only occasional involvement of the arterial side of the system. The initial phase was acute edematous phlebitis in which the vein wall was greatly thickened and hydropic and the cells of the wall were swollen. This seemed to progress to a secondary reactive phase in which the vein wall underwent a reactive reparative process as evidenced by presence of many cells both in and about the vessel wall. In serial sections the perivascular reaction was nodular, involving segments of the vessel rather than its entire extent. In the sclerotic phase the cell aggregates were gone and there were varying degrees of sclerotic thickening of the vein wall.

The vascular pattern of reaction in the cases of lupus erythematosus was identical with that seen in some cases of active rheumatoid arthritis. It was similar to that found in periarteritis nodosa, except that the latter predominately involves the arterial side of the circulation.

In the synovia from patients with lupus erythematosus the

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(3) Acta path. et microbiol. scandinav.: 28:152-156, 1951.
(4) Ann. Rheumat. Dis. 10:16-21, March, 1951.

vascular changes were identical with those in the muscle and nerve sections, but in addition the connective tissue stroma was involved early with diffuse swelling. With progression of the vasculitis to the cellular reactive stage there was a stromal fibroblastic response and numerous young fibroblasts appeared. As the process subsided, the stroma returned to normal or residual sclerosis of vessels and fibrotic thickening of the connective tissue stroma remained. Synovial lining cells were little affected.

Muscle and nerve degeneration and vascular change could not be correlated in degree. It could not be decided whether the one was the cause or the consequence of the other, or whether they were simultaneous concomitant responses to a common stimulus. It could not be ascertained whether the reaction pattern was secondary to damage by an extraneous factor, or whether hyperergic response was causing injury to the system.

[In my experience, the vascular lesions are not so uniform as indicated here. Clinically, joint manifestations are frequent, but how often this is organic, as demonstrated here, is not certain.—Ed.]

Nature of Neurofibromatosis and Related Lesions, with Special Reference to Certain Lesions of Bones: Illustrating Influence of Intrinsic Factors in Disease When Development of Body Is Abnormal. Keith Inglis[5] (Univ. of Sydney) proposes that the predisposition to neurofibromatosis rather than the condition itself is inherited. The predisposition is wide in application, influencing development of (1) a great variety of lesions on the basic intrinsic factor level which exists from the fertilization of the ovum to the appearance of neural tissue in the developing embryo; (2) a less varied group of lesions at the neural intrinsic factor level which exists during development of neural tissue in the embryo, and (3) a still more restricted group of lesions at the neurofibromatosis level. Lesions of all three levels may exist in one person and any lesion may lead to others of a secondary nature. Thus, assessing the etiologic significance of some lesions in a particular patient may be quite difficult.

Man, 34, with generalized neurofibromatosis had many nodules in the skin and subcutaneous tissue, a deep-seated tumor beneath and, underlying all, collapsed lower thoracic vertebrae with resulting kyphosis. Histologically the skin lesions represented neurofibroma and café au lait patches. The deeply situated tumor had the structure of malignant neurofibroma. No recognizable neurofibromatous

(5) J. Path. and Bact. 62:519-530, October, 1950.

tissue was found in any section of the diseased vertebrae; cause of softening of the vertebral bodies remained in doubt. The malignant lesions were linked at the neurofibromatosis level, the skin lesions at the neural intrinsic factor level and the bone lesions at the basic intrinsic factor level.

Intrinsic factors may play a part in the etiology in patients or their close relatives, who, though free from neurofibromas, have one or more lesions belonging to the neurofibromatosis complex, such as neurilemmoma, meningioma, fibroma, lipoma, angioma, tumors of the glioma group in the brain or cord, optic nerve tumors, glial hyperplasia, epilepsy, cutaneous pigmented patches, spinal curvature, congenital pseudarthrosis of the tibia, small size of bones, hydrocephalus and spina bifida.

[This adds further information, from a recognized authority, on a disease whose nature is still obscure.—Ed.]

Benign Giant Cell Synovioma. C. J. E. Wright[6] (Univ. of Leeds) reviewed 85 cases, 55 in females. Average age at onset was 36 and average duration 2 years 7 months. The commonest site was the hand (86 per cent), particularly the digits, with occurrence as common on the extensor as on the flexor aspect. There was a history of trauma at the site of growth in 19 cases.

The tumor may arise from tendon sheaths, joints or bursae. Except when it involves the large joints, it tends to be largely encapsulated, roughly ovoid or rounded and usually characteristically lobulated. Tumors rarely attain a large size, particularly on the hand, and range from a few millimeters to 2-3 cm. in diameter. A distinctive feature is lobulation; there may be surface nodularity or the tumor may consist of a number of separate nodules held together by strands of fibrous tissue. Although the tumors usually appear encapsulated superficially, there is often absence of encapsulation on the deep aspect, where tumor tissue blends with synovial membrane. Tumors on the flexor aspect of digits arise from the synovial membrane of the tendon sheath and, in the course of growth, owing to lack of space within the synovial sac, burst through the outer coverings of the sheath as an encapsulated tumor which remains attached to the sheath. Tumors on the extensor aspect arise from interphalangeal joint synovium.

Benign giant cell synovioma situated elsewhere than on

(6) Brit. J. Surg. 38:257-271, January, 1951.

the hand is rare. In the knee the tumor differs only in that it is almost invariably papillary; owing to the relatively large joint space, growth is into the cavity rather than outward.

The most characteristic diagnostic feature histologically is not the giant cells but the differentiation into synovium-lined spaces within the tumor substance. There are all gradations from the typical benign form to a more cellular variety of intermediate malignancy, which in turn is closely related to the malignant synovioma.

Local infiltration which is rare was seen in five cases. Recurrence was found in 23 (44 per cent), and in none was there metastasis. Tumors which recurred were almost invariably of the more cellular variety. Incomplete excision of the synovial attachments is probably the most important cause of recurrence. Tiny lobules of tissue only tenuously attached to the main mass may be left behind. Local excision is indicated for every tumor, whether primary or recurrent, provided this is anatomically possible. All patients should be followed for at least six months, since most recurrences appeared within this time.

The lesion is considered truly neoplastic. Blood cholesterol investigations in 17 patients failed to reveal evidence of hypercholesteremia. The lipid accumulation and siderosis so commonly found in the tumor are of degenerative or traumatic origin and purely local, related in part to the phagocytic powers of the synovial cell.

[This follows a principle already established for giant cell tumors of bone.—Ed.]

SKIN

Pemphigus: Histopathologic Study. Walter F. Lever[7] (Harvard Univ.) reviews the pathologic changes in 65 cases of pemphigus, classified according to six types. In pemphigus vulgaris acutus, the earliest histologic change in the skin consisted of intercellular edema in the lower rete malpighii, with disappearance of intercellular bridges. Cleftlike spaces developed in the epidermis, usually just above the basal layer. Bullae formed between one or two layers of basal epithelial cells and the remainder of the epidermis. They often changed gradually into suprabasal clefts at the periphery. At the time

(7) A.M.A. Arch. Dermat. & Syph. 64:727-753, December, 1951.

of bullae formation, a mild to moderately severe inflammatory infiltrate was present around the vessels of the upper corium, between the basal layer cells and within the bullae. Among these cells eosinophils were numerous. The epidermal cells around the cavities of the bullae showed loss of intercellular bridges. Some of these cells became detached singly and in small groups within the bullae (acantholysis). Such cells often showed degenerative changes. The floor of the bullae was sometimes sharply irregular due to upward growth of papillae covered by a single layer of epidermal cells. Occasionally there was a downward growth of epithelium between the papillae. After the bullae had broken and erosions formed, the basal layer of cells remained adherent to the dermis in many places. Such areas sometimes showed considerable irregular upward proliferation of papillae and downward growth of epidermal strands. Evidence of healing was noted at the margin of several erosions. Those with upward growth of papillae showed considerable papillomatosis, acanthosis and hyperkeratosis in the area of healing. The inflammatory infiltrate varied in severity and eosinophils were usually few or absent.

In pemphigus vulgaris chronicus the earliest change in the skin consisted of a small subepidermal vacuole. Bullae formed from the vacuole and were located, at least in part, below the epidermis. Their margins were rounded, and the epithelial cells forming the lateral wall were considerably stretched. The epithelial cells in the roof of bullae showed intercellular edema, and in older lesions this was often severe with dissolution of the cells, leaving only the horny layer. There was no acantholysis. Inflammatory infiltrate containing numerous eosinophils was present in the underlying corium and within the bullae. Erosions usually showed no epidermal cells at their base except at points where sweat ducts or hair follicles led into the corium. When epidermal cells remained on the surface, more than one layer was present. Proliferation of papillae and downward growth of epidermal strands were not observed.

In the malignant type of pemphigus vegetans the early lesion showed acantholysis and formation of intraepidermal, chiefly suprabasal, bullae. Older lesions, which clinically were characterized by vegetations studded with pustules, showed papillomatosis, hyperkeratosis and irregular downward growth of thickened strands of epidermis, extending into the

corium. Inflammation was extensive, eosinophils making up a large percentage of the cellular infiltrate. Intraepidermal abscesses composed almost entirely of eosinophils were present, sometimes located within the epidermal strands deep in the corium. In older vegetations which clinically showed no pustules, there were no longer any eosinophil abscesses but considerable papillomatosis and hyperkeratosis, which gave the lesions a verrucous appearance. Inflammatory infiltrate was moderate or slight, and there were few eosinophils.

In the benign type of pemphigus vegetans, the early lesions consisted of newly formed pustules, largely in the suprabasal position, and acantholysis in the lower epidermis. Older lesions resembled those of malignant vegetations with many intraepidermal or eosinophil abscesses and considerable epidermal hyperplasia.

The earliest histologic change in pemphigus foliaceus consisted of acantholysis in the upper epidermis, leading to formation of a cleft in the superficial, often subcorneal, location. Occasionally tense bullae with rounded margins formed in the same location, but usually flaccid bullae were formed or the uppermost epidermis became detached without bullae formation. The basal layers of epidermis remained attached to the dermis. Older lesions showed acanthosis, mild papillomatosis and hyperkeratosis, with an increase in thickness of the granular layers. The amount of parakeratosis varied widely. Follicular hyperkeratosis was present in some sections.

Pemphigus erythematosis is an abortive form of pemphigus foliaceus. In two cases the lesions progressed to become typical pemphigus foliaceus. The histologic changes in the nonprogressive lesions resembled those in pemphigus foliaceus.

In benign mucous membrane pemphigus sections of the skin, foreskin, conjunctiva and mouth all showed subepidermal bullae. No degenerative changes were noted in the epidermal cells.

Xanthelasma Palpebrarum: Tumor of Sebaceous Glands. Eugene Wolff[8] considers xanthelasma palpebrarum a tumor or overgrowth of the unicellular sebaceous glands found normally in the basal layer of the human epidermis. The fatty material in xanthelasma palpebrarum is not ordinary fat although it contains fatty acids. It is probably a cholesterol

(8) Brit. J. Dermat. 63:296-302, Aug.-Sept., 1951.

ester. Like normal sebaceous glands it does not stain black with osmic acid but takes a gray coloration. The basal cells, stain red with Scharlach R. and purplish with the Liebermann-Burchardt-Schultz method, are birefractive in polarized light. The fatty cells can be found in normal human epidermis, are especially numerous at the inner side of the eyelid because of the special character of its skin and are more frequent in females than in males. They are homologous with secreting

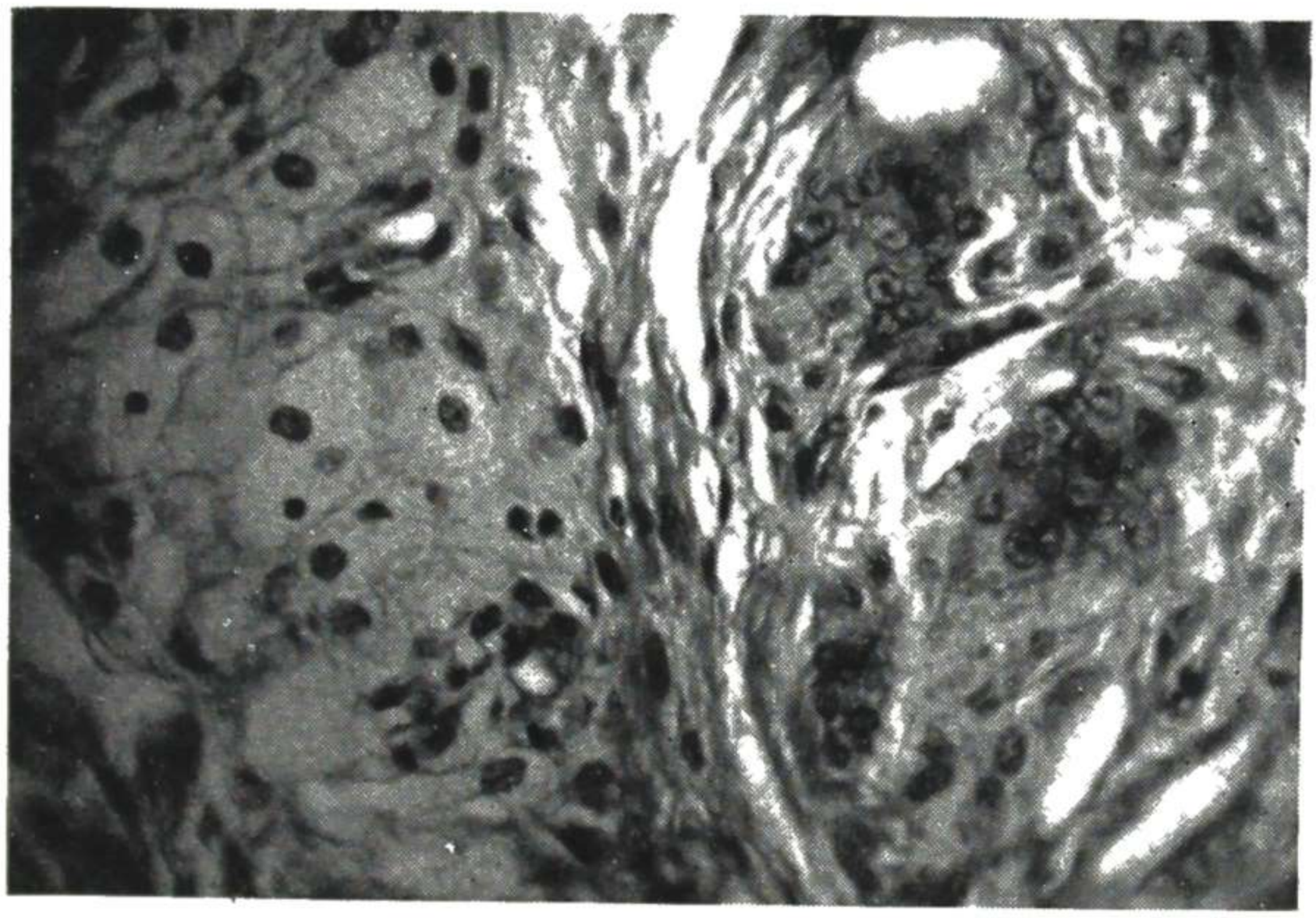

Fig. 81.—So-called Touton giant cells; × 300. (Courtesy of Wolff, E.: Brit. J. Dermat. 63:296-302, Aug.-Sept., 1951.)

cells of ordinary sebaceous glands which arise as downgrowths of basal cells. These unicellular sebaceous glands are the same as Masson's clear cells.

In xanthelasma palpebrarum, downgrowths of the basal layer of the epithelium are gradually converted into foam cells, and their nuclei become rounder, paler and usually contain one well marked nucleolus. This transition eventually forms the Touton giant cell with its cytoplasmic network and nuclei identical to those of foam cells (Fig. 81).

It is because of the special characters of the skin of the inner side of the eyelid that xanthelasma palpebrarum is so common there.

Dermatofibrosarcoma Protuberans: Report of 39 Cases is made by George T. Pack and Edward J. Tabah[9] (Memorial Hosp., New York City). The patient is usually a middle-aged adult with a growth which, though painless, has slowly in-

(9) A.M.A. Arch. Surg. 62:391-411, March, 1951.

creased in size for years. The small intracutaneous nodularity expands; if more than one nodule occurs there is eventual fusion to form a dense fibrotic plaque. After one to four years one or more projecting nodular masses appear on the surface of the plaque. The tumor then grows more rapidly and may enlarge to the size of a man's fist within a few weeks or months. Half these patients applied for treatment one to five years after noting the lesion.

The tumor is more common on the trunk than on the extremities. In this series all were firmly attached to the skin and most were confined to the dermis and subcutaneous tissue. Origin may be multicentric but development is usually from a single point. On cut section the lesion is vitreous and resistant and composed of a grayish white homogeneous tissue which may be red at the core. Encapsulation is due to a condensation of fibrous tumor tissue at the periphery. The neoplasm infiltrates on a horizontal plane through the corium and subcutaneous fat. Extension of the tumor in fine linear projections beyond its apparent margins may be responsible for the common surgical error of too limited and conservative excision with subsequent regrowth.

Microscopically the appearance is that of a cellular fibroma, which in some regions closely resembles a well differentiated fibrosarcoma of low grade malignancy. In many cases diagnosis is more readily based on clinical history and appearance than on microscopic examination. The cellular portions consist of fusiform cells arranged in whorls or bundles sometimes around a fine central capillary or lacunar vessel. Mitoses are usually rare but occurrence varies with the cellularity and rapidity of growth of the various parts of the tumor. With Masson's trichrome or Mallory's connective tissue stain it may be established that the basic structure of the neoplasm is a fibroblast or connective tissue cell. Because of hemorrhage and deposition of hemosiderin the inexperienced observer may confuse the lesion with melanoma.

Most of these lesions do not metastasize, although metastases to lymph nodes and viscera have been reported. The tumor is thought malignant because of its tendency to recur locally after a presumably wide excision. Irradiation has not been successful; radical surgical excision is the treatment of choice.

Differentiation in the Rodent Ulcer Group of Tumors. B. Lennox and A. L. Wells[1] (Hammersmith Hosp., London) used four criteria for classification of 150 tumors: (1) palisading, (2) fluid formation, (3) whorls and (4) pigmentation. Presence or absence of these characteristics was readily determined. They were constant throughout the same tumor and largely responsible for variation in appearance of different tumors. Palisading was present in 109 tumors. Melanin pigmentation to some degree, associated with the presence of melanoblasts, was noted in 52 cases. One feature indicative of fluid formation in tumors was the presence of small cystic spaces. Such an appearance within a rodent ulcer is due not to local degeneration of tumor cells or stroma but to fluid formed by the living tumor cells. As there is no excretory route the fluid produces a cystic pattern. Fluid may pass between the surrounding cells into the stroma. Such passage may stretch the intercellular bridges, occasionally resulting in a pseudoprickling effect. Evidence of fluid production was found in 87 tumors. Whorl formation existed in 54 tumors as small sperical groups of concentrically arranged flattened cells with an eosinophilic core. These parakeratotic pearls may be of the same nature as those of squamous cell carcinoma but are smaller, better defined, contain less central keratin and lack prickle cells.

The exposed area of the face below the eye was the site of growth in half of 150 patients; the temple was involved in 27, and the region of the eye in 25. Multiple tumors were present in 11 patients.

Various clinical manifestations, including therapeutic result, were not correlated to the presence, absence or combination of the four histologic features of classification. Behavior of rodent ulcers depends to some extent on degree rather than type of differentiation.

[This and the following article show the continuing interest of the British in this topic. Particularly important is the lack of real criteria for prognosis.—Ed.]

Histologic Classification of Rodent Ulcers and Its Bearing on Prognosis. A. C. Thackray[2] analyzed data on 200 patients followed for 10 years or more. Only 11 tumors were of probable sweat gland origin. Most of them appeared to be of pilar

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(1) Brit. J. Cancer 5:195-212, June, 1951.
(2) Ibid., pp. 213-224.

origin, and these were divided into two main groups on the basis of cell type and arrangement. There were frequent transitional and combined forms. In one type the cells were spindle shaped, with relatively little cytoplasm, and resembled the cells of the hair matrix. The other variety was composed of smaller cells with relatively more cytoplasm. They had a reticular arrangement, often with small clear intercellular spaces, and resembled the cells of the outer sheath of the hair root. Occasionally this type contained parakeratin pearls. The tumors were also divided according to their growth habits into circumscribed, intermediate and infiltrative. Small lesions were usually of the circumscribed type. Mitoses were absent in half the tumors and were numerous in 27 per cent.

Primary surgical excision of 63 lesions was followed by recurrence of 12. Microscopic examination of representative sections of the excised lesion was dependable in determining whether removal had or had not been complete. Radiotherapy was used on 121 ulcers, 82 of which were cured by a single course; 20 were radioresistant. The type of growth was of importance in estimating the effect of radiation. Circumscribed tumors usually responded, but infiltrative lesions often were resistant. Palisade formation was more often pronounced in the radiosensitive than in the resistant ulcers. Presence of pigment, cysts, mucus or mitoses had no apparent effect on response. Except to indicate the habit of growth, the histologic features and cell characteristics in rodent ulcers are not reliable criteria in assessing the probable outcome of radiotherapy.

NERVOUS SYSTEM AND EYE

Neurologic Lesions of Erythroblastosis Fetalis in Relation to Nuclear Deafness. William B. Dublin[3] (Univ. of Colorado) examined seven brains from newborn infants with erythroblastosis fetalis, with particular attention to the auditory pathway. Kernicterus may be unassociated with erythroblastosis fetalis but occurs predominantly in that condition. The basic requirements for the appearance of kernicterus seem to be (1) jaundice, (2) injury of brain parenchyma and (3) penetration of the blood-brain barrier by bile pigment.

Grossly the dura was icteric in proportion to the general-

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(3) Am. J. Clin. Path. 21:935-939, October, 1951.

ized jaundice in each case. The pia arachnoid was hyperemic. Cerebral swelling was present. Cut section showed hyperemia, sometimes with petechiae or larger hemorrhages, especially in the white matter. Massive subarachnoid or intraparenchymal hemorrhage was seldom noted. When kernicterus was present, foci of bright golden pigmentation affected certain structures. The most frequent centers pigmented were the lenticular nuclei and hippocampi, with the nuclei throughout the brain stem, dentate nucleus and various cerebellar folia sometimes involved. The process may extend downward from the medulla to include the gray matter of the upper cervical cord. Peripheral nerves were not involved.

Microscopically, hyperemia and edema were evident. Parenchymal damage and reaction to injury varied with the stage, acuteness and severity of the process. Alterations were more widespread than indicated by the gross pigmentation, and microscopic examination disclosed involvement of the cerebral cortex and white matter. Nerve cells showed degeneration varying from cloudy swelling to pyknosis. Later the shrunken cytoplasm appeared homogeneous and was often acidophilic. The cell became small, dark and structureless. Oligodendrocytes were swollen and proliferating. A small number of lymphocytes and macrophages appeared about the blood vessels. There was capillary thickening and proliferation.

The grossly evident golden brown pigmentation was microscopically seen best in frozen sections on comparatively fresh tissue. Much or all of it was sometimes removed by paraffin embedding. The pigment was found principally in large ganglion cells. Foci of pigmentation between the large ganglion cells appeared to represent the cut ends of swollen pigmented nerve fibers. A few pigmented granules were occasionally seen in macrophages. Hemosiderin was sometimes present, coincidental with foci of hemorrhage. The nature of the golden brown pigment is unknown. It is related to bile pigment but does not turn green when reduced in formaldehyde solution as does bilirubin. The mechanism of cerebral injury in erythroblastosis is one of anoxia resulting from intravascular hemolysis.

The distribution of lesions is the same as that in any anoxic disorder. Changes were found bilaterally in the auditory pathway. The dorsal and ventral cochlear nuclei of the medulla

showed cell injury, and in several cases few normal ganglion cells remained. The superior olivary nuclei revealed alteration of moderate severity. Gross and microscopic golden pigmentation of the cochlear and superior olivary nuclei was found. The trapezoid body and lateral lemnisci seemed to show reduction in number of fibers probably due to secondary degeneration of the processes of the injured nerve cells. Injury of the inferior collicular nuclei was mild. Moderately severe changes were noted in the medial geniculate bodies and some general loss of myelin in the auditory radiation. Mild to moderate changes were present in the auditory cortex, corresponding to the other cortical damage.

It is concluded that lesions of the important nuclear stations of the perceptive auditory pathway are the basis for deafness that may follow erythroblastosis fetalis.

Cerebral Lesions in Congenital Heart Disease: Review of Autopsies on 162 Cases is presented by Morgan Berthrong and David C. Sabiston, Jr.[4] (Johns Hopkins Univ.). Complications in congenital heart disease have become of particular practical importance because of the development of procedures which make possible successful rehabilitation of patients with cyanotic or serious acyanotic heart disease. The most bizarre cardiac malformations, which were common in patients dying during the first month of life, and those cases so complicated that they could not be satisfactorily classified, were not included in the study. Of the 162 cases selected, 135 were cyanotic and 27 acyanotic in type. There were cerebral lesions in eight patients with acyanotic heart disease. Several different types were represented and they were most often of a nonthrombotic origin. When cerebral lesions were related to thrombosis in the acyanotic patients, other obvious causes for thrombosis or embolism such as heart failure or atherosclerosis were present. Four of the 10 patients with coarctation of the aorta had cerebral aneurysms.

Of the 135 patients with cyanotic heart disease, 25 showed cerebral infarction. Most of the infarcts had resulted from vascular occlusion. The specific thrombus in a cerebral vessel responsible for the infarction was not often found. Venous thrombi either seen or inferred from hemorrhagic infarcts were commoner than arterial occlusion. Thrombi were seen in cerebral veins or dural venous sinuses in five instances and

(4) Bull. Johns Hopkins Hosp. 89:384-406, November, 1951.

hemorrhagic infarcts indicated a probable origin from venous thrombosis in nine others. Thrombi were found in the carotid vessel three times, in the middle cerebral artery four times and in the smaller cerebral or meningeal arteries three times. Infarcted areas sometimes corresponded to the distribution of a specific vessel. Anemic infarcts especially tended to involve areas following the distribution of a cerebral artery, such as the middle cerebral or branch of the lenticulostriate artery. Often an origin of cerebral emboli could not be definitely established. Nonbacterial vegetation on, or adjacent to, stenotic or malformed orifices was the probable source of cerebral emboli in six patients. Thrombi developing in peripheral veins and reaching the brain as paradoxical emboli through cardiac defects were not suggested by the findings in any case. Thrombotic lesions at the site of vascular surgery, presenting at least the anatomic possibility of being the source of cerebral emboli, were present in 3 of 15 patients dying in the immediate postoperative period. In certain cases of recent infarction due to cerebral vein thrombosis in situ, the process became evident in the early postoperative course.

Fifteen of the 19 recent cerebral infarctions were in patients dying after operation. An important factor in development of the specific vascular occlusion, whether it developed as a thrombus in situ or as cerebral emboli from thrombosis in other locations, was the polycythemia present in most patients with cyanotic heart disease. With the presence of polycythemia in cyanotic heart disease and its predisposition to vascular thrombosis, certain other conditions, however slight or transitory, may result in prompt intravascular thrombosis. Syncopal attacks with fall in blood pressure and probably reduction of cardiac and cerebral blood flow as well as capillary dilatation from anoxia may be the initiating cause of thrombus formation. The occurrence, however brief, of dehydration, anoxia and shock during or after operation, increased the tendency to thrombosis. Another possible influence is that of the blood platelets, which are commonly increased in number after significant surgical procedures. Actual platelet counts were not carried out in this study.

Focal necrosis of cerebral tissue may result from cerebral anoxia in the absence of vascular occlusion. Such lesions are usually small and appear as diffuse or regional atrophy with loss of ganglion cells, the severe forms ending as local-

ized glial scars. Five patients showed recent or old microscopic lesions compatible with the result of anoxia. In addition, others had small perivascular hemorrhages of the cerebral tissue which were typical of recent diffuse anoxia. Of eight showing petechiae, six died postoperatively and the anoxia could be attributed to complications provoked by surgery or anesthetic procedures.

Functional impairment of the central nervous system may exist with no corresponding morphologic change observed by ordinary methods of examination. Some patients with congenital heart disease had neurologic manifestations which led to a clinical diagnosis of cerebral lesions before death, but routine postmortem examination did not demonstrate any morphologic alterations in the brain. Systemic infarcts in organs other than the brain were present in 8 of the 25 patients with cerebral infarcts. Recanalization of pulmonary thrombi was present in 14 of the 23 patients with tetralogy of Fallot and cerebral lesions. Brain abscess in 5 of the 162 patients was in general agreement with the usual incidence found in patients with cardiac malformations.

[This is of practical importance in view of the increasing number of surgical operations for congenital heart disease. In the light of disclosures in the next article, it is of especial interest that vascular occlusion in some of the cases was inferred rather than demonstrated.—Ed.]

Infarction of Brain without Thrombosis: Analysis of 100 Cases with Autopsy. Samuel P. Hicks and Shields Warren[5] (Boston) found no mechanical occlusion of the cerebral vessels in 60 of 100 cases of fatal cerebral infarction seen at autopsy. Cases were selected in which stroke was the principal cause of death or was one of the prime causes of death and in which the infarct or infarcts were so large that the blood vessels supplying them could be adequately studied grossly. Only those cases were included in which the lesion was clearly the result of reduction or occlusion of blood flow in one or more major cerebral vessels. With rare exceptions, the major infarction was two to several days old.

Thrombosis of the cerebral vessels accounted entirely for infarction in 33 cases and partially for infarction in 7. No thrombosis was found in 60, and in 67 some explanation other than thrombosis was required. Cardiovascular disease was common in the series. Some degree of coronary arterioscle-

(5) A.M.A. Arch. Path. 52:403-412, November, 1951.

rosis was present in 73 cases and heart failure in 44. Thrombi were found in the heart at autopsy in only 15 cases; valvular disease was present in 7 of these. In five of the seven, embolism probably caused the cerebral vascular occlusion. Hypertension was present in only half of the cases of nonthrombotic infarction with severe to moderate arteriosclerosis. In most of the nonthrombotic cases, systemic circulatory failure was excluded as a cause.

Arteriosclerosis may play two important roles in the genesis of cerebral infarction. The most obvious is that a sclerotic vessel may tend to become thrombosed. This was the explanation in the cases of thrombosis. The other role is that of partial obstruction with the blood flow reduced to the extent that a thrombus in a small branch of a sclerotic vessel may precipitate infarction. Systemic circulatory failure, by slowing the blood flow, contributes to formation of cerebral vascular thrombi. Of the 40 patients with thrombosis, 23 had heart failure; 11 of these had thrombi in the heart or mitral stenosis. The relation of the hypertensive state to the genesis of apoplexy appears significant. High blood pressure was present in 70 patients.

In the majority of the nonthrombotic cases, an intrinsic focal reduction of blood flow seems to account for most of the lesions. Since there were also necrosis, evidence of stasis and sometimes diapedesis in small arteries and venules as well as capillaries, arterial constriction must have been the cause.

Hemorrhagic apoplexy and infarction are variations of a single underlying process, that of ischemia. Interstitial edema which develops after vascular constriction may be a progressive and serious complication. It aggravates the ischemia, which in turn results in further edema. In the brain a comparatively slight vascular ischemia may thus be converted into a serious irreversible process.

[It has been thought for many years that some cases of apoplexy are the result of thrombosis and that the thrombosis is particularly likely to occur when there is arteriosclerosis. The evidence presented here favors the view that slowing of the blood flow contributes to the formation of thrombi in the cerebral vessels, which appears to be true of thrombosis in general. Of particular importance is the report of cases in which no vascular occlusion could be demonstrated. The care of the examinations lends strong support to this view, but it must be realized that there is a limitation to what can be discovered by morphologic study. Infarcts of the brain have been shown to be primarily hemorrhagic, as is true in many other situations in the body.—Ed.]

Experimental Allergic Encephalomyelitis. Bernard N. Halpern, Ivan Bertrand and François Lhermitte[6] (Paris) produced disseminated encephalomyelitis in the monkey, guinea pig and dog by repeated parenteral injection of a mixture of 10 Gm. fresh sheep or rabbit brain and 0.1 Gm. phenol in 10 cc. physiologic saline solution, emulsified with 10 cc. aquaphor® and 10 cc. paraffin oil containing 25 mg. killed BCG. The mixture was incubated at 60 C. for 45 minutes. There are no reports of production of similar lesions in the dog, which appears to be the experimental animal of choice because of its striking symptoms and ease of humoral studies. The lesions consisted mainly of perivascular infiltrations (of neutrophils in the more acute stages and lymphocytes in the chronic) associated with demyelinization of perivascular parenchyma. The picture differed somewhat in the various species. The lesions could not be induced when paraffin oil containing killed organisms was replaced by oil in which they had been merely macerated.

The lesions have been attributed to an allergic response to some nervous system substance. Although circulating antibodies have been demonstrated, it has not been possible to reproduce the disease by serum transfer. The authors produced some lesions by injection of affected brain tissue or tissue from the inflamed site of injection into other healthy animals, but they were not at all similar to the characteristic disease. Because of their effect on capillary permeability, antihistaminic agents in high doses were given to some animals in conjuction with injection of brain tissue. Many died, due perhaps to intercurrent infections which were induced by the antihistamines, but typical lesions of encephalomyelitis were greatly reduced or absent.

Such experimental lesions seem closely allied to the demyelinating encephalomyelitis in man which follows immunization against rabies and to the similar conditions in exanthematous diseases. The morphologic appearance of the lesions is similar to that of multiple sclerosis.

Encephalitic Form of Metastatic Carcinoma. Carcinomatous extension to the nervous system may manifest itself in many ways, e.g., as a solitary metastasis to the brain or as multiple metastases of varying number and size. Less com-

(6) Presse méd. 58:684-687, June 14, 1950.

mon is the encephalitic form which may closely simulate other types of encephalitis. Leo Madow and Bernard J. Alpers[7] (Jefferson Med. College) report four such cases in which metastatic carcinoma of the brain was diffuse, scattered, unassociated with nodule formation and infiltrated the parenchyma, perivascular spaces (Figs. 82-84) and meninges. Inci-

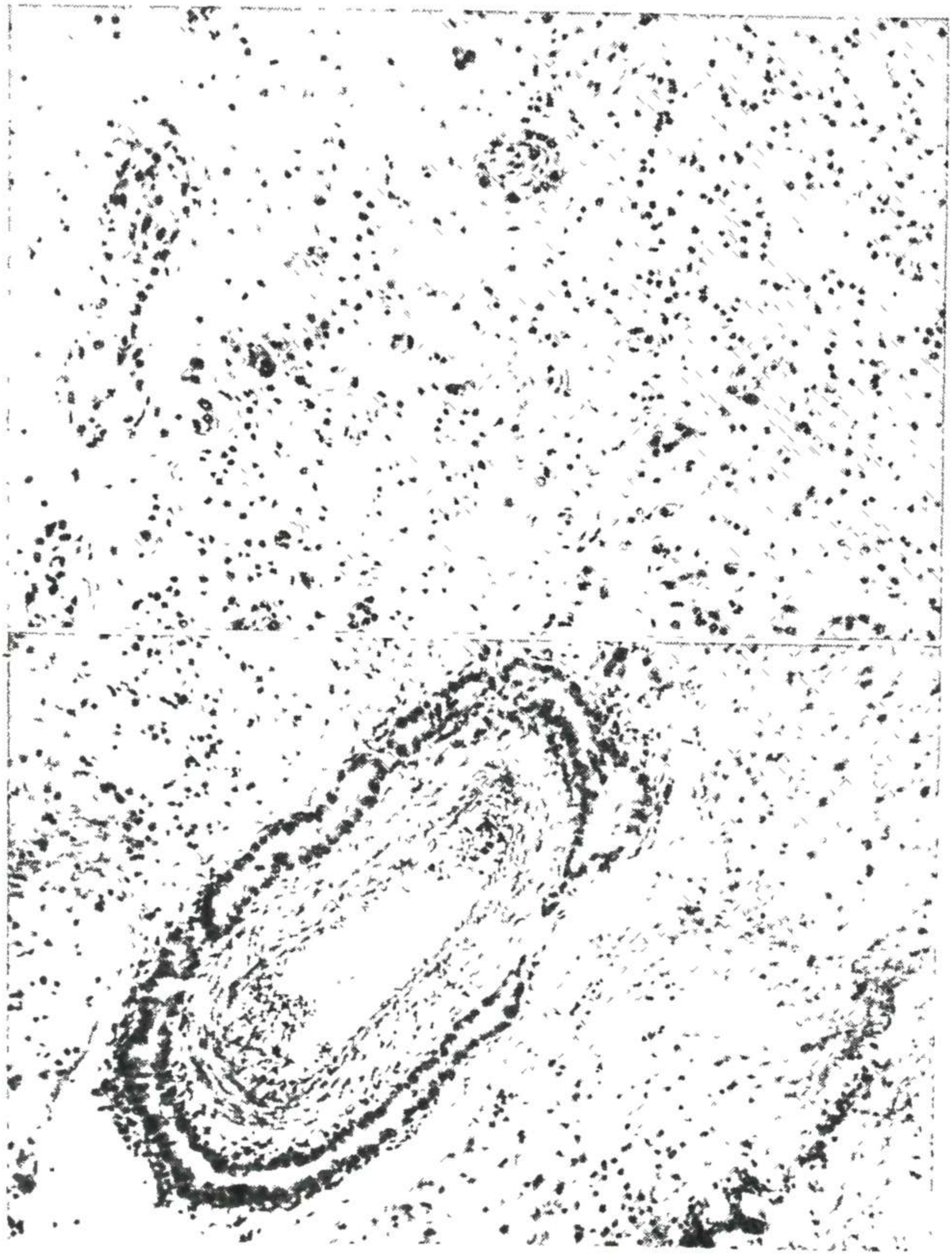

Fig. 82 (top).—Section at junction of gray and white matter showing predominant perivascular distribution of carcinoma cells.

Fig. 83 (bottom).—Larger blood vessel showing same perivascular arrangement, without spread to surrounding tissue.

(Courtesy of Madow, L., and Alpers, B. J.: A.M.A. Arch. Neurol. & Psychiat. 65:161-173, February, 1951.)

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(7) A.M.A. Arch. Neurol. & Psychiat. 65:161-173, February, 1951.

dence of this condition among all metastatic carcinomas seen by the authors was 3.8 per cent.

Gross inspection of the brain reveals nothing of significance. The meninges may be cloudy and thickened locally or diffusely over the hemispheres and brain stem. Any part of the brain or brain stem may be invaded by the process. No large nodule is visible to the naked eye. Microscopically the meninges or brain tissue may reveal direct extension of carcinoma cells, associated with perivascular infiltration by them.

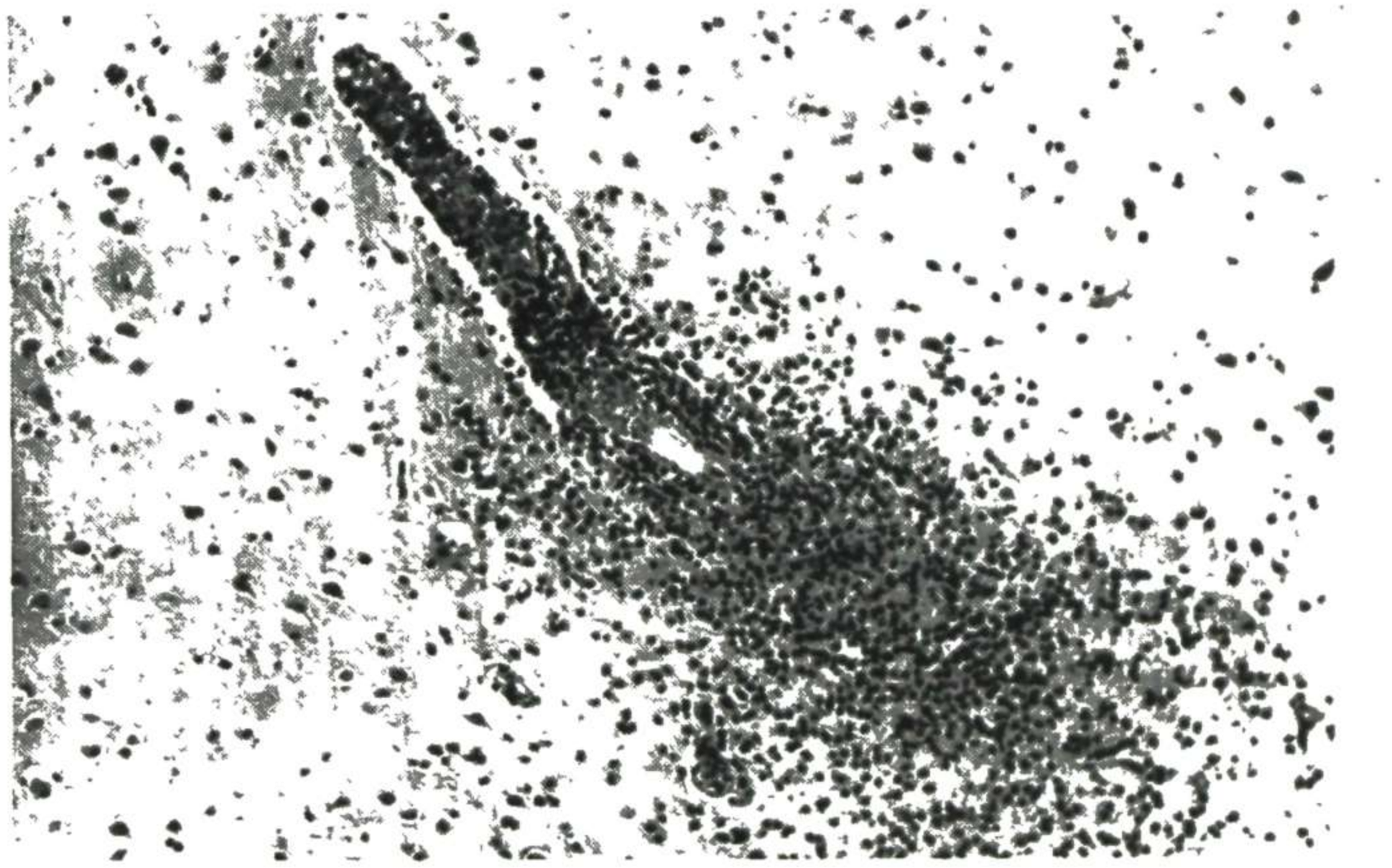

Fig. 84.—Reaction of polymorphonuclear leukocytes in cortex to meningeal infiltration by carcinoma cells. (Courtesy of Madow, L., and Alpers, B. J.: A.M.A. Arch. Neurol. & Psychiat. 65:161-173, February, 1951.)

There may be perivascular infiltration alone. Ganglion cells show no specific changes. The spinal cord may be involved in the same manner as the brain.

The clinical picture associated with carcinomatous encephalitis has no distinctive feature. The condition should always be suspected in middle-aged or older patients with what appears to be an encephalitic process of undetermined origin.

Tuberculous Meningitis; Correlation of Therapeutic Results with Pathogenesis and Pathologic Changes. — *General Considerations and Pathogenesis.*—Oscar Auerbach[8] (Halloran Veterans' Admin. Hosp., Staten Island, N. Y.) reports observations on 108 cases of tuberculous meningitis found at 2,333 autopsies on patients dying of some form of tuberculosis, before the introduction of streptomycin. Ages varied

(8) Am. Rev. Tuberc. 64:408-418, October, 1951.

from 7 months to 67 years. Meningitis was present in 42.2 per cent of the children with fatal tuberculous infections but in only 2.9 per cent of persons over age 12 (considered the upper limit of childhood). In all fatal cases of meningitis, males outnumbered females 2:1. Incidence of meningitis was much greater among Negroes than among white persons.

A progressive primary complex was present in 12 of the 41 children. In 25, the primary complex was either in an advanced stage of healing or was completely healed. The lungs of only two of the adults were free from tuberculous changes other than a healed primary complex. Chronic pulmonary tuberculosis was present in 34 adults, and 22 of these also had lesions characteristic of dissemination secondary to an active extrapulmonary tuberculous process. Acute miliary tuberculosis was the only lesion in 26 adults. In both groups of adults with dissemination characteristically extrapulmonary, miliary foci were numerous and were at different stages of anatomic development, indicating that there had been multiple seedings. Urogenital and skeletal tuberculosis was present in 50 adults and 5 children.

Tuberculous meningitis in children is most often related to spread from a primary pulmonary complex. This spread occurs early in development of the primary complex, and the primary lesion may heal afterward, eliminating the source of dissemination. Tuberculous meningitis in adults is usually secondary to an active extrapulmonary tuberculous process. In the presence of chronic pulmonary involvement, lesions characteristic of dissemination from extrapulmonary foci are often found in adults who have tuberculous meningitis.

Pathologic Changes in Untreated and Treated Cases. — Auerbach[9] compared the pathologic changes in patients treated with streptomycin with those in untreated patients. In the latter, a gelatinous exudate was found in the subarachnoid space, the greatest concentration usually being in the region of the optic chiasma. Focal lesions were seen in the leptomeninges in well advanced cases. They followed the blood vessels and had a predilection for the sylvian fissure. The ependyma and choroid plexuses of the ventricles were often involved. Microscopically, the process was usually uniform. A fibrin network existed between the pia and arachnoid membrane. In addition to an acidophilic homogeneous fluid,

(9) Am. Rev. Tuberc. 64:419-429, October, 1951.

this network contained polymorphonuclear leukocytes, red blood cells, macrophages and lymphocytes. In older foci there was caseation with epithelioid and giant cells. The blood vessels showed cellular infiltration of the subendothelial zone, fibrinoid transformation of the media and circumscribed tubercles in the adventitia.

In patients treated with streptomycin for prolonged periods, the gelatinous exudate had become thickened, dull gray and fibrotic, obliterating the subarachnoid space. It contained areas of caseation. Occasionally the fibrosis covered the foramens of Luschka, obliterating the communication between the ventricular system and subarachnoid space. In these instances dilatation of the lateral ventricles was present. When death was directly or indirectly due to tuberculous meningitis, caseation was far more pronounced than in untreated patients. The caseous areas appeared to arise from the fibrinous exudate so prominent in the untreated patients. Proliferation of granulation tissue was pronounced in treated patients. Granulation tissue containing epithelioid cells and fibroblasts apparently developed from the perivascular connective tissue of the meninges. Langhans' cells were usually much less numerous than is customary in tuberculous granulation tissue. The cellular elements formed wide collars of varied size around the blood vessels and differed from those seen in untreated patients, in whom foci were usually present in the adventitia in a single localized area. When streptomycin had been given for over a year, areas of caseation were surrounded by either granulation tissue containing abundant collagen fibrils or hyalinized connective tissue. After two years, most of the exudate was replaced by hyalinized connective tissue. Blood vessels showed fibrous thickening of the intima, with decrease in the size of the lumen. Such an obliterating endarteritis was a characteristic finding in vessels bordering caseous areas.

Presence of dense hyalinized connective tissue is due partly to the natural healing tendency of a tuberculous process of long standing but also, perhaps, to the action of streptomycin. Since repeated seedings to the meninges are not a factor in the course of tuberculous meningitis in children, opportunity for successful streptomycin treatment appears to be greater than in adults. Streptomycin prolongs the natural course of

the disease and results in extensive healing changes within the exudate.

[These studies give valuable information on pathogenesis of tuberculous meningitis and effects of streptomycin. The controls are well set up, but it must be evident to experienced pathologists that tuberculous meningitis may become cicatrized. The hazard associated with use of streptomycin is shown in the following article.—Ed.]

Necrosis of Basal Nuclei in Cases of Tuberculous Meningitis Treated with Streptomycin. According to published reports, the pathologic lesions of tuberculous meningitis treated with streptomycin are characterized by proliferative arteritis of the small vessels originating from the circle of Willis, with foci of ischemic necrosis in the brain secondary to the vascular occlusion. Autopsies on 13 patients have shown focal areas of this type. Time between onset of meningitis and death varied from 21 to 270 days. Amount of streptomycin given and routes of administration also varied. R. H. Rigdon[1] (Univ. of Texas School of Med.) reports clinical and pathologic data on three cases in which ischemic areas of necrosis were found.

Case 1.—Boy, aged 14 months, with tuberculous meningitis for five weeks, was treated with streptomycin intramuscularly and intrathecally for 15 days. At autopsy the brain was covered with a thick gelatinous exudate. The ventricles were dilated. The reaction in the meninges was characterized by focal areas of caseation and many tubercles composed of giant and epithelioid cells. The walls of some small vessels were degenerated and infiltrated by inflammatory cells. The lumens of other vessels often were greatly decreased by a proliferative cellular process originating from the intima. Focal necrosis was present in the basal nuclei and near the cortex of the parietal lobes. Macrophages were numerous in some of these foci, whereas in others there was little cellular reaction. No tuberculous type reaction was observed within the large areas of degeneration. The ischemic areas of necrosis were considered secondary to the vascular lesions in the meninges.

Case 2.—Boy, 3, had tuberculous meningitis for 83 days. At autopsy the meninges presented a typical gross appearance. The entire ventricular system was greatly dilated, and many small tubercles were scattered over the wall of the ventricle. There was a large yellowish brown, spongy focus of necrosis in the left temporal lobe, lateral to the basal nuclei. It was infiltrated by macrophages and microglia, and there was also much gliosis. The walls of some of the small arteries were diffusely infiltrated by inflammatory cells. Many of the large arteries showed proliferation of the intima with pronounced diminution of the lumen. One vessel showed recanalization of the occluded lumen.

Case 3.—Woman, 38, had tuberculous meningitis for 185 days and

(1) Tuberculology 12:135-141, March, 1951.

was treated with streptomycin for 106 days. At autopsy there was a thick, irregular, relatively firm exudate at the base of the brain. The entire ventricular system was dilated. Focal necrosis was present in the basal nuclei. Microscopically, the meningeal exudate showed a proliferative and an exudative type of tuberculous reaction. The walls of some of the arteries in the subarachnoid space were degenerated and infiltrated by inflammatory cells. The lumens were greatly decreased by proliferation of cells from the intima. Some arteries without degeneration of the wall showed proliferation of the intima. The areas of degeneration in the basal nuclei were infiltrated by macrophages and some showed extensive gliosis. None showed a reaction in any way suggestive of tuberculosis. All were typical of ischemic necrosis. The spinal cord also showed areas of ischemic necrosis. The primary focus of infection within the central nervous system was not demonstrated.

The lives of these three patients were apparently prolonged by therapy. The foci of necrosis in the brain were similar to those previously described. It seems likely that these ischemic lesions result from the vascular occlusion demonstrated in the vessels of the meninges. The small arteries arising from the major ones around the base of the brain where the exudate is most extensive are often terminal vessels which supply the cortex and basal nuclei. Vascular occlusion in such vessels would predispose to focal ischemic necrosis. The necrosis secondary to vascular occlusion in tuberculous meningitis may occur in many areas of the brain but is most common at the base of the brain, especially in the area of the basal nuclei.

Histopathologic Study of 11 Cases of Poliomyelitis, Including 1 in Newborn, is reported by Malcolm Fowler[2] (Univ. of Adelaide). Eight patients had lesions in the cerebral cortex. The anterior central gyrus was affected in seven, superior frontal in six and paracentral lobule in three. The lesions usually occurred in the layers containing Betz cells and consisted of focal or diffuse areas of microglia with a variable proportion of polymorphonuclear leukocytes. Occasionally the inflammation was spread out over a large area, involving the entire depth of the cortex with the exception of layer 1. Betz cells were commonly involved in some areas. Occasionally the small pyramidal neurons were affected in areas where Betz cells remained well preserved. The affected neurons either showed chromatolytic changes with cytoplasmic swelling or were deeply stained and shrunken, with a dense nucleus. In one instance, Betz cells adjacent to the injury showed increased

(2) M. J. Australia 2:613-628, Nov. 10, 1951.

lipofuscin pigment in the cytoplasm. Capillaries were sometimes increased in the focal areas of inflammation and degeneration. Many dead neurons were represented by microglial foci. Lymphocytic infiltration of the overlying meninges and of the underlying vessels of the white matter accompanied the cortical lesion. Perivascular cuffing was also found in the gyrus cinguli, pyramidal layer of the hippocampus, gray matter of the uncus, amygdaloid nucleus, head of the caudate nucleus, claustrum and internal capsule, corpus callosum, fornix and putamen. The olfactory bulbs were unchanged.

Perivascular cuffing was present in the lateral nuclear mass of the thalamus in nine cases, and occasionally the involvement was more extensive with infiltration of microglia and polymorphonuclear leukocytes, edema and neuronophagocytosis. The medial nuclear mass contained foci of infiltration in two. In the subthalamus of two patients neuron damage and cellular infiltration occurred in the subthalamic nucleus, and in one of these and in a third patient there was also involvement of the zona incerta.

In most patients, cuffing of vessels, tissue infiltration and occasionally neuronophagia were found in the substantia nigra. In the patient with greatest injury in the substantia nigra, entire groups of nigral cells were destroyed, but the damage was never as diffuse as is often found in encephalitis lethargica. In the red nucleus of seven patients, changes consisted of focal or diffuse infiltration in one or both nuclei. In five of these the lesions were confined to peripheral areas, four of which were concentrated on the medial halves of the nuclear edge. Severe neuronal destruction was often present. Microglial infiltration of the tegmental reticular formation was observed in all but two patients and both of these had perivascular cuffing. The cerebellar cortex of the vermis was infiltrated in one patient, and near the inflammation some Purkinje cells had disappeared. Infiltration was observed in the dentate nucleus in five patients and in two others perivascular cuffing was present. Mononuclear infiltration was seen in all but one when the nuclei of the cerebellar roof were examined. Three patients had large dense foci and neuron destruction in the pontile nuclei.

The most severe damage was found in the reticular formation, vestibular nuclei and nucleus ambiguus in the eight patients with primary bulbar involvement. The nucleus am-

biguus was totally necrotic in several patients and nearly destroyed in others. Changes in the vestibular nucleus approached a similar degree of damage. In these regions, presence of polymorphonuclear leukocytes was characteristic of the early stages of the disease. Vascularity was increased as well as neuronophagia with large numbers of well developed *Gitterzellen*. The tractus solitarius showed lesions, usually mild, in five patients.

The spinal cord was studied in five patients. The extent of involvement of the anterior horn was usually most severe at the cervical and upper thoracic levels. In all, such levels had 50 per cent or more and in one complete destruction of the motor neurons in the anterior horn. The posterior horns usually showed mild patchy involvement which appeared unrelated to severity of the lesion in the anterior horn. Clark's columns and the intermediolateral columns were affected at some level in three patients.

The newborn infant appeared normal at birth. Dyspnea and right facial paresis developed 12 days later. At autopsy, changes of the nervous system were confined to lesions at or below the level of the pons. Structures involved were the motor nucleus of the fifth nerve of one side, facial nucleus and nucleus ambiguus bilaterally, reticular formation of the medulla and to a slight degree the pontile reticular formation. Almost all cells of the motor nucleus of the trigeminal nerve were necrotic but the sensory nucleus was normal. There was complete absence of inflammatory cells, except for very occasional cuffing with lymphocytes. Neurons either had completely disappeared or showed various stages of disintegration. The cytoplasm of necrotic cells stained deeper with eosin than is normal, and the nucleus was usually dense and homogeneous. Often, both nucleus and cytoplasm were shrunken. Other cells were pale and swollen with a finely vacuolated cytoplasm. The substance of Nissl usually had completely disappeared. No intracellular inclusions were detected. The cord and olfactory bulbs were not available.

[The value of this paper to the general pathologist is in the emphasis on the widespread and topographic distribution of the lesions in poliomyelitis.—Ed.]

Clinical Manifestations and Pathologic Anatomy of Pinealoma. Heinrich Kalm and Rolf Magun[3] (Univ. of Hamburg) report four cases. In all patients the initial symptom

(3) Deutsche Ztschr. Nervenk 164:453-468, 1950.

was diabetes insipidus, which was followed by general hypothalamic symptoms such as cachexia, asthenia, impotence and lowering of the basal metabolic rate. Symptoms due to increased intracranial pressure, especially severe vomiting, appeared only four months after onset of disease and papilledema was mild, perhaps owing to dehydration or slow progression. Neurologic signs of invasion of the lamina quadrigemina (paralysis of upward gaze, pupillary disturbances, diplopia and nystagmus) and signs due to invasion of the cord were manifest 5-24 months after onset.

Grossly, there was a filling of the third ventricle leading to an internal hydrocephalus and compression of the lamina quadrigemina. Extensive metastases had spread along the adventitial layers of the subependymal vessels of the third ventricle and had broken through at various places, creating nodular metastases. In addition, seeding of pinealoblasts along the flow of cerebrospinal fluid produced an ependymoblastoma with metastases as far down as the cauda equina in one patient. Microscopically, the whole gamut of maturation of pinealoblasts was seen, but cells in all stages seemed to have retained the ability to divide. However the cells spreading along adventitia were all pinealoblasts and apparently only these have the capacity to metastasize and mature at the site of metastasis. Thus, this tumor would be of low grade malignancy, although metastasis occurs rather early. In three patients in whom lumbar puncture was performed, many lymphocytes were found in the cerebrospinal fluid. In retrospect these may have been pinealoblasts, which are similar to lymphocytes. Cytologic study of such "lymphocytes" in patients presenting a similar clinical picture may aid diagnosis.

[This study keeps open the question as to the exact nature of tumors of the pineal body.—Ed.]

Wernicke's Disease: Clinical and Pathologic Study of Nine Cases. Russell S. Boles and Russell S. Boles, Jr.[4] call attention to the importance of recognizing this disease clinically as health may be restored by adequate treatment with thiamine and other B complex vitamins. The disease occurs frequently in alcoholics and may be overlooked in persons with hepatitis, cirrhosis, pyloric stenosis, dysentery or malnutrition in pulmonary tuberculosis. Some disturbance of psychic function may be expected in all cases, usually initially. Ano-

(4) Gastroenterology 19:504-515, November, 1951.

rexia, anxiety, restlessness, loss of memory, lack of co-operation and even hostility should suggest the disease. Disturbances of consciousness follow. In the later stages stupor and coma are often present and sometimes obscure the neurologic findings. Various types of ophthalmoplegia, particularly nystagmus, are common. Ataxia is infrequent.

In two of nine cases observed by the authors the condition was recognized clinically and administration of thiamine produced prompt recovery. Diagnosis was made at autopsy in seven. In two, acute symptoms of Wernicke's disease appeared postoperatively, after subtotal gastrectomy in one and after pneumonectomy in the other. In one of the fatal cases fatty cirrhosis was present and in five cases fatty dystrophy of the liver.

Examination of the brain revealed changes in the mammillary bodies in all fatal cases. The mammillary bodies appeared small and brown. Microscopically, there were loss of cells, edema, ring hemorrhages and proliferation of astrocytic glia and microglia. Extensive varicosity and endothelial proliferation of blood vessels were commonly associated with the gliosis. In one instance the interpeduncular fossa was infiltrated by polymorphonuclear leukocytes, macrophages and erythrocytes. Other periaqueductal and periventricular structures were sometimes involved. The thalamus in four cases showed areas of cell loss and proliferation of glial elements. In about half the cases other parts of the brain were also involved. The dorsal vagal nuclei, olivary nuclei and vestibular nuclei occasionally showed cellular degeneration, edema, perivascular hemorrhage and gliosis. In one case there were scattered areas of satellitosis in the cerebral cortex and dilated perivascular spaces scattered throughout the brain. Perivascular collections of oligodendrogliocytes were prominent in the basal ganglions.

[When the description of the morphologic lesions is considered, one wonders at the presumed beneficial effects of vitamin treatment.—Ed.]

Pathology of the Retinopathy of Prematurity: Retrolental Fibroplasia was studied by Parker Heath[5] (Massachusetts Eye and Ear Infirm.). The material which depicts early pathologic change was obtained at autopsy; that illustrative of later stages was obtained by enucleation from patients suspected of harboring neoplasms. He found that the occurrence of retinop-

(5) Am. J. Ophth. 34:1249-1259, September, 1951.

athy of premature birth is proportional to the degree of prematurity. This bilateral disease originates at the oral zone of the retina. Although clinical recognition may be delayed for months, microscopic evidence of the disease can be found at birth or shortly thereafter.

The histopathology can be divided into three stages. In the first, there are edema, dilated capillary channels and hemorrhage in the incompletely differentiated oral attachment of the retina. This was not present in all meridians but was more often found in the temporal regions. Endothelial proliferation, budding and neovascular tissue production are characteristic. Uveal hyperemia is also present. This stage is specific for the disease and is reversible. The retinas posterior to the ora were differentiated according to fetal age. Ganglion cells were present, as well as outer and inner nuclear layers. The rods were well represented.

The second stage is that of extension of the pathologic process into the vitreous, which undergoes angiofibrous organization and ultimately causes separation of the retina. Uveal hyperemia is not present. A clinically apparent retrolental fibrous dentate membrane forms with retinal separation.

The third stage is that of pathologic repair and atrophy. Microscopic findings are variable and nonspecific. A completely separated gliosed and fibrotic retina, old hemorrhages, pigment, cholesterol and mineral deposits are found. Clinically, one frequently encounters glaucoma and, with the late atrophic changes, a shrunken globe.

[Several articles on other aspects of retrolental fibroplasia appeared in the 1951 YEAR BOOK OF EYE, EAR, NOSE AND THROAT, pages 82-90. —Ed.]

CLINICAL PATHOLOGY

ARTHUR HAWLEY SANFORD, M.D.

The following article presents the experiences with laboratory procedures of four clinical pathologists who work together. Because of the general character of the subjects discussed, this abstract may serve as an introduction to this part of the YEAR BOOK.

—A. H. SANFORD.

Laboratory Aids in Diagnosis and Treatment. According to Max M. Strumia, John J. McGraw, Jr., Albert B. Sample and Robert A. Donato[1] (Bryn Mawr, Pa.), clinical pathology is a relatively new specialty, which owes its birth and growth to development of the basic medical sciences. The great bulk of the work of the clinical pathologist deals with living patients; his task is to help them to live rather than to determine why they die. The clinical pathologist must accept his role of consulting partner with the clinician, and the clinician must learn to use the clinical pathologist as a daily consultant if the patient is to derive maximal benefit.

There is an ever growing tendency to put patients through a laboratory mill with the expectation that they will come out at the other end with diagnosis, prognosis and treatment stamped on a variety of laboratory reports. One of the most important points is proper selection of tests, based on thorough history and physical examination, which usually suggest the proper approach. This rational approach is better than blind use of a number of tests or spotty use of a few isolated and unrelated tests over a long period. A series of single tests done over a period of days is often more inconclusive than a group of tests in which the various results may be interpreted in their relation to one another. Therefore groups of diagnostic procedures are recommended (1) for broad general surveys of apparently healthy persons or those with ill defined complaints and (2) for patients in whom clinical studies have suggested a definite type of lesion. Since the more tests done, the lower the cost and the greater the benefit to the patient, Bryn Mawr Hospital offers groups of tests (metabolic, prenatal, liver and kidney function, electrolyte balance, etc.) at half the cost of individual tests in the specific group.

Among the newer laboratory procedures helpful to the clinician is estimation of blood volume. The most accepted

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(1) M. Clin. North America 35:1579-1592, November, 1951.

method for determination of the plasma volume is the dye method, using Evans' blue dye. Blood volume is generally estimated by determining the plasma volume and at the same time the relative volume of packed red cells. The most important practical application of the blood volume determination is in estimation of pre- and postoperative transfusion requirements. Another practical application is in the measure of anemia and of tolerance to transfusion of patients with cardiac failure. Among important hormone analyses are tests for protein-bound iodine, which may be of value in determining the status of thyroid function, and pregnancy tests utilizing frogs. Electrophoretic analysis, a valuable tool in the study of protein mixtures, has been used extensively in research. The clinical laboratory finds electrophoretic methods useful in diagnosis and evaluation of liver disease, lupus and multiple myeloma and in studying changes in blood proteins in various clinical conditions. Practical flame photometers are now commercially available and in clinical laboratories have proved valuable in study of electrolyte balance.

HEMATOLOGY

New Method for Determination of Fibrinogen in Small Samples of Plasma is described by Oscar D. Ratnoff and Calvin Menzie[2] (Johns Hopkins Univ.).

METHOD.—Venous blood was drawn from the antecubital vein and immediately transferred to Pyrex centrifuge tubes containing 0.2 ml. oxalate mixture for each 5 ml. blood. The oxalate mixture consisted of 0.8 mg. potassium oxalate and 1.2 Gm. ammonium oxalate in each 100 ml. solution.

Pyrex glass was crushed in a mortar so that the diameter of the largest particles was about 0.5 mm. The crushed glass was used repeatedly and washed between each use with chromic acid.

To a 40 ml. round-bottomed Pyrex centrifuge tube were added about 0.5 ml. crushed glass, 10 ml. of 0.85 per cent NaCl solution and 0.05 ml. thrombin solution (Parke, Davis & Co.) containing 1,000 National Institutes of Health units/ml. Finally, 0.5 ml. plasma was pipetted into the tube, which was then agitated with an oscillatory motion. After about 10 minutes the tube containing clotted fibrin was centrifuged for 5 minutes at 2,000 rpm in an angle centrifuge. After the supernatant fluid was discarded, 10 ml. NaCl solution was added to the fibrin clot. The clot was carefully expressed against the wall of the tube with a glass rod. The fibrin was again separated by centrifugation for three minutes, washed once more

(2) J. Lab. & Clin. Med. 37:316-320, February, 1951.

with 10 ml. NaCl solution and recentrifuged for three minutes. Then 1 ml. of 10 per cent NaOH solution was added to the centrifuge tube containing crushed glass and fibrin. The tube was heated in a boiling water bath for 10 minutes. After the tube cooled, 7 ml. water was added, followed by 3 ml. of 20 per cent sodium carbonate solution and 1 ml. of Folin and Ciocalteu phenol reagent (Hartmann Leddon Co.). A blue color resulted which was fully developed

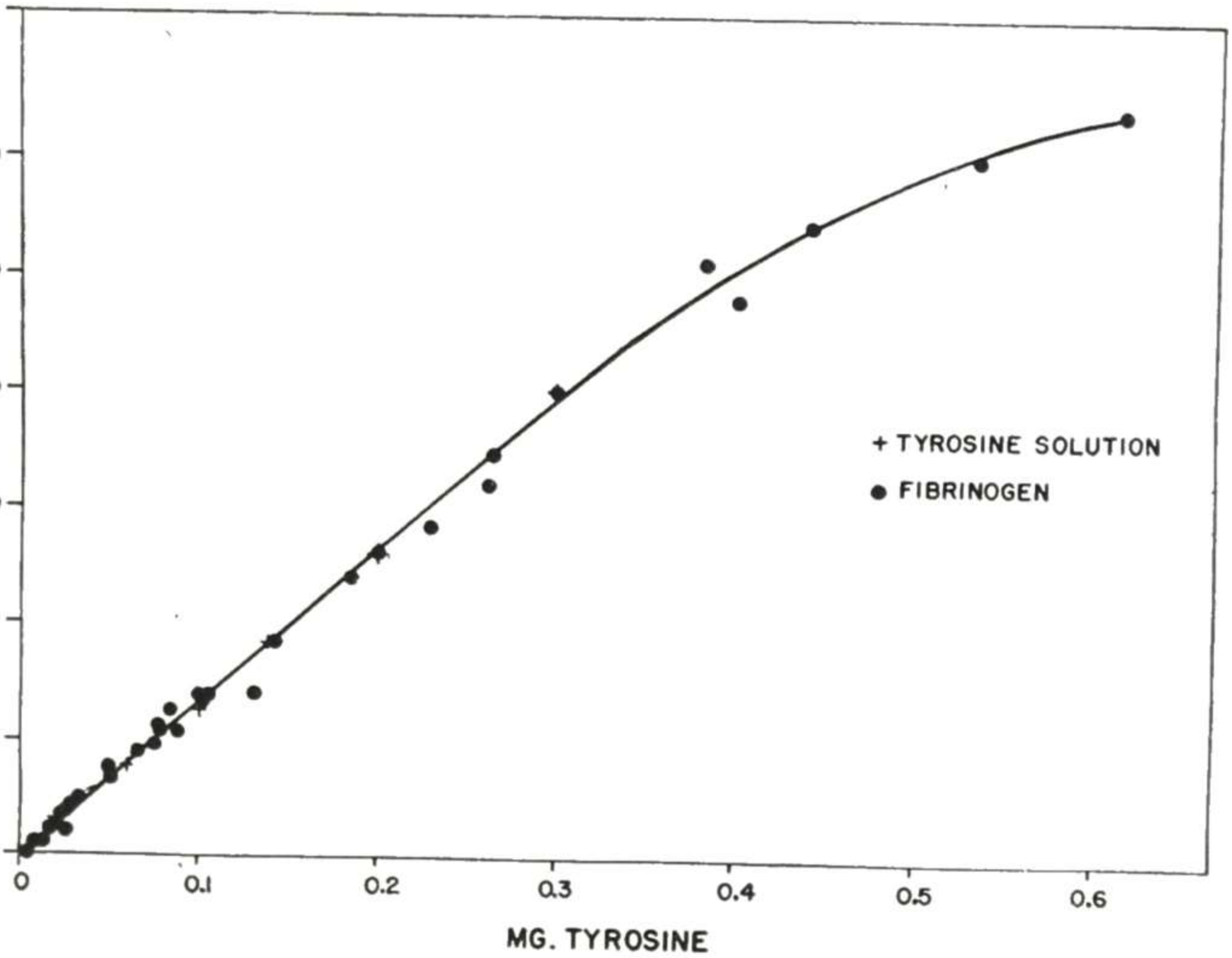

Fig. 85.—Calibration curve for determining relation between optical density and fibrinogen concentration. (Courtesy of Ratnoff, O. D., and Menzie, C.: J. Lab. & Clin. Med. 37:316-320, February, 1951.)

within 10 minutes. A 1 ml. aliquot colored solution was diluted with 3 ml. water. A blank was prepared by exactly duplicating the procedure outlined except for addition of plasma. Intensity of the color was read against the blank in a Coleman Junior spectrophotometer at a wave length of 650 mμ, with cuvets which had an internal diameter of 10 mm.

Amount of fibrin in the plasma tested was estimated by comparing intensity of the developed color with that of 1 ml. standard solution of tyrosine. This standard contained 200 mg. tyrosine/L. of 0.1 N HCl. One ml. tyrosine solution was pipetted into a 40 ml. centrifuge tube and in succession 1 ml. NaOH solution, 6 ml. water, 3 ml. sodium carbonate solution and 1 ml. phenol reagent were added. A blue color resulted and 1 ml. colored solution was diluted with 3 ml. water; after 10 minutes, intensity of the blue color was read against a blank which was prepared in the same way as the standard except that 1 ml. water was substituted for the tyrosine. Optical density of the developed color was directly proportional to concentration of fibrinogen or tyrosine until an amount equivalent

to 0.35 mg. tyrosine was reached. When intensity of color developed in an unknown solution was greater than that produced by 0.35 mg. tyrosine, the equivalent amount of tyrosine was estimated with the aid of a calibration curve.

In developing the calibration curve (Fig. 85), various amounts of tyrosine were diluted with water to 7 ml. Then 1 ml. of 10 per cent NaOH solution, 3 ml. of 20 per cent sodium carbonate solution and 1 ml. phenol reagent were added in succession. After 10 minutes, 1 ml. of this colored solution was diluted with 3 ml. water and intensity of color read against a blank. Optical densities of colors developed in solutions of 0.02-0.3 mg. tyrosine are plotted with cross marks.

A sample of oxalated plasma was diluted to varying degrees with NaCl solution and tyrosine-like activity of the fibrinogen in 0.5 ml. of each dilution of plasma was determined by the technic described. The tyrosine-like activity of one such solution was plotted arbitrarily on the tyrosine curve. Activity of the other dilutions was then plotted relative to this first point in proportion to degree of dilution. It was thus possible to determine the relation between optical density and concentration of fibrinogen beyond the point where optical density was directly proportional to its tyrosine-like activity.

Amount of fibrinogen in the plasma samples was calculated by multiplying the weight of tyrosine giving an equivalent amount of color by the factor 11.7. When the amount in 100 ml. plasma was reported, the calculated answer was multiplied by 1.08 to correct for the dilution of plasma by the anticoagulant solution.

With this technic, concentration of fibrinogen in plasma averaged 272 mg./100 ml. in adult men and 294 mg./100 ml. in adult women.

Effect of Carbon Dioxide on Blood Coagulation. In samples taken from the forelimb of eight dogs (1) without application of a tourniquet and (2) after application of a tourniquet for 5-10 minutes, René Fontaine, Paul Mandel, Amiot and L. Holderbach[3] (Strasbourg) demonstrated a significant increase of prothrombin activity, sometimes up to twofold, after stasis. In no case did the hematocrit reading change. For correct prothrombin determination, therefore, blood samples should be drawn without a tourniquet.

After semiclosed ether drop anesthesia in six dogs for 10 minutes, cyanosis was allowed to progress and blood samples were taken at peak. Only slight changes were observed in coagulation and clot retraction times or in the cell volume, but prothrombin activity was definitely increased. Four other dogs had the same changes under similar conditions, but after intubation and intensive oxygenation, prothrombin activity

(3) Presse méd. 59:1025-1026, July 18, 1951.

returned to normal, thus further indicating the need for prevention of anoxia during anesthesia.

Sensitized Clotting Time: Simple Test for Bedside Study of Clotting Mechanism is reported by Geza de Takats[4] (Univ. of Illinois).

METHOD.—Venous blood, 1 ml., is added to 4 μg. heparin in 0.1 ml. isotonic sodium chloride solution in a 13 × 100 mm. test tube which has been thoroughly cleaned and dried. The tube is corked and, after gentle inversion twice, allowed to stand for 12 minutes, after which it is inverted every half-minute until the blood stops flowing, as in the Lee-White method of venous coagulation time.

In normal young subjects and hospitalized patients with chronic vascular and nonvascular disease, clotting time was 22-28 minutes; when the test was run in duplicate, maximal deviation was not more than 2 minutes. Serial determinations run daily on postsurgical patients revealed two distinct patterns: (1) rise of heparin-retarded clotting time between the third and fourth days, and (2) no rise, or even a fall. The postoperative rise may be as high as 48 minutes. After severe trauma or massive hemorrhage, considerable elevation in clotting time may be observed. This is also true in the convalescent phase after acute thrombosis or acute coronary occlusion.

New Method of Determining Coagulation Time of Blood based on increasing viscosity and decreasing flow of clotting blood is described by Anders Kristenson[5] (Stockholm).

PROCEDURE.—About 10 ml. venous blood is placed in a rectangular vessel which is part of a special pendulum (Fig. 86). Skin, needle and inner surface of the container are oiled with 2 per cent olive oil-ether solution, the ether being allowed to evaporate completely. The container is covered with a closely fitting lid, the upper side of which consists of moist filter paper to prevent drying of blood during the experiment. The temperature is maintained at 35-40 C. The pendulum is adjusted so that it completes a double swing in about two seconds, and its excursion is limited to 10 degrees on each side of the zero position. A series of four to five swings in succession are recorded on a paper film each minute.

At first, movements of the pendulum are quickly moderated due to blood flow in the vessel. As coagulation progresses and blood flow diminishes, inhibition of excursion decreases (Fig. 87). By measuring the amplitude of the first and fourth swings at one minute intervals, a coagulation profile can be obtained.

Clinical tests gave varying results. Injection of heparin caused quick prolongation of coagulation with slow return

(4) J. A. M. A. 146:1370-1372, Aug. 11, 1951.
(5) Acta med. scandinav. (supp. 259) 140:9-17, 1951.

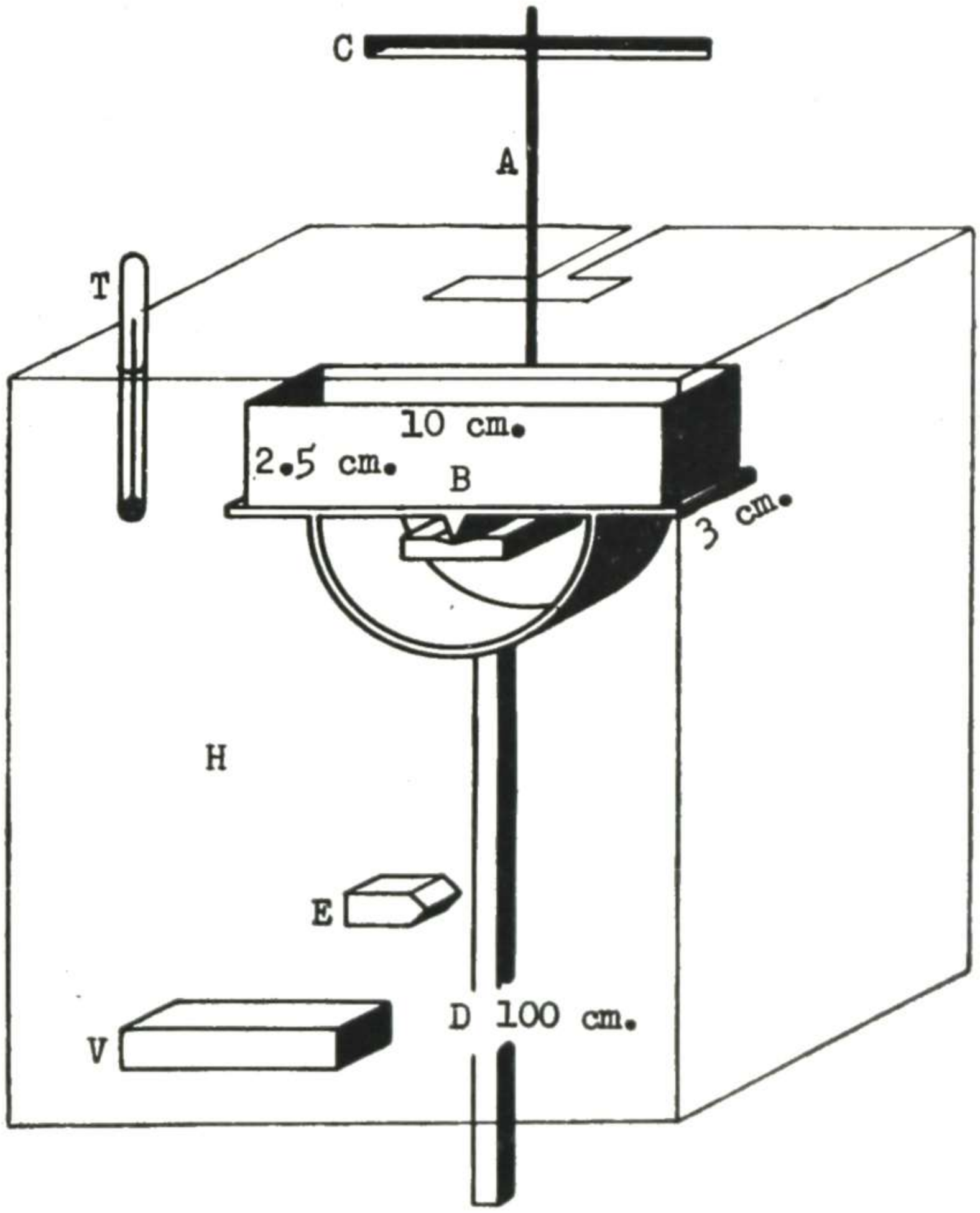

Fig. 86.—Pendulum arrangement. *A*, pointer, movements of which are registered by camera (*C*); *B*, rectangular vessel attached to pendulum (*D*); *E*, stop; *H*, cardboard hood; *T*, thermometer; *V*, electric heater. (Courtesy of Kristenson, A.: Acta med. scandinav. (supp. 259) 140:9-17, 1951.)

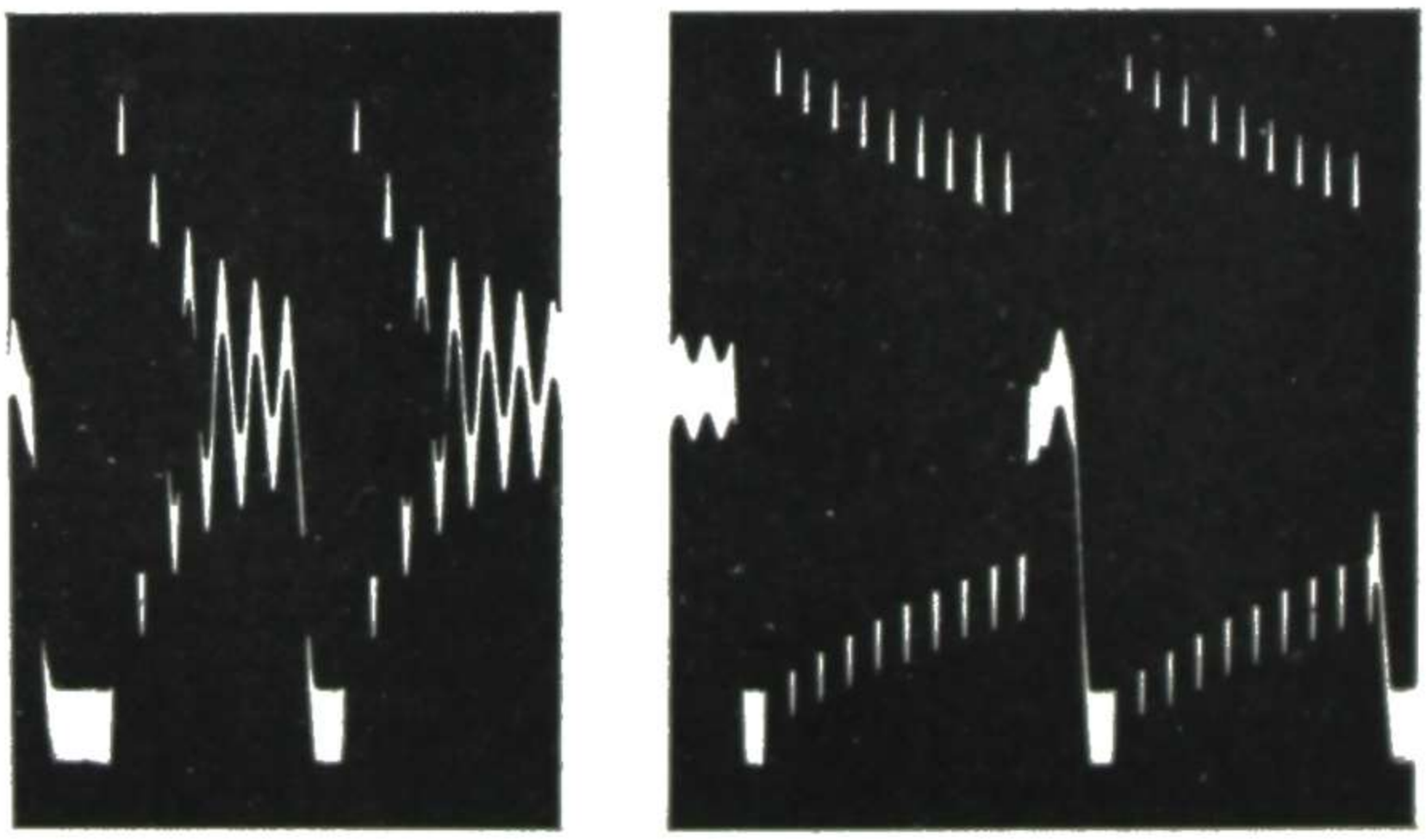

Fig. 87.—Registered pendulum movements. Curves obtained at beginning (fluid blood) and end (coagulated blood) of experiment. (Courtesy of Kristenson, A.: Acta med. scandinav. (supp. 259) 140:9-17, 1951.)

to normal. Dicumarol® gave varying effects. A prolonged prothrombin time may accompany a normal coagulation profile, and occasionally a normal prothrombin time accompanies a profile that indicates reduced coagulation capacity. In severe pulmonary tuberculosis and acute epidemic hepatitis, tendency to coagulation was reduced. In other diseases studied the coagulation profile was within normal limits.

Circulation Time as Clinical Test. H. Kopelman[6] (Postgraduate Med. School of London) studied circulation times using saccharine solution and the vital capacity in patients with congestive cardiac failure before and after treatment. In those with uncomplicated congestive failure due to hypertension, ischemic heart disease or chronic rheumatic valvular disease, circulation time was prolonged and vital capacity reduced. After treatment and improvement, circulation time progressively fell as vital capacity increased.

In patients whose congestive failure was complicated by long histories of recurrent and chronic bronchitis and who had clinical evidence of emphysema or fibrosis, there was some initial increase in circulation time and conspicuous reduction in vital capacity. With improvement, circulation time fell rapidly to a low normal value but vital capacity rose little or not at all. About half these patients also had hypertension.

In those with acute nephritis or acute uremia, circulation time was normal or only slightly prolonged, whereas vital capacity was reduced in all. With improvement, circulation time remained normal and vital capacity rose to its presumed previous level.

Further investigation showed that considerable reduction in arm-to-tongue circulation time can occur with little change in the cardiothoracic ratio. Although this ratio may remain much higher than the accepted normal, circulation time may be within normal limits. In 11 cases, the difference between the arm-to-tongue and the right heart-to-tongue circulation times varied between 3 and 6 seconds and was not related to the right auricular pressure measured by saline manometer. In 18 cases the minute cardiac output, determined by the Fick principle, was compared with the arm-to-tongue circulation time. With normal circulation times, cardiac output was higher than in those cases in which circulation time was prolonged.

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(6) Brit. Heart J. 13:301-308, July, 1951.

All these factors must be taken into account to determine the significance of the circulation time. Serial readings of the circulation time and the vital capacity and differentiation of cases into the groups described may help in evaluation of the relative parts played by these factors and their underlying cause.

Direct Method of Measuring Circulating Blood Volume of dogs is described by S. R. Mukherjee and S. Rowlands[7] (Univ. of Edinburgh).

PROCEDURE.—Erythrocytes are labeled with radioactive phosphorus and resuspended in inactive plasma to form whole blood. A standard suspension of erythrocytes is prepared from which the total activity of the injection can be estimated. Radioactive blood is injected and circulating blood sampled. The circulating blood volume is deduced through comparison of the radioactivity of the samples with that of the standard suspension.

The circulating blood volume of 21 normal healthy dogs of different ages and sexes and of mixed breeds was determined by this procedure. Results were the same as those obtained when standard technics for determining the blood volume were used. Average was 9.5 ± 1.7 ml./100 Gm. body weight. With some modifications, this method could give a reliable estimate of blood volume in human patients within 30 minutes of injection of radioactive erythrocytes.

Rapid Method for Clinical Total Blood Volume Determination Using Radioactive Iodinated Human Serum Albumin (RIHSA) is described by J. Bradley Aust, Shelley N. Chou, James F. Marvin, Edwin L. Brackney and George E. Moore[8] (Univ. of Minnesota). Human serum albumin is iodinated with I^{131} in an essentially neutral buffer. One cc. of RIHSA contains 5 mg. human serum albumin and 2 mg. salt. Metabolic studies have indicated that 24 hours after administration, about 55-65 per cent is still in the circulation; since it is gradually metabolized, free I^{131} is liberated and may be taken up by the thyroid. This can be prevented by blocking the thyroid with stable iodine in the form of Lugol's solution, 10 drops three times daily for a week, which is begun at least 24 hours before administration of RIHSA.

Figure 88 shows the design of the three tube Geiger counter constructed for this test. A thin-walled brass tube is centrally placed to hold the cuvets. The counter is mounted in

(7) Lancet 2:98-101, July 21, 1951.
(8) Proc. Soc. Exper. Biol. & Med. 77:514-518, July, 1951.

a lead cylinder to reduce background count. A coaxial cable connects the counter to a Berkeley scaler, model 1000B, which is controlled by a predetermined time interval clock. Heparin is used as an anticoagulant.

PROCEDURE.—From every batch of RIHSA delivered, a sterile

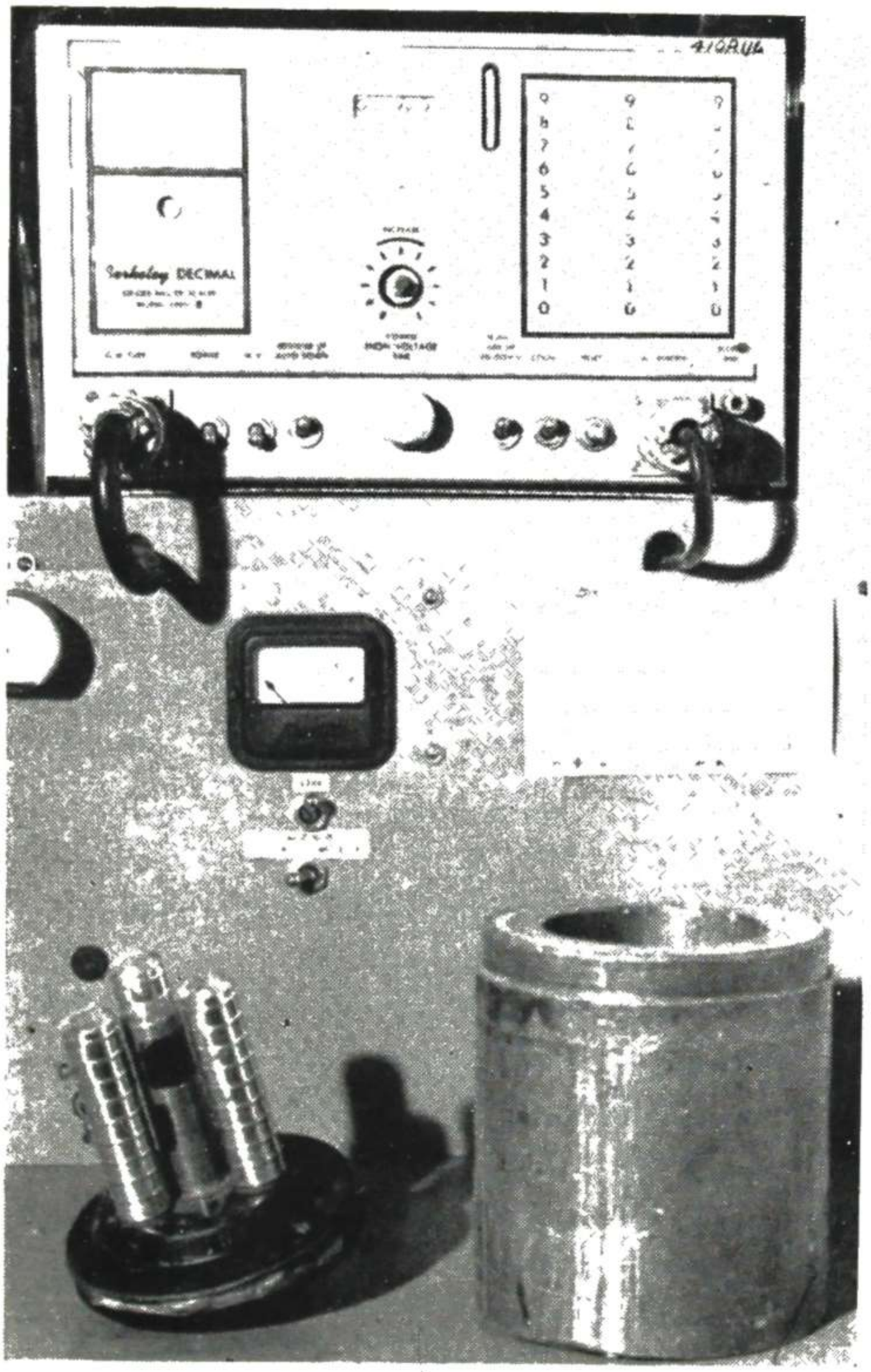


Fig. 88.—Three tube Geiger counter. (Courtesy of Aust, J. B., *et al.*: Proc. Soc. Exper. Biol. & Med. 77:514-518, July, 1951.)

tracer solution is prepared so that 10 cc. contains 60 μc. I^{131}. One cc. tracer solution is further diluted to 100 cc., of which 10 cc. is used as the standard. This contains 0.6 μc., which will give about 7,500 counts/five minutes in the triple thyrode counter. By means of calibrated syringes, each patient is given 10 cc. tracer solution intravenously, which contains 30-60 μc. I^{131}, depending on the decay factor; 10 minutes after administration, blood is withdrawn from the antecubital vein of the opposite side with a heparinized syringe and 10 cc. immediately delivered into a selected 10 cc. cuvet.

The cuvet is inverted several times to assure homogeneous suspension of the blood corpuscles and then put into the triple thyrode counter and counted for five minutes. Total blood volume can be calculated as follows:

$$TBV = \frac{\text{counts/5 min. standard} \times \text{cc. tracer solution given}}{\text{dilution of standard} \times \text{counts/5 min. specimen}}$$

Blood volume determinations may be repeated by giving additional doses of RIHSA after residual activity in the blood stream has been determined.

Blood Volume of Normal Female as Determined with P^{32}-Labeled Red Blood Cells. Nathaniel I. Berlin, Grace M. Hyde, Robert J. Parsons, John H. Lawrence and Shirley Port[9] (Univ. of California) measured blood volume in 16 normal females with P^{32}-labeled red blood cells. Blood volume was 64.4 cc./total red cell volume 27.0 cc. and plasma volume 37 cc./kg. body weight. These values for total red cell volume do not differ notably from those observed in a similar group of males when difference in total body fat in the two sexes is considered.

Cryoglobulinemia: Influence of Cryoglobulin on Suspension Stability and Sedimentation Rate of Erythrocytes. E. Hugh Luckey, Ella Russ and David P. Barr[1] (Cornell Univ.) isolated a chemically pure, apparently homogeneous cryoprotein from a patient with myeloma and evaluated its effect on the suspension stability and sedimentation rate of red cells. The patient was a man, 58, with sensitivity to cold, Raynaud's phenomenon, bleeding from mucous membranes, retinal hemorrhages, progressive deafness and arthritis. These symptoms were attributed partly to presence of a cold precipitable protein in the circulating blood in concentrations of 5.4-9.8 Gm. per cent.

Blood from this patient at 26 C. was compared with that of a patient with cold agglutination and autohemagglutination due to an unknown cause in titer of 1:1,000,000. Microscopic and photographic examination revealed that the phenomena of aggregation of red cells were entirely different in the two (Fig. 89). The abnormality in the blood of the patient with cold agglutinins but no cryoglobulins was easily identified as autohemagglutination with diffuse clumping of red cells and no precipitate formation. In the patient with cryoglobulinemia but no cold agglutinins, the apparent agglu-

(9) Proc. Soc. Exper. Biol. & Med. 76:831-832, April, 1951.
(1) J. Lab. & Clin. Med. 37:253-263, February, 1951.

tination at room temperature was due to precipitation of cryoglobulin with physical enmeshing of red cells and mechanical approximation of red cells by the precipitate and was regarded as pseudoagglutination. Under the microscope at room temperature, three different physical states were observed in the oxalated blood of the patient with cryoglobu-

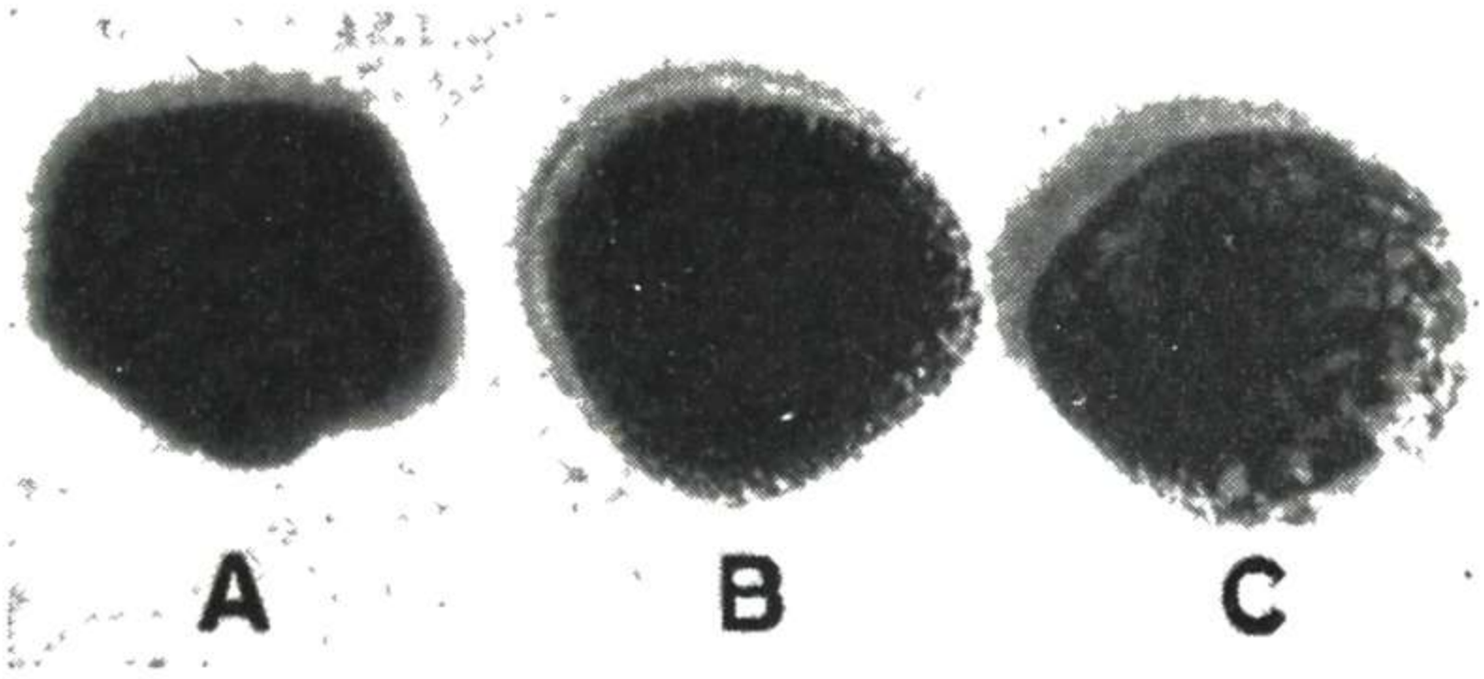


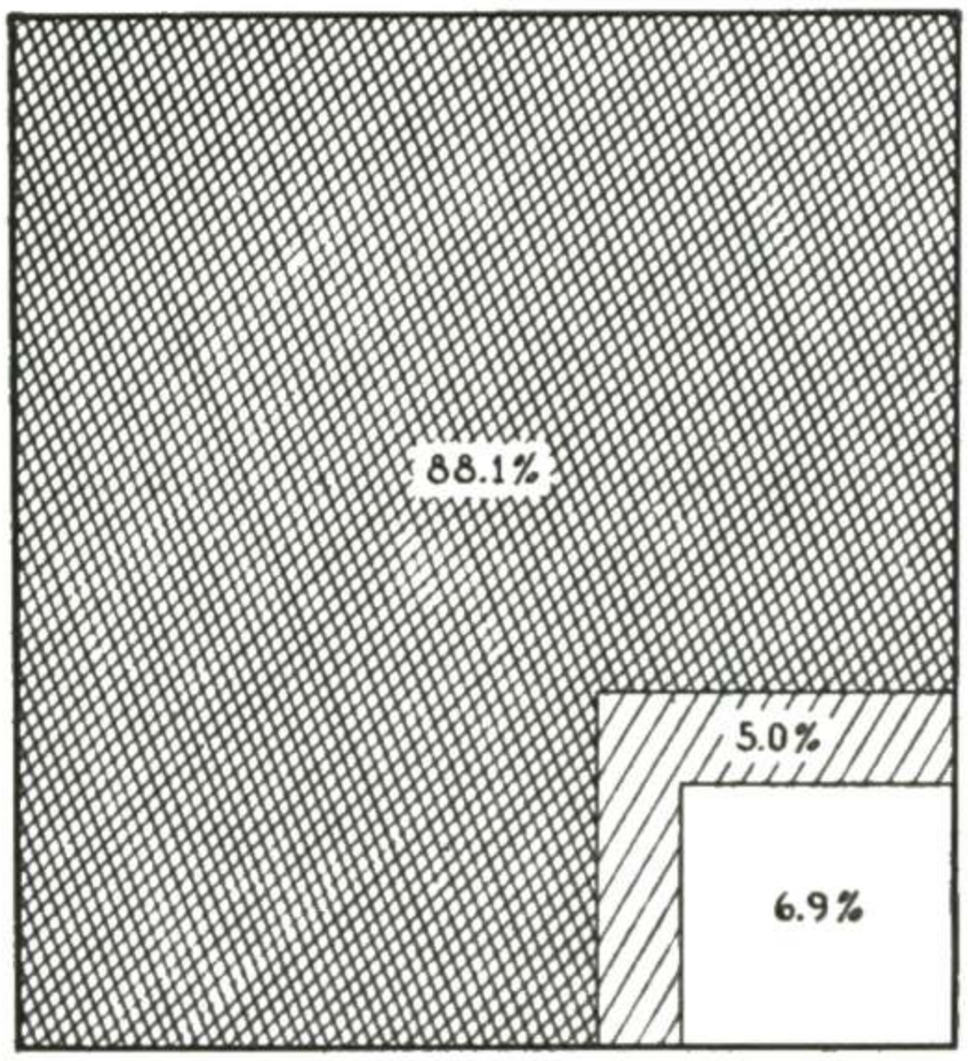
Fig. 89.—Comparison of drops of blood, with Heller-Paul solution as anticoagulant. *A,* normal; *B,* blood with cold agglutination titer of 1:1,000,000; *C,* blood containing cryoglobulin. (Courtesy of Luckey, E. H., *et al.*: J. Lab. & Clin. Med. 37:253-263, February, 1951.)

linemia: (1) aggregates of red cells in the midst of cryoglobulin precipitate; (2) rouleau formation in juxtaposition to the masses of cryoglobulin precipitate, and (3) normally distributed cells at variable distances from the area of cryoglobulin precipitate. On warming of the slide to body temperature and above, there was loss of the appearance of agglutination and disappearance of the cryoglobulin precipitate. As the blood of the patient with cryoglobulinemia was progressively diluted with normal saline solution, the cryoglobulin precipitate and aggregates of red cells became progressively less evident. The red cells from the patient with cold autoagglutination were still aggregated with dilutions of a million parts with normal saline solution.

There was striking acceleration of the corrected sedimentation rate of citrated blood from the patient with cryoglobulinemia both at room temperature and at 37 C. and with all anticoagulants at 37 C. Further experiments showed that the increased sedimentation rates were not due to intrinsic properties of the red cells or to fibrinogen. Removal of cryoglobulin from the plasma significantly decreased the corrected sedimentation rate of resuspended cells. The simple addition of cryoglobulin to normal blood profoundly increased the sedimentation rate.

The observations give further support to the long-recognized fact that the suspension stability and sedimentation rate of erythrocytes are affected by the protein content of the suspending fluids.

Clinical Significance of Increased Rouleau Formation in Smears of Peripheral Blood. The special value of rouleau formation in detection of multiple myeloma is well recognized, but Edwin D. Bayrd[2] (Mayo Clinic) states that its



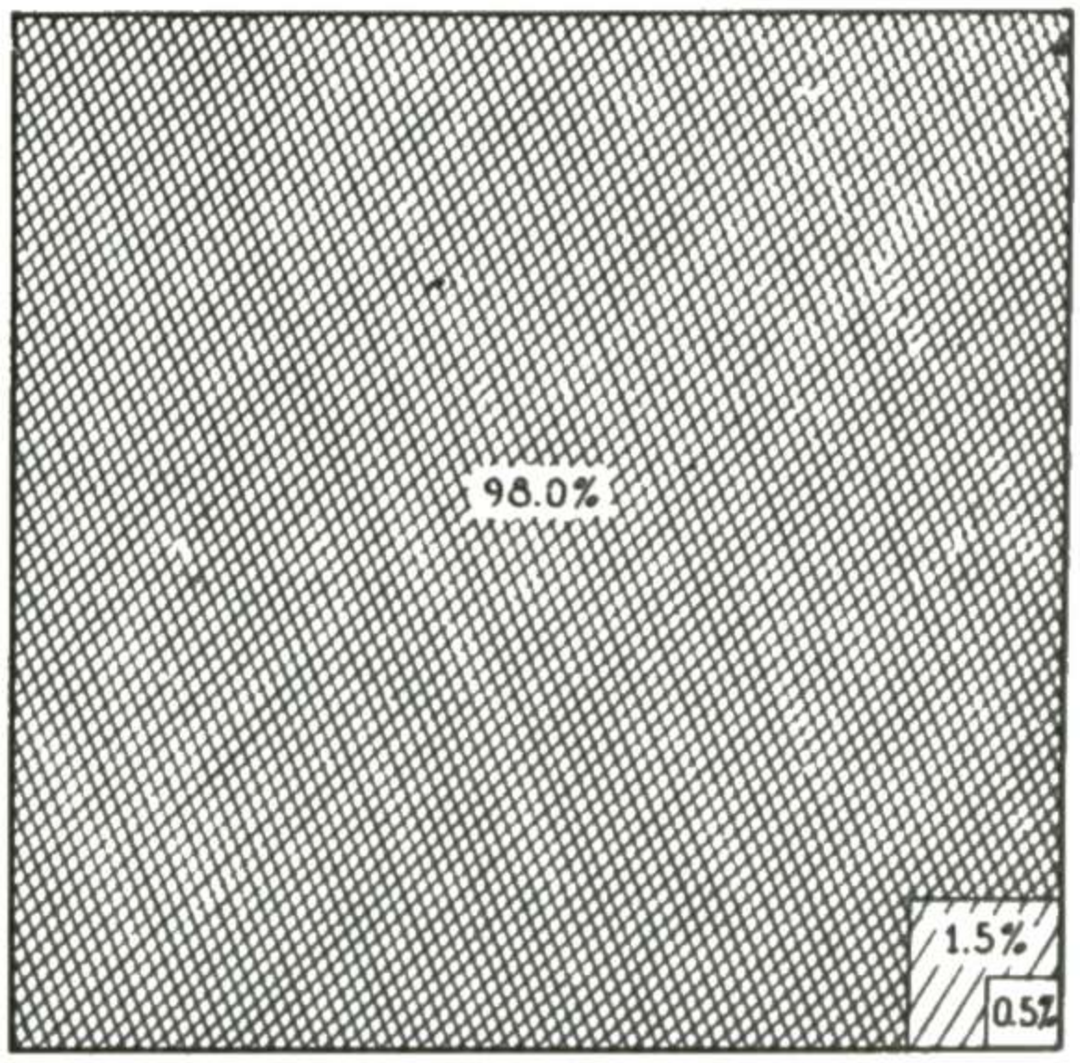


Fig. 90 (left).—Proportion of patients with grade 1 or 2 rouleau formation having significant organic disease (crosshatch), questionably significant organic disease (diagonal lines) or no recognized organic disease (clear space).

Fig. 91 (right).—Proportion of patients with grade 3 or 4 rouleau formation having significant, questionably significant or no recognized organic disease.

(Courtesy of Bayrd, E. D.: Am. J. Clin. Path. 21:777-784, August, 1951.)

significance in other conditions is generally unappreciated. Rouleau formation in 414 cases was graded (1 to 4) and correlated with the diagnosis, sedimentation rates, total serum protein values and albumin-globulin ratios.

All but 30 patients had a significant organic disease (Figs. 90 and 91). In 15 of the 30 patients the excessive rouleau formation occurred in the summer months, when drying of smears is slower than in other months and formation of rouleaux somewhat enhanced. Of the 384 with organic disease, 135 had neoplasms, 67 infections, 36 disturbed hepatic function, 34 a collagen disease and 24 renal disease. The rest had miscellaneous disorders. Of those with neoplasm, 85 had carcinoma, 33 malignant lymphoma and 17 multiple myeloma.

Of the 45 patients with grade 4 rouleau formation, only 8.8 per cent had sedimentation rates less than 100 mm./hour

(2) Am. J. Clin. Path. 21:777-784, August, 1951.

(Westergren). There was an unequivocable tendency for the albumin-globulin ratio to fall below normal but in more than a fourth of the patients the ratio remained within the normal range.

The simultaneous occurrence of appreciable myeloid immaturity and excessive rouleau formation is presumptive evidence for underlying neoplasm but against leukemia. When macrocytosis was noted with associated high grade rouleau formation, hepatic disease was present.

Method for Obtaining Large Yields of Human Platelets as By-product of Blood Collection is described by Gustave Freeman[3] (Children's Med. Center, Boston). Blood-collecting and -transfusing sets made of translucent plastic material and containing a column of ion-exchange resin (sulfonated polysterene divinyl benzene copolymer) for making blood incoagulable by decalcification were used. Needles were coated with tris (2-hydroxyethyl) dodecylamine. The resin column, contained in 28 mm. tubing, was suspended between the donor needle and the blood receptacle by small caliber tubing. The ion exchanger consisted of 50 Gm. Dowex-50 beads on the sodium cycle.

After blood was collected, the resin container and a few centimeters of attached tubing were cut free and the contents washed with unbuffered 0.085 per cent NaCl in distilled water into a silicone-lined bottle. About 10 ml. saline was added at a time and the resin container kneaded to free blood elements attached to the resin beads. The total volume of saline used equaled the volume of blood collected. About 88 per cent of the platelets filtered from the blood were recovered.

Platelets eluted from resin appear normal by visualization in the phase microscope and when added to oxalated, platelet-poor plasma reduce the prolonged clotting time to normal.

Remarks on Technic of Eosinophil Counting. C. G. Bergstrand, B. Hellström and B. Jonsson[4] (Karolinska Inst.) state that counting of eosinophils in a chamber after staining by Rud's method gives reliable results for both venous and capillary blood.

Method.—The tip of the finger or heel is pricked with a sharp lancet so that bleeding is free and abundant. The first few drops of blood are discarded and the next drawn into the pipet. The

(3) Science 114:527-528, Nov. 16, 1951.
(4) Scandinav. J. Clin. & Lab. Invest. 2:341-345, 1950.

blood is carefully blown into a tube from the pipet (Ellermann's system for diluting blood) and mixed with the staining solution by shaking the tube. The staining solution contains 1 ml. of 1 per cent magdala red, 6 ml. acetone, 14-20 drops of 10 per cent sodium carbonate and 45 ml. water. Proportion of blood to staining solution is 1:20. A mixing time of 30 seconds gives most satisfactory results. The Jessen counting chamber with a depth of 0.4 mm. and a

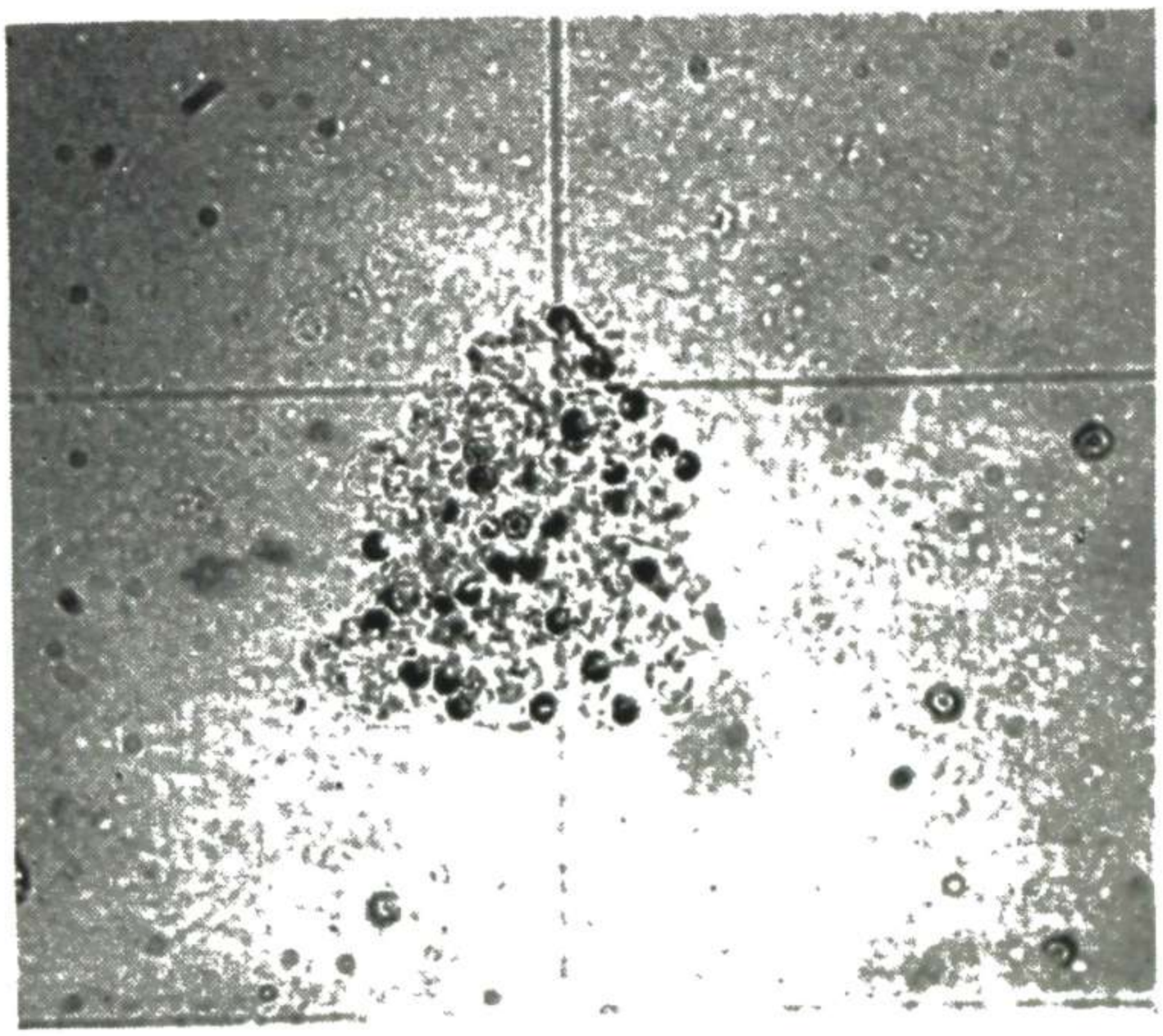


Fig. 92.—Agglutination of eosinophils after mixing blood with staining solution for five minutes; reduced from × 220. (Courtesy of Bergstrand, C. G., *et al.*: Scandinav. J. Clin. & Lab. Invest. 2:341-345, 1950.)

volume of 10 cu. mm. provides enough cells to be counted in a single chamber.

An important detail in the procedure is the length of time the blood is mixed with the staining solution. When shaken too long the eosinophils agglutinate (Fig. 92); this is probably the source of error noted by other investigators. Accuracy of the method was calculated on the basis of double estimations, and was 7 per cent when ½ cu. mm. undiluted blood was examined. The error varied somewhat with the number of cells in the specimen. For values below 50 cells/cu. mm., more than 0.5 cu. mm. blood should be counted. In any case the error can be diminished by counting a greater volume.

Variation and Error in Eosinophil Counts of Blood and Bone Marrow were studied by William R. Best and Max Samter[5] (Univ. of Illinois). The differential count has a large margin of error and is not suitable for enumeration of eosino-

(5) Blood 6:61-74, January, 1951.

phils in the peripheral blood. It is of value in study of eosinophils in marrow because volumetric enumeration is unreliable and atypical staining characteristics of certain young forms make their identification with chamber methods difficult. There are several satisfactory staining methods.

METHODS.—Dunger described an eosin-acetone stain, which has been modified by Thorn: 5 cc. of 2 per cent aqueous eosin, 5 cc.

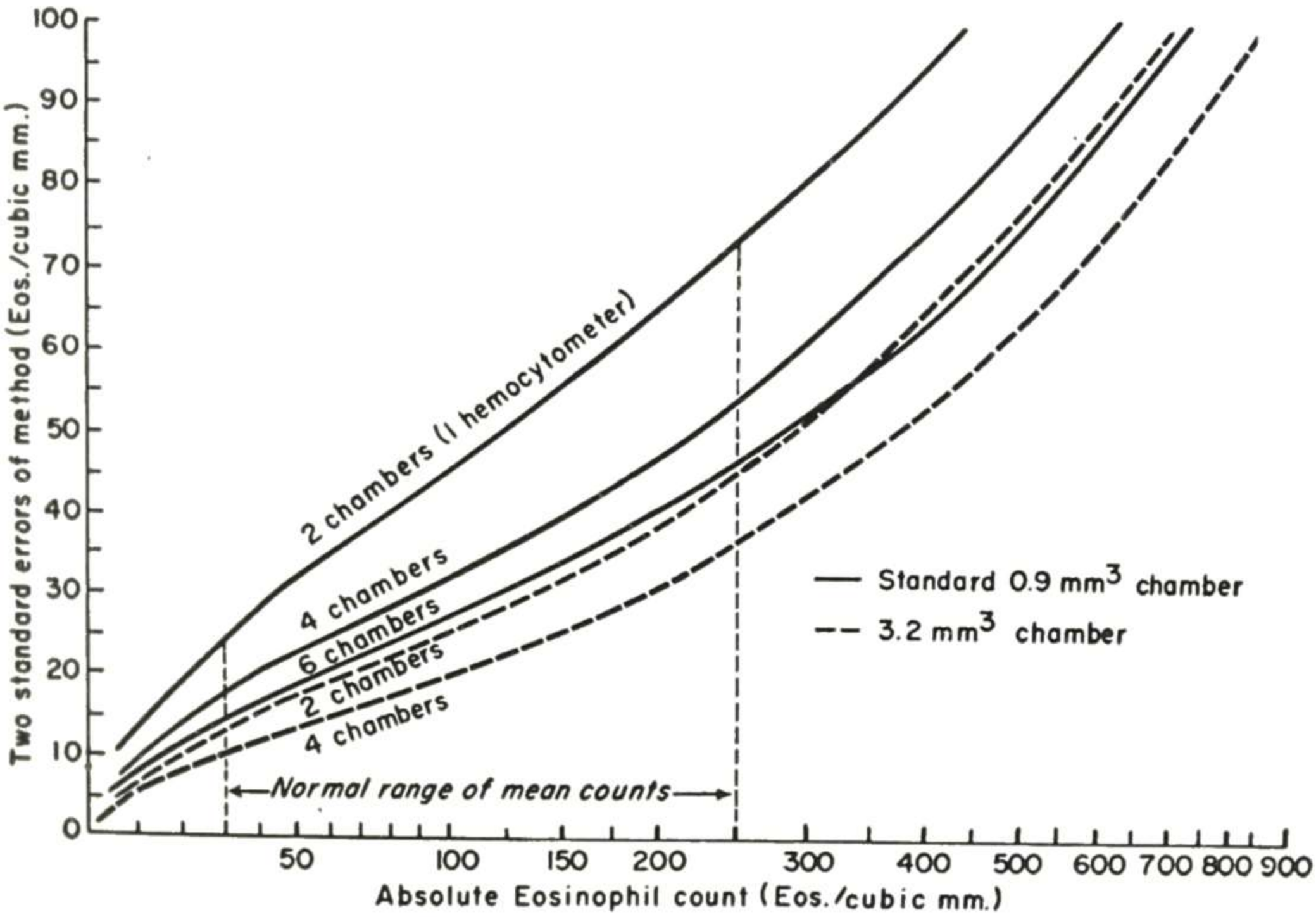


Fig. 93.—Chance theoretical error of chamber eosinophil counts. (Courtesy of Best, W. R., and Samter, M.: Blood 6:61-74, January, 1951.)

acetone and distilled water to make 100 cc. After preparation the mixture is filtered and may be refrigerated. Blood drawn into the mixture must not be shaken more than 30 seconds or lysis will occur. Staining is optimal in about three minutes.

Randolph proposed a stain in which two stock solutions are used: (1) 0.1 per cent methylene blue in 50 cc. propylene glycol and 50 cc. distilled water; (2) 0.1 per cent phloxine in 50 cc. propylene glycol and 50 cc. distilled water. Equal portions of each should be mixed and filtered for each day's use. Optimal staining requires at least 20 minutes after mixing. In contrast with the eosin-acetone stain, filled chambers may be stored under a moist Petri dish cover several hours before cells are counted, a procedure which is helpful if several subjects are being studied simultaneously.

A 1:10 dilution is preferred for counting. Blood is drawn to the 1.0 mark of a standard white cell pipet and stain to the 11.0 mark. Since the larger the chamber, the more eosinophils will be counted with a resultant increase in accuracy, a special large Levy counting chamber 0.2 mm. deep with a ruled area of 4 × 4 mm. is recom-

mended. Cells should be counted in the entire ruled area of both chambers. Increase in the number of chambers will improve validity of the count.

Berkson and associates have proposed a formula by which the errors of chamber counts due to chance distribution and to allowable inaccuracies of equipment manufacture may be

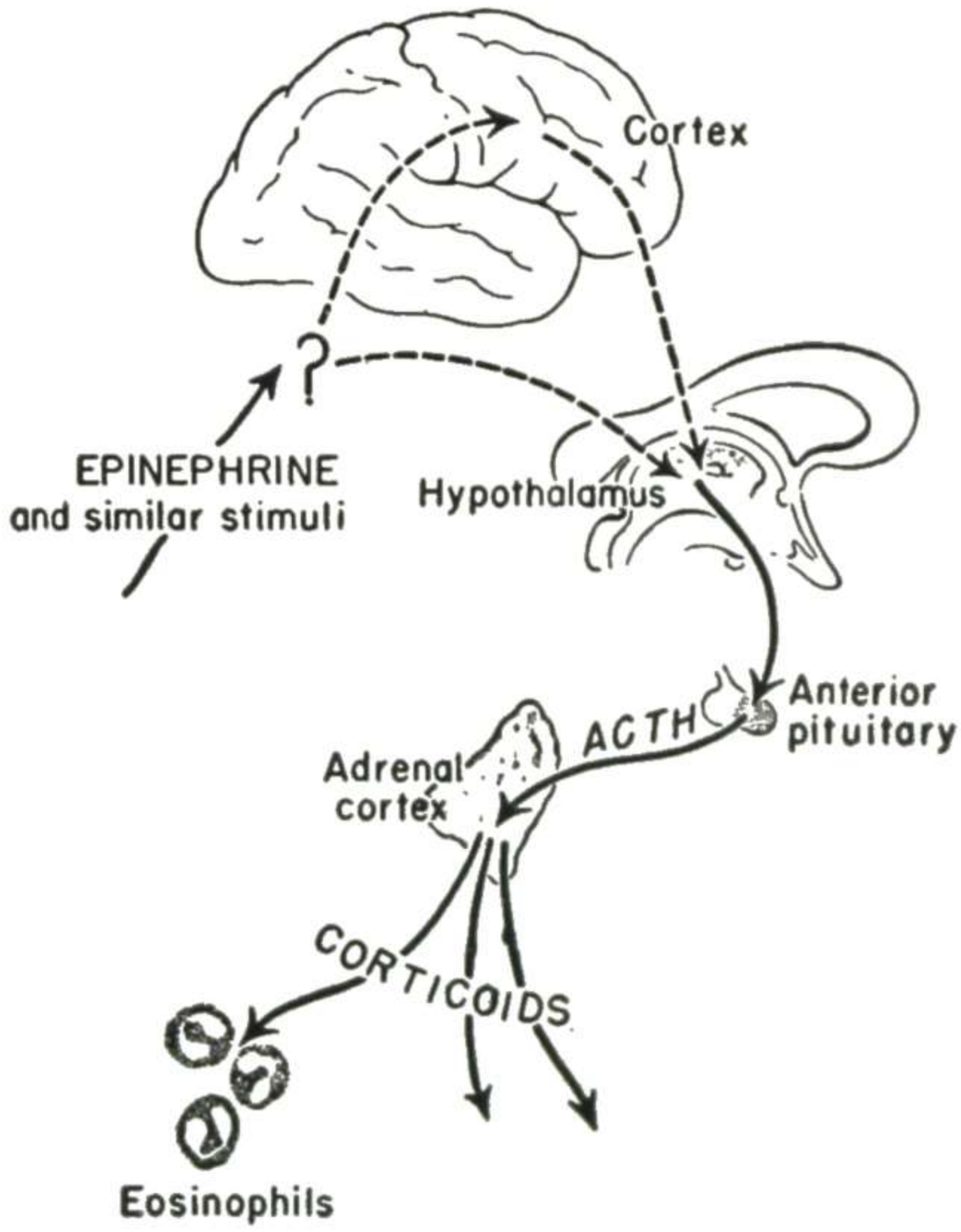


Fig. 94.—Hypothalamic-pituitary-adrenal chain of epinephrine eosinopenia. (Courtesy of Best, W. R., and Samter, M.: Blood 6:61-74, January, 1951.)

determined. The coefficient of variation equals the square root of the quantity:

$$\frac{(92)^2}{\text{no. cells counted}} + \frac{(4.6)^2}{\text{no. chambers used}} + \frac{(4.7)^2}{\text{no. pipets used}}$$

This formula can be applied to eosinophil counts regardless of stain, dilution, chamber size or number of chambers used. It does not allow for technical errors of measuring, mixing or chamber filling which vary with the technician's proficiency. With this formula, curves were plotted defining two standard errors of the method for several chamber combinations according to the mean eosinophil level (Fig. 93).

Maximal eosinopenia due to ACTH or cortisone occurs at about four hours. Eosinopenia of 50 or more per cent four hours after subcutaneous or intravenous administration of

epinephrine (0.2 mg. or more) or an equivalent oral dose of ephedrine (45 mg.) may imply adequate function of the hypothalamic-pituitary-adrenal chain (Fig. 94). Failure to obtain adequate response may be due to: (1) inaccuracies of the test owing to chance and physiologic variation; (2) refractoriness to a single dose of ACTH, epinephrine or ephedrine; (3) the patient might be tested while subjected to stimuli inherent in his disease or environment which are known to cause release of adrenocorticotrophic hormones. The relatively slight additional stimulation of the test may then be sufficient to induce further eosinopenia, a phenomenon observed while testing patients under stress or acutely ill.

Observations on Eosinophil Count in Man: Proposed Test of Adrenal Cortical Function. Bernard Fisher and Edwin R. Fisher[6] (Univ. of Pittsburgh) performed 514 eosinophil counts on 149 normal healthy male and female subjects under various conditions. Results were 5-887 eosinophils/cu. mm. blood. For clinical purposes a normal range of 25-300 would seem optimal. There was no significant difference between fasting and nonfasting subjects. There was a significant fluctuation or diurnal variation in both groups. Results were so variable after injection of adrenal cortical extract into 25 subjects that no conclusions could be drawn as to its effect on circulating eosinophils. Administration of adrenalin® caused a significant decrease in circulating eosinophils. There was no apparent relation between total circulating eosinophil and total leukocyte values.

METHOD.—A free flow of capillary blood was obtained by puncture wound of the finger with a sharp beveled, scalpel blade. The blood was drawn to the 1 mark on a standard leukocyte pipet after the first drop was removed from the skin with dry cotton. Hinkleman's solution (0.5 Gm. yellow eosin, 0.5 cc. Formalin [concentrated], 0.5 cc. of 95 per cent phenol, sufficient distilled water to make 100 cc.), the diluent and stain were then drawn to the 11 mark. The pipet was shaken vigorously and, after a few minutes, shaken again. Four standard Neubauer type counting chambers were carefully charged. Eosinophils were then counted on the four chambers, including all 16 sq. mm. Average of the four chambers was calculated and half of this multiplied by the factor 50 to obtain the number/cu. mm. blood.

Circulating Eosinophils in Children in Health and Disease. Katharine Hain[7] (Cornell Univ.) analyzed 361 counts of circulating eosinophil done on 75 children with various

(6) Am. J. M. Sc. 221:121-132, February, 1951.
(7) Pediatrics 7:408-414, March, 1951.

conditions. Eosinophil counts were determined by the Thorn modification of the Dunger method in which eosinophils are stained bright red with an acetone-eosin stain whereas the other leukocytes are almost colorless.

The range of circulating eosinophils for 18 normal children was 109-359. Among 12 patients with nonfebrile miscellaneous disease, including Cushing's syndrome, chronic hemolytic anemia and rheumatoid arthritis, counts were 84-599. In three patients with mild febrile illness, counts were 170-341. In 29 patients with acute febrile illness, counts ranged from 0 to 97. In 10 patients with various allergic manifestations the range was 393-2,665. Patients with miscellaneous diseases subjected to the epinephrine, insulin or ACTH test had a pronounced drop in circulating eosinophils. The counts returned to previous levels after the tests.

The mechanism responsible for decreased eosinophil count is obscure, but apparently the decrease reflects the adequacy of the adrenal cortical reserve. Under carefully controlled conditions, the eosinophil count serves as a simple, practical test of this reserve.

Fibrinolysis and Eosinophil Count. The suggestion has been made that fibrinolysis is produced through the liberation of adrenaline. S. C. Truelove[8] (Radcliffe Infirm., Ox-

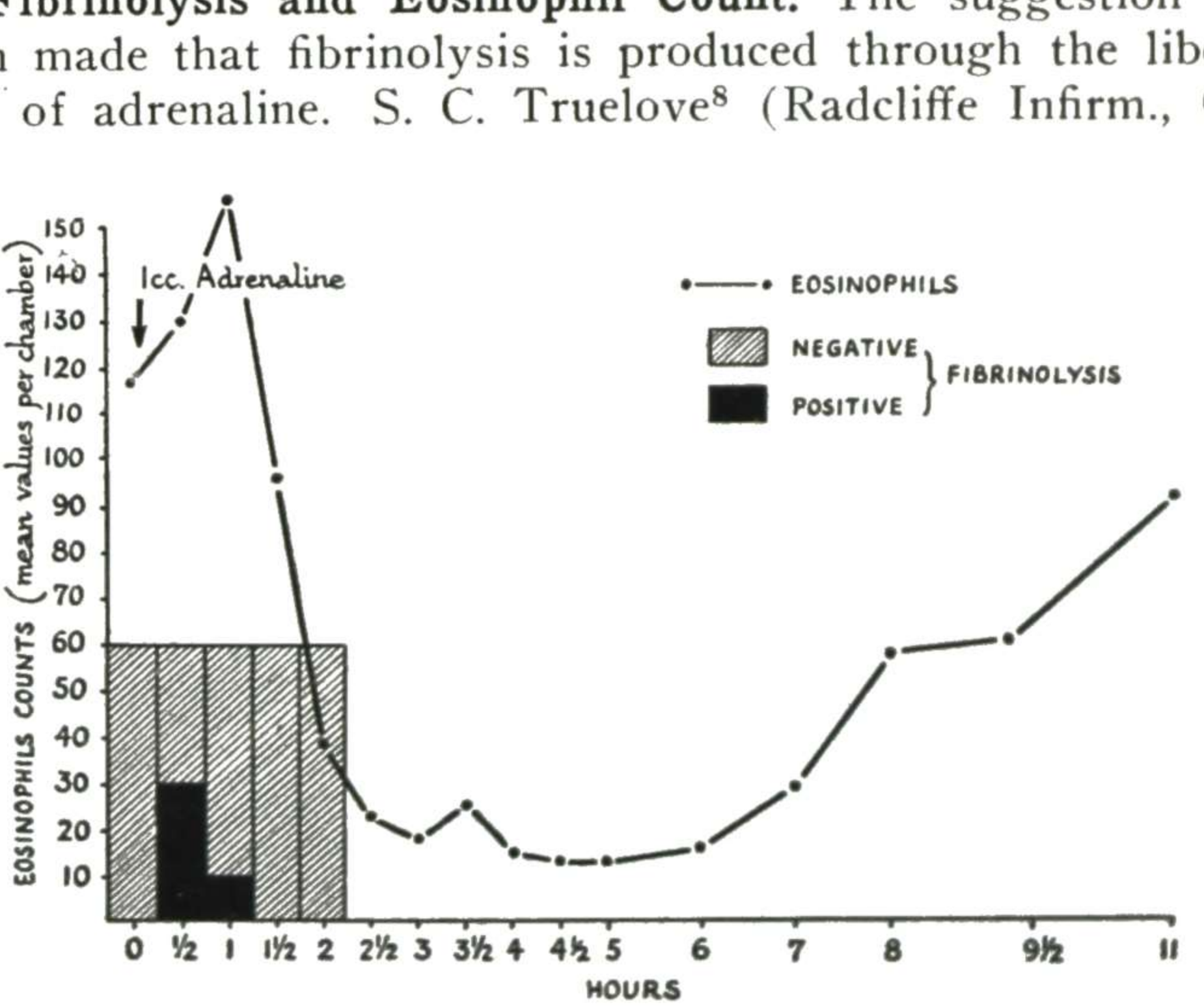


Fig. 95.—Effect of epinephrine on fibrinolysis and eosinophil count. (Courtesy of Truelove, S. C.: Clin. Sc. 10:229-240, May, 1951.)

(8) Clin. Sc. 10:229-240, May, 1951.

ford) examined this hypothesis by performing experiments on human volunteers. Subjects were given 1.0 mg. epinephrine subcutaneously. In all, fibrinolysis occurred after the injection, maximal time being about ¼-½ hour after it was begun. Eosinopenia was observed in all. Figure 95 shows the typical effect of the experiment. Fibrinolysis followed either exercise or suggestion under hypnosis, but neither was associated with eosinopenia. Smaller doses, insufficient to produce fibrinolysis, were also followed by eosinopenia. It seems unlikely that a high level of circulating adrenaline is the usual agent which induces fibrinolysis. Large doses of nor-epinephrine failed to provoke it. Delayed eosinopenia appears to be a more sensitive index of circulating adrenaline than fibrinolysis.

New Diluting Fluid for Eosinophil Count is described by T. Manners[9] (Royal Infirm., Sunderland). This fluid evaporates slowly and mixes easily. It stains eosinophils pink, does not lyze them but lyzes other leukocytes and erythrocytes.

METHOD.—The diluting fluid consists of 50 Gm. urea, 0.6 Gm. trisodium citrate, distilled water to 100 ml. and 5 ml. of 2 per cent aqueous phloxine. This solution should be centrifuged as soon as it is made and the upper 100 ml. supernatant fluid carefully pipeted into a thoroughly clean receptacle. Eosinophils tend to collect around any floating particle of dust or foreign material, thus giving an uneven distribution of dust in the counting chamber, which predisposes to erroneous counting.

Blood is drawn from the second or subsequent drop of a finger-prick to the 1 mark of an ordinary white-cell-counting pipet and the diluent added to the 11 mark. The pipet is shaken two to three minutes, and four of the Fuchs-Rosenthal counting chambers are filled by capillarity. The counting chambers are set aside in a moist chamber made by placing a few sheets of filter paper soaked in water in the bottom of a large Petri dish. After 15 minutes nearly all leukocytes other than eosinophils are lyzed and the count is performed under the 16 mm. objective with a 10× eyepiece. The number of eosinophils per cubic millimeter is ascertained by dividing the total number of cells in all counting chambers by 1.28.

Eosinophil Counting: Modification of Pilot's Method is described by J. C. W. MacFarlane and G. W. Cecil[1] (London). Pilot's fluid, which contains 50 ml. propylene glycol, 40 ml. distilled water, 10 ml. 1 per cent phloxine in water and 1 ml. 10 per cent sodium carbonate in water, was modified by the addition of 1 unit heparin/ml. solution. The heparin prevented formation of clumps of cells which could not

(9) Brit. M. J. 1:1429-1430, June 23, 1951.
(1) Ibid. 2:1187-1189, Nov. 17, 1951.

be broken by prolonged shaking of the pipet. With this modification accurate eosinophil counts could be made by waiting 15 minutes after collection and dilution of the blood to allow for proper lysis of cells other than eosinophils and staining of the eosinophils. The waiting period can be accomplished while the blood and counting fluid are in the pipet or in the counting chamber.

Diurnal Rhythmic Changes in Blood Eosinophil Levels in Health and Certain Diseases. Franz Halberg, Maurice B. Visscher, Edmund B. Flink, Kenneth Berge and Fred Bock[2] (Univ. of Minnesota) determined absolute values of circulating eosinophils in 17 normal male subjects. There was a regular diurnal rhythm in the eosinophil level in circulating venous blood. In the morning between 6:30 and 9:30 a.m., eosinophils decreased from 430 to 251/cu. mm. in "controlled" subjects. In general, when oral temperature was low, eosinophil value was high; this negative correlation, however, was not high over the entire period. There was no relation between barometric pressure, environmental temperature, relative humidity or meal times and eosinophil values.

Studies were carried out on eight hospitalized patients with various disorders related to the adrenal cortex and/or pituitary gland. Those with Addison's disease, bilateral adrenalectomy or hypopituitarism failed to show the normal diurnal eosinophil rhythm. This finding may be of diagnostic value.

The observations suggest that the adrenal cortical hormones play a role in eosinophil level fluctuation in the normal subject. Studies of the magnitude and time of eosinophil level swings may contribute to the knowledge of the dynamics of endocrine function.

Changes Observed in Mature Granulocytes Stained by Feulgen's Method in Different Age Groups. Noel Adler[3] (London) studied the blood films of 50 healthy men by the Feulgen method for detection of desoxyribonucleic acid. The subjects, aged 50-96, were divided into five age groups. Feulgen-negative granulocytes were counted and average concentration of nuclear chromatin estimated in the Feulgen-positive cells.

In a normal Feulgen blood picture erythrocytes are invis-

(2) Journal-Lancet 71:312-319, August, 1951.
(3) Lancet 2:293-294, Aug. 18, 1951.

ible; monocytes are Feulgen negative with a faint meshwork in the pale pink nuclei; small lymphocytes show an abundance of deeply stained coarse granules, situated mainly at the circumference of the nucleus (Fig. 96); large lymphocytes are Feulgen negative, and an intermediate and more immature

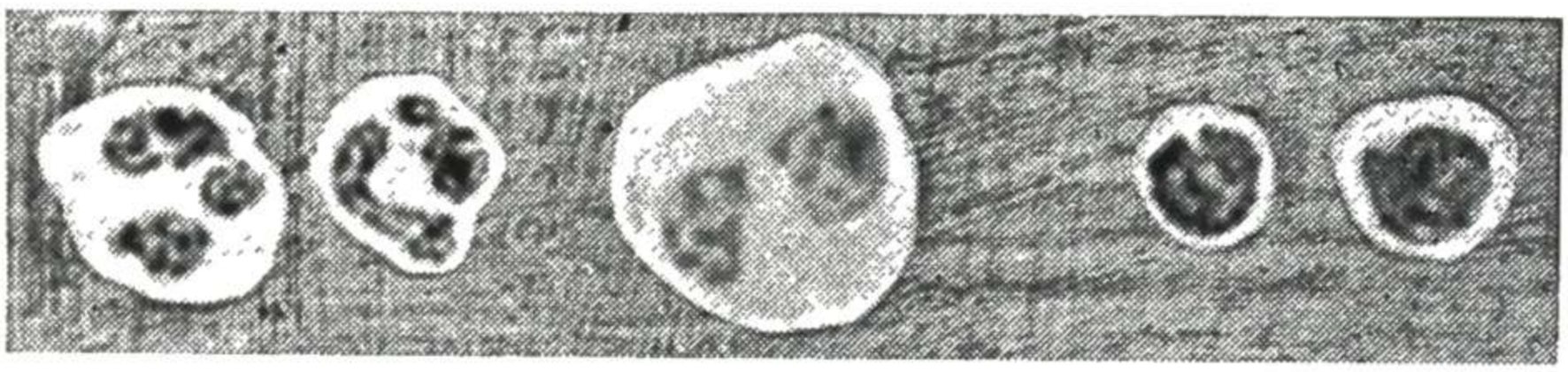


Fig. 96.—Left, Feulgen-positive cells with much nuclear chromatin; center, granulocyte showing only moderate Feulgen staining; right, two small lymphocytes showing positive Feulgen reaction. (Courtesy of Adler, N.: Lancet 2:293-294, Aug. 18, 1951.)

group contains moderately Feulgen-positive nuclei with Feulgen-negative nucleoli.

Results showed progressive increase of Feulgen-negative and decrease of Feulgen-positive nuclear material with age. The change was not uniform and there were variations in every age group. The greatest contrast was between the extremes of age. Counting of both Feulgen-positive and -negative cells may serve as an indication of intracellular changes in old age.

New Case of Pelger-Huët Nuclear Anomaly. Fifty families throughout the world have been reported to have this anomaly, which is transmitted by a dominant, but not a sex-linked, characteristic. In persons with this anomaly the nuclei in the circulating blood are of stab form, with coarse chromatin structure, or typically bisegmented and formed by two round parts which are connected by a small band (Fig. 97). The anomaly is often overlooked and the smear interpreted as an idiopathic shift to the left. It has been found in the rabbit; breeding experiments have shown that rabbits heterozygous with respect to this trait are otherwise normal, but those that are homozygous die in utero or early in infancy, or have many congenital deformities. Homozygous human beings are obviously very rare, but in heterozygous persons, increased susceptibility to infection, especially tuberculosis, is the only anomaly reported.

Five members of the family reported by Georg V. Knorre[4] have the anomaly. They comprise three generations

(4) Ztschr. ges. Inn. Med. 5:273-274, 1950.

—a grandmother, two adult daughters and two children of one daughter. One daughter has a child who is free from the anomaly; the other has had three children (two with the anomaly), one of whom died in infancy. All patients are clinically normal, but the grandmother has had several abortions and only two of six pregnancies resulted in children who grew to adulthood. The peripheral smears of all five are almost identical. The basophils show normal, though somewhat plump, nuclei; the monocytes have normal nuclei, but the

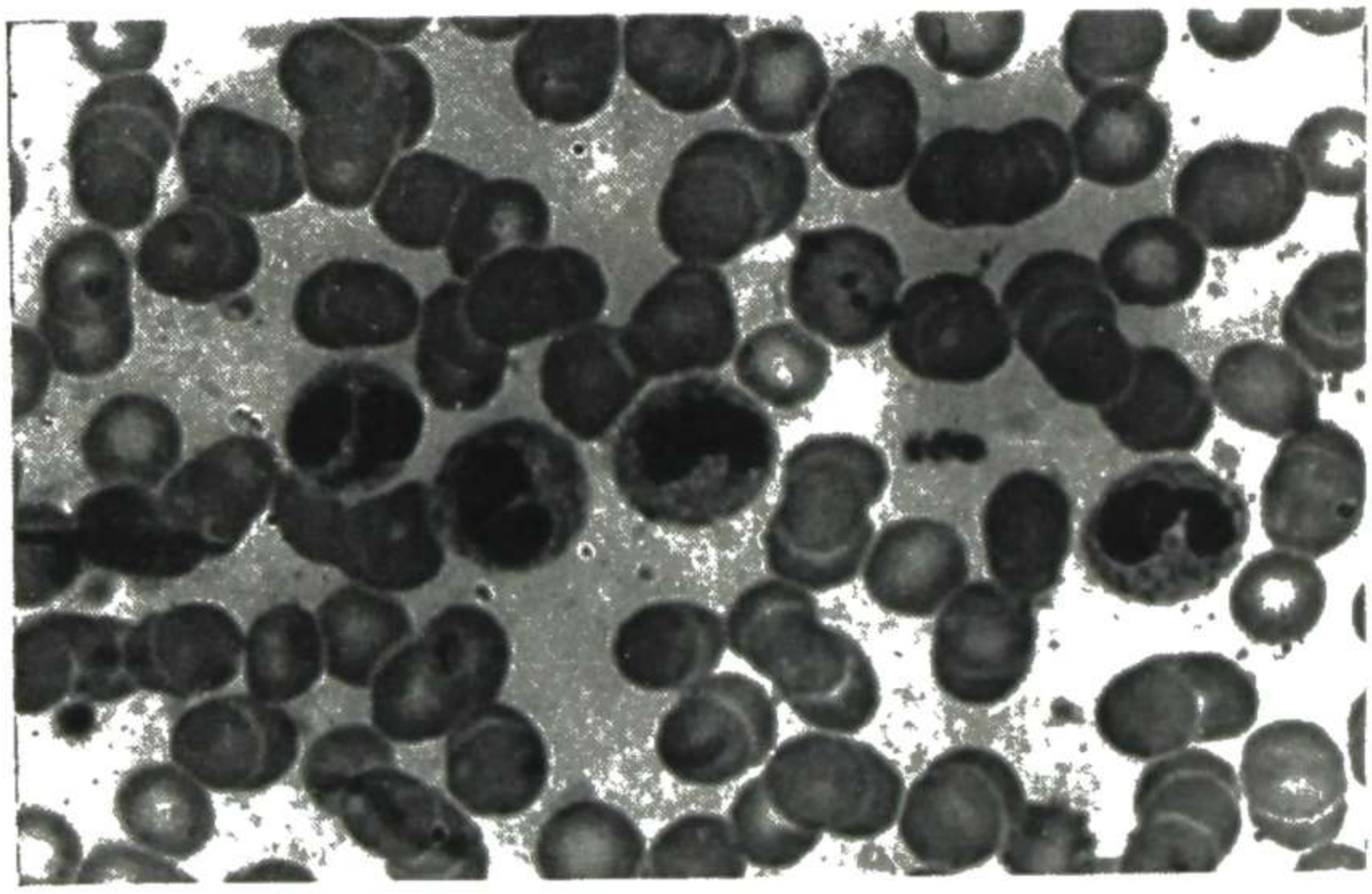


Fig. 97.—Appearance of blood in Pelger-Huët nuclear anomaly. (Courtesy of Knorre, G. V.: Ztschr. ges. Inn. Med. 5:273-274, 1950.)

eosinophils show the same anomaly as the neutrophils. In a few bisegmented cells cytoplasmic granulation is increased, although it does not reach the magnitude of toxic granulation. Bone marrow of only the grandmother was studied; it shows the typical clumped chromatin in the nuclei of the neutrophil series, including the myelocytes, but not the promyelocytes. The more mature eosinophils contain similar chromatin, but some of the eosinophilc myelocytes have a fine nuclear structure. The basophilic cells are normal.

[Huët, in an article entitled "Ueber eine bisher unbekannte familiare anomalie der leukocyten" (Klin. Wchnschr. 11:1264-1266, July 23, 1932), cited Pelger's description of the blood picture but gave no reference.—Ed.]

Normal Megakaryocyte Concentration in Aspirated Human Bone Marrow was investigated by Franklin G. Ebaugh, Jr., and Robert M. Bird[5] (Cornell Univ). Marrow from 23 healthy young adults had a mean megakaryocyte concentration of 6.1 ± 1.6/10,000 granulocytes. Of the three principal

(5) Blood 6:75-80, January, 1951.

cell types in marrow, the megakaryocyte content seemed to parallel most closely the granulocytes.

METHOD.—Sternal marrow was aspirated with no. 18 gauge needles. About 0.2 ml. marrow was withdrawn into a clean, dry 5 ml. glass syringe and immediately discharged on a clean glass slide. No anticoagulation was used. Duplicate dilutions were made in certified Thoma white cell pipets, Türck's solution being the diluting fluid. The dilution was 1:10. Before counting, the pipets were shaken by hand for three minutes. Megakaryocytes were enumerated in an 0.2 mm. deep, double chamber Fuchs-Rosenthal hemocytometer; total number of nucleated cells was determined with a conventional 0.1 mm. deep, improved Neubauer hemocytometer. The smears were stained by Wright's method and 500 cell differential counts performed.

Simple Office Procedure for Demonstrating Lupus Erythematosus Cells in Peripheral Blood is described by Hazel B. Mathis[6] (New York Univ.). With this procedure, results were consistently reproducible. Use of marrow or blood from other sources was unnecessary.

METHOD.—About 5 cc. venous blood is placed in a sterile centrifuge tube containing 3 drops of liquid heparin (liquaemin® sodium, Organon, Inc.; 1 cc. equals 10 mg.). After the sterile cotton plug is replaced, the tube is gently shaken to ensure adequate mixing and allowed to stand upright at room temperature until the blood cells have settled to the bottom. This usually requires about 30 minutes. A long-stemmed pipet is then used to transfer the supernatant plasma portion and the extreme uppermost level of the underlying cells to a second sterile centrifuge tube. It is maintained at 37.5 C. for 45 minutes or at room temperature for 2 hours.

The incubated plasma is centrifuged at 1,500-2,000 rpm for three minutes. Supernatant plasma is removed and all but about 0.5 cc. discarded. The latter is retained for use as a diluent. The sediment is completely removed with the pipet and 1 drop placed on the surface of a glass slide. (Slides should be kept in 95 per cent alcohol and wiped dry just before being used.) A second slide is placed on top and gentle pressure maintained until the drop of sediment is compressed into a thin layer. The slides are then drawn apart in opposite parallel directions. Several slides are prepared. The interior of the centrifuge tube is searched for small cartilage-like masses composed mainly of collections of packed platelets. The masses are picked up with a small wire loop, placed on a slide and emulsified with a drop of the plasma diluent. A film is then made as described. All preparations are air dried and stained. Best results have been obtained with tetrachrome (MacNeal) stain, which is applied for 1½ minutes and an equal volume of distilled water added for an additional two minutes. Slides are washed under running water and drained dry. Films are examined under "high dry" power, L.E. cells being more readily found at the periphery of the film.

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(6) Blood 6:470-473, May, 1951.

Demonstration of L.E. Cell without Use of Anticoagulants. Williford Eppes and Ethel Ludovic[7] (State Univ. of Iowa) describe a method.

METHOD.—Venous blood, 20 cc., from patients with systemic lupus erythematosus, was defibrinated by agitation in a small jar containing a paper clip. It was then transferred to a serologic tube and centrifuged for five minutes at 1,800 rpm. Since the buffy coat was indistinct, the top of the cellular layer was pipetted off and recentrifuged in a Wintrobe hematocrit tube. Smears were made of the buffy coat. Blood from the same patients was drawn into a syringe coated with silicone and transferred to a serologic tube similarly coated. This was centrifuged at 1,000 rpm for two minutes. Smears were made of material pipetted from the region of separation of cells from plasma.

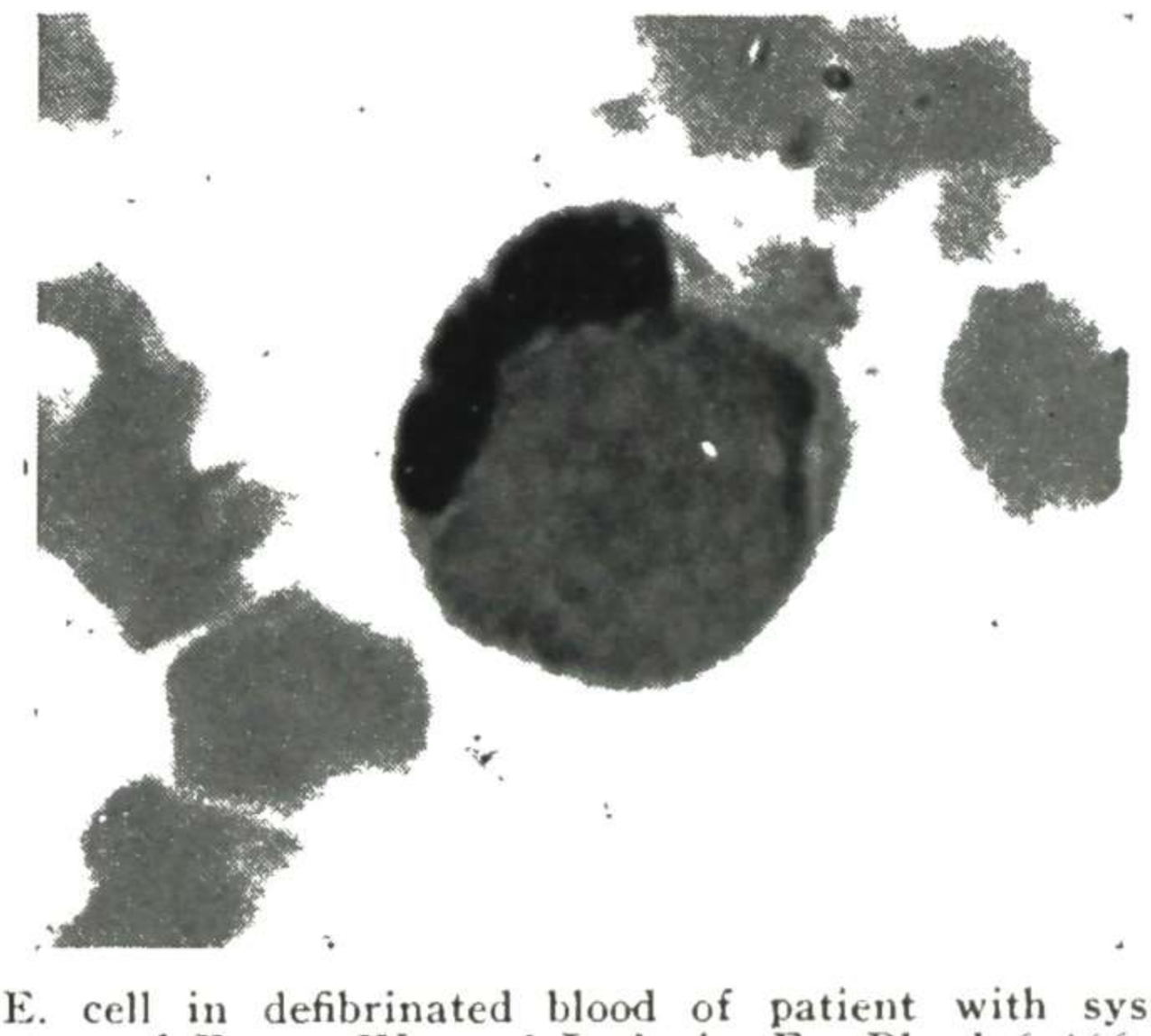

Fig. 98.—L.E. cell in defibrinated blood of patient with systemic lupus erythematosus. (Courtesy of Eppes, W., and Ludovic, E.: Blood 6:466-469, May, 1951.)

On the smears of the buffy coat of concentrated defibrinated blood, neutrophilic leukocytes were often grouped in large clusters of 10-50 cells and L.E. cells were observed frequently in these clusters and also found singly (Fig. 98). Small numbers of L.E. cells and rosettes were found in unmodified blood concentrated in silicone-coated tubes. Specimens from controls showed no L.E. cells or clumping of neutrophils.

[Dr. Eppes in a personal communication (Sept. 16, 1951) called attention to the work of Moffatt, Barnes and Weiss reported in the *Journal of Investigative Dermatology* (14:153-156, June, 1950). These authors independently demonstrated the L.E. cell in defibrinated blood without use of anticoagulants. Although their report appeared before this article, Dr. Eppes was not aware of it. See also the studies by Barnes, Moffatt, Lane, and Weiss in the 1950 YEAR BOOK, pages 266-269.—Ed.]

(7) Blood 6:466-469, May, 1951.

Plasma L.E. Test in Systemic Lupus Erythematosus: Study of 23 Patients with Positive L.E. Tests is reported by John R. Haserick[8] (Cleveland Clinic). The test is of value for the diagnosis and investigation of systemic lupus erythematosus. Since it depends on a rather unusual serologic procedure, there is a possibility that false-positive results may be obtained or that other diseases may produce a substance

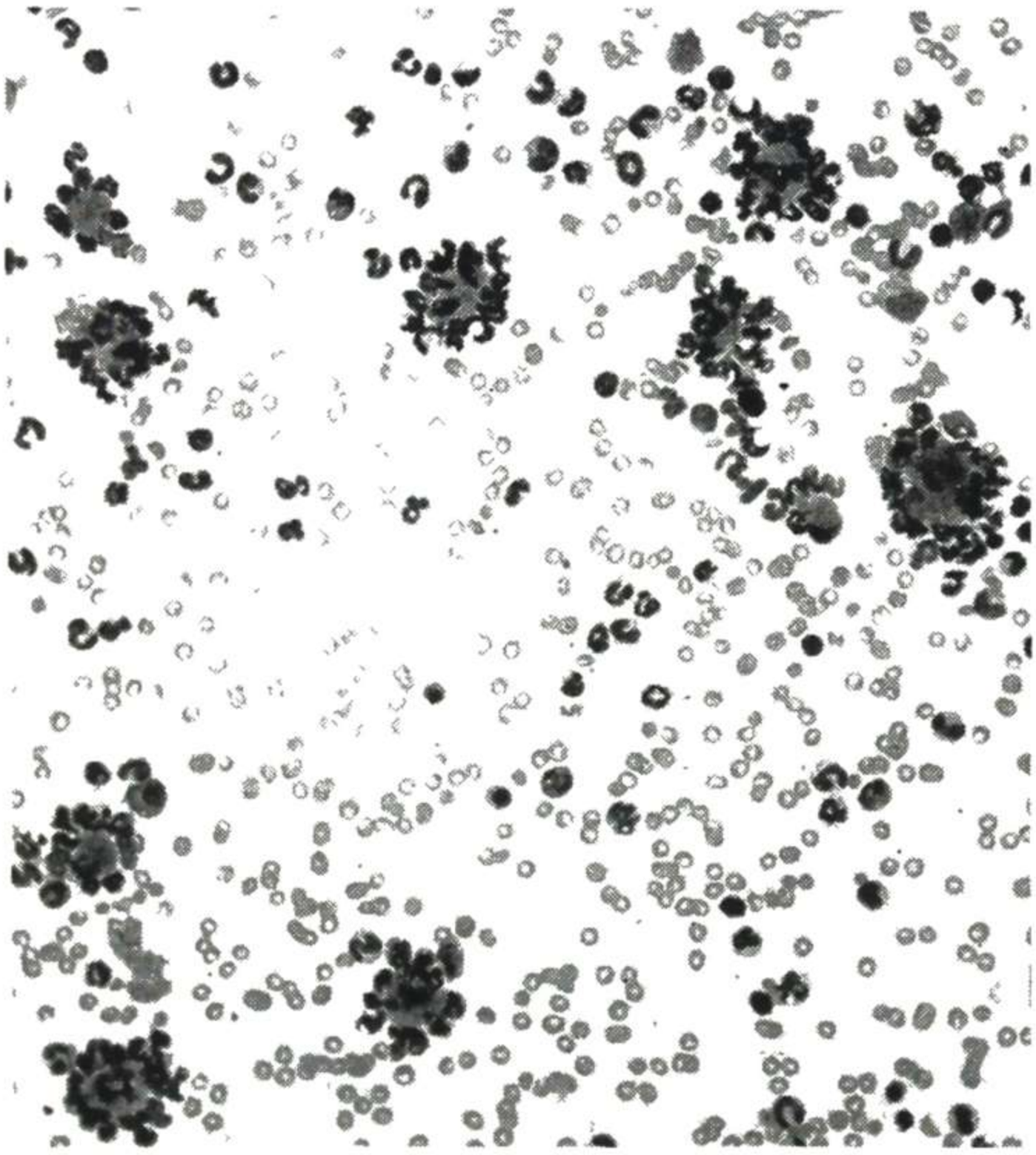

Fig. 99.—Positive reaction to plasma L.E. test. (Courtesy of Haserick, J. R.: J. A. M. A. 146:16-20, May 5, 1951.)

similar to the L.E. factor which will induce the L.E. phenomenon. Until further evidence appears to the contrary, however, the test may be considered specific. Through its use the blood factor which induces the L.E. phenomenon has been found to be in the gamma globulin fraction. Further observations have disclosed an immunologic difference between this L.E. factor and normal gamma globulin.

METHOD.—The indicator, 1 cc. heparinized bone marrow (human or dogs), is placed in a paraffin-lined tube. Plasma, 0.5 cc., is added. The mixture was agitated slowly for ½ hour and placed in a hematocrit tube. After centrifugation at 1,000 rpm for five minutes, the cellular layer is removed, smeared and Wright's stain applied. Rosettes of clumped leukocytes and/or L.E. cells indicate a posi-

(8) J. A. M. A. 146:16-20, May 5, 1951.

tive result. Rosettes of leukocytes alone indicate a positive result when dog marrow is used, for L.E. cells are rarely found in such preparations (Fig. 99).

Concentrated peripheral blood cells may be substituted for marrow as the indicator, and the mixing carried out in a watch crystal. The method can be improved by remixing the patient's plasma with the cells. (This has the effect of bringing the L.E. factor into better contact with concentrated peripheral blood cells and has provided a more simplified technic and yet consistent results.)

Controls are prepared with both the marrow and peripheral blood cell methods, a comparison thus being possible when leukocytic clumping is in doubt.

This test may correlate seemingly unrelated conditions under the diagnosis of systemic lupus erythematosus. The plasma L.E. reaction continues positive longer than that of any other laboratory test during remissions of lupus erythematosus. It is of no prognostic value. A patient with lupus erythematosus may give a negative reaction, possibly owing to an abnormally low gamma globulin level.

Heparin Tolerance Test in Lupus Erythematosus. Using de Takats' technic, Peter Borrie[9] (St. Bartholomew's Hosp.) performed heparin tolerance tests on the blood of 14 patients with chronic discoid lupus erythematosus and 10 with the acute or subacute systemic form of the disease. Blood clotting time was determined by the Dale-Laidlaw method. Systemic lupus erythematosus was associated with pronounced increase in resistance to the anticoagulant activity of heparin. This antipolysaccharide activity may have some bearing on the basic histopathologic changes in the collagen cement substance. In systemic lupus erythematosus, this phenomenon, high erythrocyte sedimentation rate, false-positive serologic reactions, "L.E. cell" and heterophil agglutinins to sheep red cells may all result from a qualitative alteration in gamma globulin. Reports indicate that cortisone and ACTH have a less striking and less constant effect on plasma globulins, "L.E. cell" and heterophil antibodies to sheep red cells than on other aspects of lupus erythematosus. Since they suppress almost all other evidence of the disease while they are being administered, the rise in the gamma globulin level may be one of the more fundamental changes in this condition.

Congenital Hemolytic Disease Due to A-B-O Incompatibility. R. William Provenzano[1] (Boston) reports two cases

(9) Brit. J. Dermat. 63:21-27, January, 1951.
(1) Connecticut M. J. 15:476-478, June, 1951.

of a hemolytic syndrome similar to erythroblastosis fetalis caused by A-B-O incompatibility. Recognition of this condition as a cause of erythroblastosis fetalis is essential for proper diagnosis and therapy in hemolytic disease of the newborn. Ample evidence has established the validity of A-B-O isoimmunization reactions, and acceptance of hyperimmune anti-A and anti-B agglutinins in group O mothers with corresponding group A or B infants is no longer questioned.

One patient had a minor hemolytic episode requiring no treatment, but the other had pneumonia and hepatitis which complicated the underlying hemolytic process, as well as a positive Coombs reaction. The exact value of the Coombs test in A-B-O incompatibilities is not yet determined. Some authors state that their patients have had consistently negative reactions to Coombs tests.

In view of these cases and those reported by others, it is recommended that all husbands of Rh-positive group O mothers be grouped routinely as an aid to early detection of actual or potential existence of this syndrome.

Trypsin-Modified Erythrocytes: Their Use as Test Cells in Acquired Hemolytic Anemia was evaluated by Martin C. Rosenthal, William Dameshek and Roberta Burkhardt[2] (Tufts College).

METHOD.—The usual methods of agglutinin detection, consisting of duplicate determination of serum agglutinin activity with both saline and 20 per cent bovine albumin as diluents, were used. Tests were performed at 37, 22 and 3 C. with the patient's own thrice-washed cells, washed group O, Rh-negative cells from a normal donor and trypsin-modified erythrocytes.

To prepare trypsinized cells, erythrocytes were washed twice with 0.85 per cent sodium chloride and made up to a 2 per cent cell suspension in saline. To each milliliter of this suspension, 0.1 ml. of a 1 per cent crude trypsin solution was added. The suspensions were incubated at 37 C. for 10 minutes, then washed with 0.85 per cent sodium chloride twice. They were then brought to their original volumes to reconstitute a 2 per cent suspension. Cells were freshly treated with trypsin before use each time. Crude trypsin (Difco 1:250) was used. A stock 1 per cent solution was made by dissolving 1 Gm. powder in a volume of 0.85 per cent sodium chloride sufficient to make 100 ml. trypsin solution. The resulting suspension was filtered through Whatman no. 30 filter paper and the clear filtrate stored at 3 C. until needed. Fresh trypsin was prepared each week.

Trypsin-modified erythrocytes were used in tests on five

(2) Am. J. Clin. Path. 21:635-640, July, 1951.

patients with acquired hemolytic anemia, all showing circulating agglutinins and a positive Coombs reaction. Trypsin-modified erythrocytes exposed to saline dilutions of these serums showed titers remarkably similar to those obtained in cells with albumin when the mixtures were incubated at 22 C. At 37 C. the titers and albumin were slightly higher, whereas at 3 C. the reverse was true.

The mechanism by which trypsin enables saline-suspended red blood cells to react with incomplete antibodies is not known. Not only do the cells show altered reactivity but they are also capable of absorbing more antibody to their surface. This probably accounts for the strength of their agglutinates and results in sharper end points. Less significance should be placed on a high titer of cold agglutinins obtained with trypsin than with the usual technics. The absence of prozone is another advantage in using trypsin-modified cells. When serial studies are performed on patients under therapy, alterations of titers are demonstrated by use of trypsin as readily as, if not better than, albumin. When multiple determinations are made, trypsin is more economical.

Acquired Hemolytic Anemia: Report of Case in Infant, with Discussion of Several Etiologic Factors Which May Have Been Operative is made by Bedford H. Berrey (Baylor Univ.) and James D. Watson[3] (Univ. of Kansas). The etiology of acute hemolytic anemia is varied, frequently complex and at best poorly understood. Hemolytic reactions have followed administration of sulfonamides, and blood transfusion and hemolytic crises have been observed in cases of atypical pneumonia. In the authors' patient, a girl aged 9 months when first seen and observed for 28 months, hemolytic anemia may have been produced by one or a combination of the foregoing factors.

During the observation period there were remissions and exacerbations, but remissions became progressively shorter. Some type of nonspecific respiratory infection was present. Cough, fever, x-ray evidence of chronic bronchitis and sinusitis, together with failure of the infection to respond to antibiotics, suggest the possibility of virus etiology. A diagnostic level of cold hemagglutinin antibodies was detected. Although no immediate adverse results followed sulfadiazine therapy, a possible relation between sulfonamide sensitivity and erythroid hypoplasia cannot be excluded.

She was immunized to Rh-positive blood, but this immunization probably played only a temporary part in the illness. Anemia de-

(3) A.M.A. Arch. Pediat. 68:10-27, January, 1951.

veloped before any transfusions, indicating that the disease was initiated before she was immunized. When the blood was found to be homozygous Rh-negative, she received only Rh-negative blood. Since this was early in the course, the Rh antibodies could not have had any part in the major portion of the disease. Autoagglutination was probably related to production of cold hemagglutinins. Use of fresh, warm blood with added 5 per cent sodium bicarbonate solution suggests that cold agglutinins may have played a primary role. She died before the Coombs test became widely known and it was not performed.

Increase in lymphocytes and definite erythroid hypoplasia were noted in the marrow at autopsy. Anoxia, present during final hospitalization, was due to the anemia and the chronic pulmonary process. The erythrophagocytosis probably represented extension into the blood stream of the active phagocytosis in the reticuloendothelial system.

[The authors present an excellent list of references in their bibliography.—Ed.]

Incidence of Sickling was 7.2 per cent in 1,000 Negro patients examined by M. Price Margolies[4] (Univ. of Pennsylvania). Only two of the group had sickle cell anemia. Review

ERYTHROCYTE SICKLING IN NEGRO POPULATION OF UNITED STATES

LOCATION	NO. TESTED	NO. POS.	%
Georgia	300	13	4.3
Michigan	400	30	7.5
Tennessee	100	5	5.0
Missouri	300	19	6.3
Louisiana	100	5	6.0
Maryland	250	16	6.4
New York	150	13	8.7
New York	213	12	5.6
New York	77	4	5.2
Alabama	1,500	122	8.1
Georgia	1,800	99	5.5
Texas	200	13	6.5
Tennessee	2,539	211	8.3
Florida	674	65	9.6
Pennsylvania	100	13	13.0
Texas	1,205	65	5.3
North Carolina	100	14	14.0
Illinois	1,263	119	9.4
South Carolina	719	57	7.9
West Virginia	275	18	6.5
Louisiana	692	45	6.5
New York	226*	18	8.0
Pennsylvania	1,000	72	7.2
	14,183	1,048	Mean 7.25

*Sickling tests were done on 226 consecutive newborn infants and their mothers. Studies on infants are omitted, as inclusion of both would make one group reflect the other.

(4) Am. J. M. Sc. 221:270-272, March, 1951.

of all the available reported studies on the incidence of erythrocyte sickling reveals an incidence of 7.25 per cent in the United States (table), 9.35 per cent in Central and South America and 11.1 per cent in Africa—a world incidence of 9.01 per cent. The rarer occurrence of sickling in the United States as compared with Central and South America may be attributed to the less pure strain of Negro blood found in this country.

Sickle Cell Anemia: First Case Reported from Egypt is described by A. S. Abbasy[5] (Alexandria) in a white girl, 13, whose chief complaints were pallor and weakness and who had had recurrent crises consisting of anorexia, fever and colicky abdominal pains for three years. At these times there were tenderness of the limbs and joints, jaundice, extreme fatigue and exhaustion. She had no definitely negroid features, and admixture of Negro blood was reasonably excluded through six ancestral generations. The red blood cell count was 2.50 million and hemoglobin 45 per cent. Sickling preparations on the patient's red cells were positive and the washed red cells sickled again when suspended in their own serum. Tests on 18 relatives showed sickling only in the cells of the father. A paternal uncle may have died of this disease.

Only a few other cases have been reported in white persons of Greek, Italian or Sicilian ancestry. The patient's family originated in Algeria, where three cases were diagnosed in natives by Smith on the basis of the anatomic changes observed in the spleen.

Studies on Abnormal Hemoglobins.—*Their Demonstration in Sickle Cell Anemia and Other Hematologic Disorders by Means of Alkali Denaturation.*—By electrophoresis and denaturation, Karl Singer, Amoz I. Chernoff and Lily Singer[6] (Michael Reese Hosp.) identified three types of hemoglobin, designated type N (normal adult), type F (fetal) and type S (sickle cell).

REAGENTS.—(1) Exactly N/12 KOH or NaOH (pH 12.7) refrigerated in paraffin-lined bottles. (2) Precipitating solutions: 800 ml. of 50 per cent saturated $(NH_4)_2SO_4$ plus 2 ml. 10 N HCL.

PROCEDURE.—An approximate 10 per cent hemoglobin solution is prepared from fresh oxalated or clotted blood obtained by venipuncture. The cells are washed once with normal saline, shaken for five minutes with 1.2-1.8 volumes of distilled water and 0.4 vol-

(5) Blood 6:555-558, June, 1951.
(6) Ibid., pp. 413-428, May, 1951.

umes of toluene C.P., and the mixture is centrifuged at 3,000 rpm for 20 minutes. The upper two layers are discarded, the clear red solution filtered and adjusted to about 10 per cent concentration by adding distilled water. The exact hemoglobin concentration is then determined.

To a serologic test tube containing 1.6 ml. alkaline reagent and kept in a water bath at 20 C. for several minutes, 0.1 ml. hemoglobin solution is added; the pipet is rinsed six times and the tube gently shaken for 10 seconds. A stop watch is started the moment the hemoglobin is introduced into the denaturing medium. After exactly one minute, 3.4 ml. precipitating solution is added, the test tube inverted six times and the mixture immediately filtered through a double layer filter paper.

With normal adult hemoglobin the filtrate is colorless, whereas in the presence of resistant compounds a faintly brown to deeply red color may be seen. However, use of the spectrophotometer is recommended since "colorless" filtrates may still give distinct readings (0.5-1.7 per cent of the original hemoglobin concentration). All determinations should be performed at least in duplicate and the figures averaged. When values in the range of 1.7-2.4 per cent are found, the mean of six determinations may be taken as the final result.

Tests on 100 hematologically normal persons above age 3 disclosed that normal pigment is completely denatured in one minute. Fetal hemoglobin, which is alkali-resistant, may be demonstrable until the end of the second year of life. Alkali-resistant hemoglobins were regularly encountered in sickle cell anemia (but not in the trait), in the more fully developed Mediterranean syndromes and in one of four families with hereditary spherocytosis. In addition to these hereditary blood disorders, abnormally denaturing hemoglobin fractions were observed in three instances of chronic aregenerative anemia and irregularly in patients with untreated pernicious anemia, acute and chronic leukemia and myelophthisic anemia. Only normally denaturing pigments were noted in all other kinds of anemia.

Identification by Means of Fractional Denaturation.—Singer, Chernoff and Singer[6a] hypothesize that these abnormally denaturing fractions may represent continued production of fetal pigment beyond the physiologic age limit in hereditary hemolytic syndromes and reactivation of such a mechanism in some acquired hematologic disorders. To obtain further evidence, the fractional denaturation procedure was carried out. This method parallels that already described, except that the reaction is allowed to proceed in aliquot portions of the

(6a) Blood 6:429-435, May, 1951.

hemoglobin solution over longer periods. By this means, progress can be followed by alkaline degradation of resistant hemoglobin.

A characteristic pattern was established for the fetal compound. The resistant fractions in Mediterranean anemia, acute leukemia and chronic aregenerative anemia behave like the fetal pigment. In sickle cell anemia four specimens had fetal hemoglobin whereas seven had a fetal-like compound. In two members of a family with hereditary spherocytosis, the resistant pigment was also fetal-like. These observations give further support to the hypothesis previously outlined. It is emphasized that the hypothesis is merely descriptive and is not based on knowledge of the biochemical mechanisms involved. Understanding of these mechanisms may bring about better knowledge of the fundamental processes of hemoglobin synthesis in health and disease.

Studies of Hemagglutinins in Hereditary Spherocytosis and in Acquired Hemolytic Anemia; Their Relation to Hypersplenic Mechanism. Claude-Starr Wright, Matthew C. Dodd, Bertha A. Bouroncle, Charles A. Doan and Robert M. Zollinger[7] (Ohio State Univ.) studied incomplete antibodies with the developing serum (Coombs) and trypsinized red cell technics in 185 patients with hemolytic anemia or some other blood dyscrasia and in 280 others who were either normal or had miscellaneous nonhematologic diseases. In the first group the reaction to the developing serum test was positive in 371 and to the trypsinized red cell test in 406 determinations. The serums of the second group were all negative and served as controls.

The study failed to confirm that the Coombs test is a reliable diagnostic procedure for differentiation of acquired hemolytic anemia and hereditary spherocytosis. Of the 77 patients who had positive reactions to one or both tests, 88 per cent had active hemolysis, recent history of associated hemolytic phenomenon or exhibited an inherited trait of defective erythrocytes. Of the remaining nine who had positive reactions to one or both tests, five had essential thrombocytopenic purpura. This suggests previous erythrocyte sensitization and is in line with recent concepts of the hypersplenic phenomena. The evidence indicates that the antibodies demonstrated with these technics have probably been associated pri-

(7) J. Lab. & Clin. Med. 37:165-181, February, 1951.

marily with the hemolytic phenomenon. Studies of circulating peripheral and splenic antibodies were made in 30 cases at the time of splenectomy. The generally higher titers and quantitative agglutination in the various dilutions of the splenic residual blood focused consideration on the spleen as the initiating source and perpetuator of the autosensitizing antibodies.

A hypothesis of the immunologic phases of hypersplenism, which follows, is illustrated in Figure 100. Slowing of the circulation through the spleen by any of several causes may up-

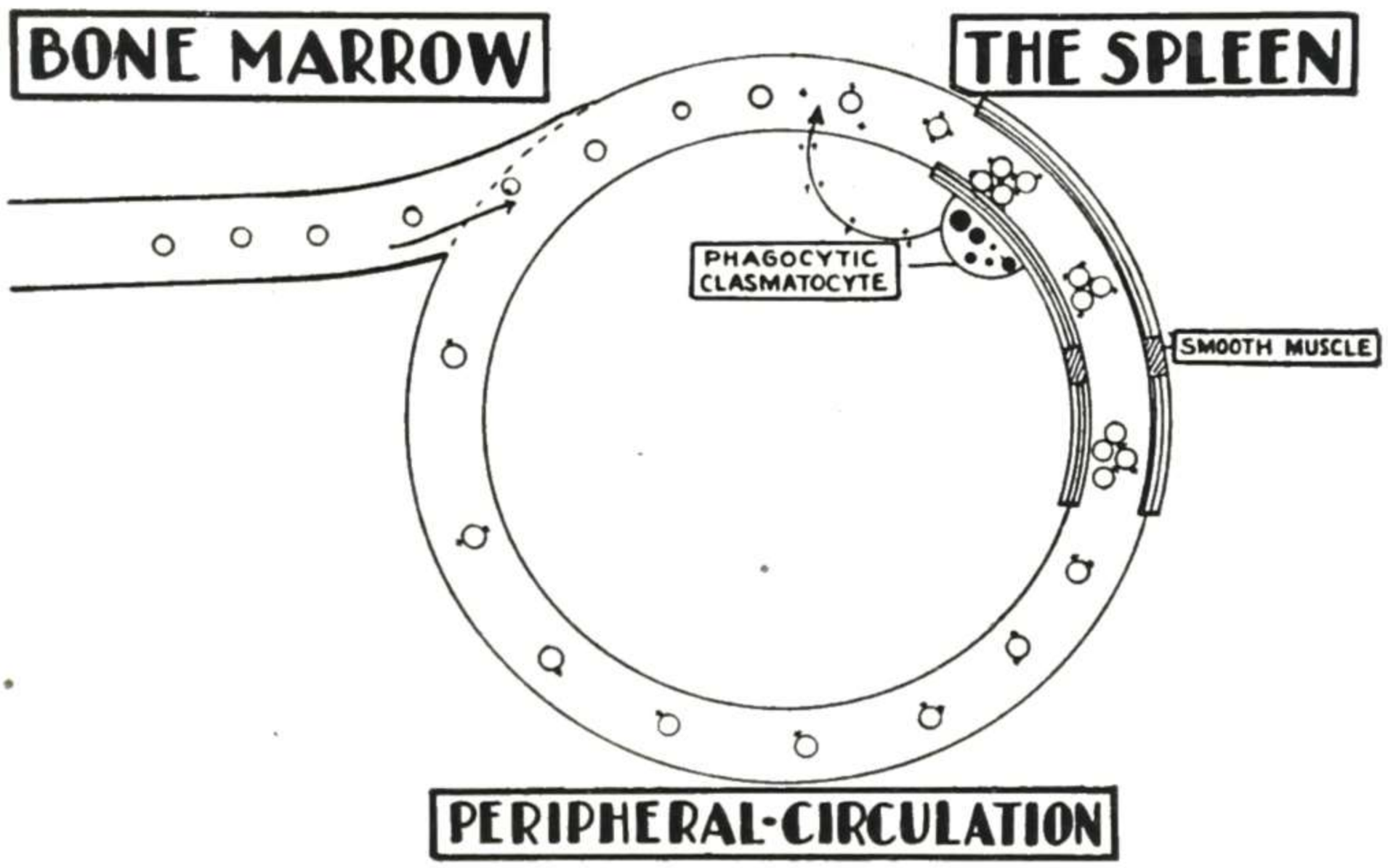

Fig. 100.—Hypothetical immunologic phases of hypersplenism. (Courtesy of Wright, C.-S., *et al.*: J. Lab. & Clin. Med. 37:165-181, February, 1951.)

set the delicate vascular equilibrium and reserve blood balance and cause changes in the quality and integrity and therefore the stability of the withheld formed elements to the degree that facilitates their phagocytosis by the reticuloendothelial macrophages. These cells have been shown to be at least one source of and to play a major role in antibody synthesis. With the increased antigenic material being supplied, production of antibodies mounts. They enter the circulation, sensitize other freshly delivered normal cells from the marrow, and these newly autosensitized cells agglutinate and then become more easily withheld in the sluggish circulation of the splenic vascular beds, establishing a vicious destructive cycle.

The concept explains the therapeutic failure and recurrence of excessive cell destruction, despite compensatory mar-

row hyperplasia after splenectomy, in terms of (1) accessory splenic tissue still present, and (2) hyperimmune circulating antibodies stimulating generalized extrasplenic reticuloendothelial hyperplasia and hyperphagocytosis again in a vicious cycle, which are not affected favorably by any known medical or surgical measure.

Infectious Lymphocytosis: Report of Two Cases Noted Following Trauma is given by Sydney Waldman and Abraham M. Frumin[8] (Mount Sinai Hosp., Philadelphia). Cases associated with trauma have not been reported previously.

In 1941 Smith first described the disease as a benign infectious contagious process associated with pronounced leukocytosis and absolute lymphocytosis. Clinical signs and symptoms can be so mild as to escape attention. Heterophil antibody test results are negative. Lymph node biopsies may show degeneration of lymph follicles and striking proliferation of reticuloendothelial cells.

In two children injured by automobiles there was mild, absolute eosinophilia, a finding noted previously by others. In both, lymphocytosis was severe. The lymphocytes were all mature and none resembled the atypical mononuclear cells of infectious mononucleosis or blast forms; usually they were small and compact. Heterophil antibody test results were negative in both patients. Absence of granulomatous lesions in the bone marrow was of additional value in excluding diagnosis of infectious mononucleosis. Careful study of the blood finding, marrow, history and physical examination ruled out acute leukemia and infectious mononucleosis.

Differential Test for Infectious Mononucleosis. Israel Davidsohn, Kurt Stern and Chiyo Kashiwagi[9] (Chicago) state that because of the multiplicity of clinical symptoms found in infectious mononucleosis (table), diagnostic errors are easily made. Though an experienced hematologist can usually diagnose or at least suspect the disease by examining a blood smear, some cases defy recognition on morphologic grounds alone. Furthermore, most diagnoses must be made without benefit of an expert hematologist. Additional diagnostic aids are the presumptive and differential tests. The presumptive test, nonspecific in character, is done by addition of serum to a 2 per cent suspension of sheep red cells followed

(8) J. Pediat. 39:455-459, October, 1951.
(9) Am. J. Clin. Path. 21:1101-1113, December, 1951.

by incubation at room temperature. The differential test, highly specific, is based on the finding that anti-sheep agglutinins in infectious mononucleosis are never completely removed by treating the serum with guinea pig kidney and are usually completely removed by beef red cells.

The presumptive test is indicated when clinical and/or

SYMPTOMS AND SIGNS IN 106 PATIENTS WITH INFECTIOUS MONONUCLEOSIS*

	No. of Patients	%
Lymphadenopathy	101	95.3
Fever	93	87.7
Pharyngitis	64	60.4
Without membrane	50	47.2
With membrane	14	13.2
Splenomegaly	51	48.1
Headache	26	24.5
Hepatomegaly	24	22.6
Prostration	11	10.4
Emesis	10	9.4
Pain in abdomen	8	7.6
In upper abdomen	6	5.7
In lower abdomen	2	1.9
Stiffness or pain in neck	6	5.7
Skin rash	5	4.7
Epistaxis	3	2.8
Icterus	3	2.8
Loss of weight	2	1.9
Diarrhea	2	1.9
Arthritic pains	2	1.9
Purpura	2	1.9
Gingivitis	2	1.9
Convulsions	1	0.9
Toothache	1	0.9
Albuminuria	14	13.2
Positive test for syphilis	3	2.8
Relapses (17 da. to 2 mo.)	7	6.5
Recurrence (1 yr.)	1	0.9

*Additional clinical findings recorded in the literature: hematuria, myositis or myalgia, hemoptysis and uterine bleeding.

hematologic findings suggest infectious mononucleosis. In the presence of such findings a titer of 1:224 or higher in the test confirms the diagnosis. Indications for the differential test are: (1) borderline titer of anti-sheep agglutinins (1:112 or less) as determined by the presumptive test in patients suspected of having infectious mononucleosis; (2) titer of anti-sheep agglutinins of 1:56 or higher in patients without clinical or hematologic evidence of the disease; (3) history of recent injection of horse serum in a patient with an anti-sheep

agglutinin titer of 1:56 or higher by the presumptive test.

Infectious mononucleosis is the only disease in which anti-sheep agglutinins have at least one-eighth the original titer remaining after absorption with guinea pig kidney, with a consequent drop not exceeding three tubes in two-fold serial dilutions, and are completely removed after absorption with beef red cells.

Negative presumptive test results obtained up to seven days after onset of symptoms may be followed by positive results when tests are repeated three to nine days later. To determine how long serologic findings may persist after clinical symptoms appear serial tests were done in 19 patients until negative results were obtained. Negative results were found in 11 patients before the end of 12 weeks after onset of illness and in 1 as early as 4½ weeks; a positive result was obtained in 1 patient as late as 18 weeks after onset of symptoms.

Description of Two Types of Hemophilia and Criteria for Diagnosis of Hemophilia. During an investigation of 49 hemophiliacs and 200 hemorrhagic episodes severe enough to cause some incapacity, D. Brian Dunn and Raymond N. Lyons[1] (Univ. of Sydney) found that there are two clinical types of the disease. The first is associated with hemarthroses due to intra-articular hemorrhage. Prothrombin consumption time

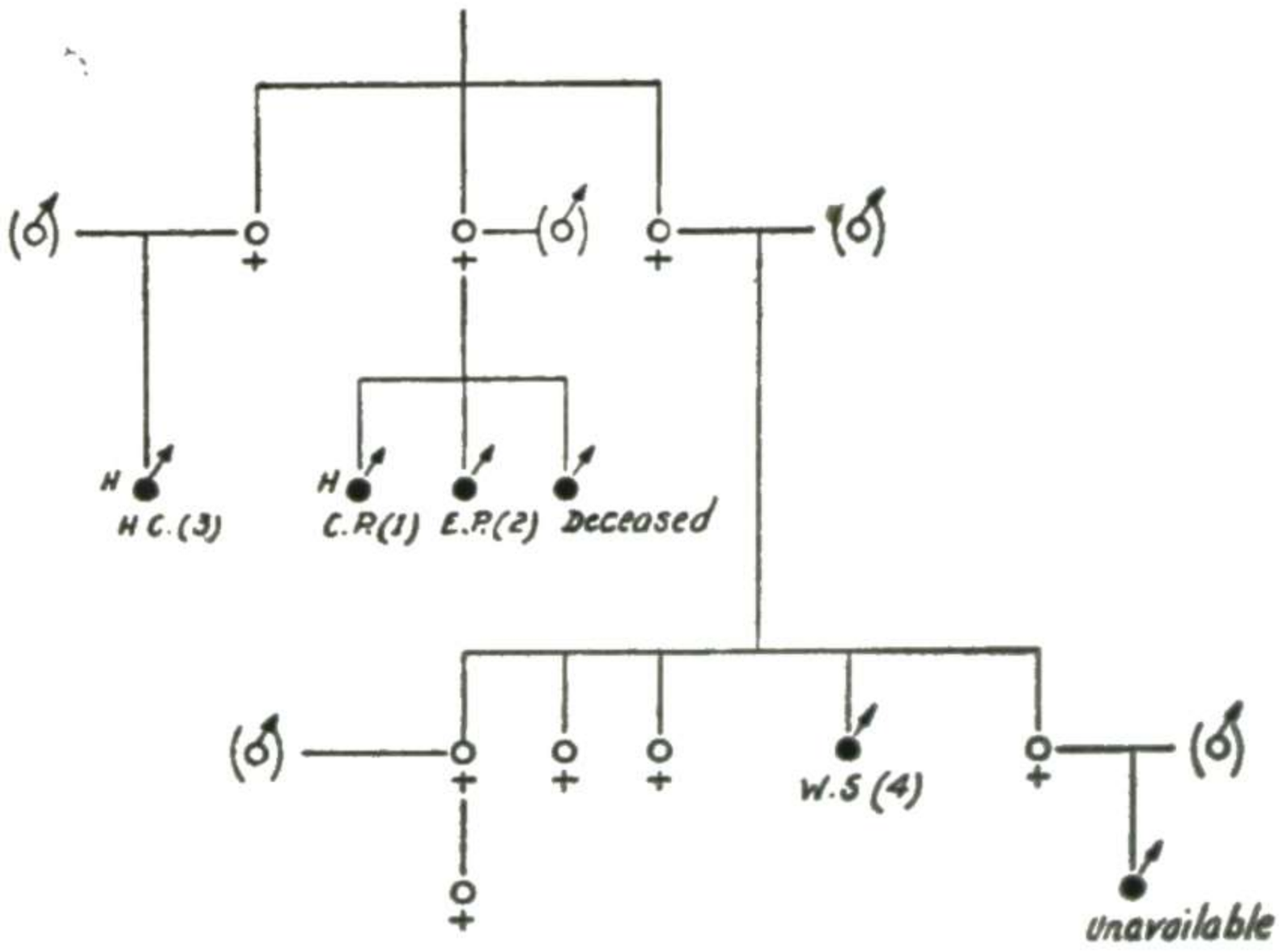

Fig. 101.—Family tree of four hemophilic patients with two types of hemophilia. *H* indicates hemarthrosis. (Courtesy of Dunn, D. B., and Lyons, R. N.: M. J. Australia 2:183-185, Aug. 11, 1951.)

(1) M. J. Australia 2:183-185, Aug. 11, 1951.

does not increase in two hours and, in the absence of certain therapies, coagulation time is between 20 and 180 minutes. In the second type hemarthroses are not present. Prothrombin consumption time increases slightly during 2 hours and clotting time ranges between 10 and 30 minutes. Of the 49 patients, 21 had the first and 22 the second type of hemophilia. The type for six was not determined. The two types are related, as indicated by the presence of the first type in one brother and a cousin and the second in the older brother and a cousin in the same family (Fig. 101).

Criteria for laboratory diagnosis of hemophilia are: coagulation time (Lee and White, 1913), prolonged; bleeding time (Duke, 1918), normal; prothrombin consumption time (Quick and Favre-Gilly, 1949), constant or increased slightly during two hours; platelet count, normal; coagulation time of recalcified plasma (Quick, 1942), high speed centrifuging over five minutes, low speed centrifuging over three minutes and decreased clotting time on standing; prothrombin concentration (Quick, 1945), normal; accessory factor V (Owren, 1947), normal concentration; fibrinogen, normal concentration.

Hemophilia Associated with Normal Coagulation Time. Clarence Merskey[2] (Radcliffe Infirm., Oxford) reviews clinical features of hemophilia in eight families. All patients had normal coagulation times, and results of other commonly used blood tests were usually normal. Clinically there was no doubt that these persons had hemophilia, but their blood possessed some ability, though not equal to that of normal blood, to correct defects of known hemophilic blood.

With serum from whole clotted venous blood or recalcified citrated plasma the normal range for prothrombin consumption index is always under 40 and often under 20 per cent. In the 14 patients of this series, this test gave abnormal readings in 11 and normal in 3. Values of 100-200 per cent are common in the usual hemophiliacs, but such readings were rare in this series. When the test was done on serum from clotted capillary (finger prick) blood, results were normal in all cases.

Titration of antihemophilic globulin was done on the blood of at least one member of each family; results showed deficiency of this substance when compared with a normal control. This was used as the final test in doubtful cases.

In these patients clinical symptoms varied in degree from

(2) Brit. M. J. 1:906-912, Apr. 28, 1951.

mild to severe but remained relatively constant in any particular family. Symptoms were those of classic hemophilia but the hemorrhagic episodes were often widely separated. Epistaxis and easy bruising were almost invariable but hemarthroses were rare.

That hemophilia can occur with normal coagulation time cannot be overemphasized. Many of the patients studied had been told that surgery could be safely undertaken. Only after severe hemorrhage and death in one patient was it realized that this advice had been incorrect. These patients must be regarded as hemophiliacs and all the serious implications recognized. The common bleeding and clotting tests should not be relied on to exclude possibility of hemophilia.

CHEMISTRY

Accuracy of Certain Common Chemical Determinations: Second Survey. J. G. Snavely and W. R. C. Golden[3] (Stamford Hosp.) submitted unknowns (nonprotein nitrogen, glucose, chloride, hemoglobin and protein) to 28 hospital and 13 commercial laboratories. Of 198 tests performed, 92 were outside the allowable limits of error. The hospital laboratories previously surveyed reported 38 per cent errors; private commercial laboratories, 48 per cent, and hospital laboratories not previously surveyed, 59 per cent. Although most results tended to approach the correct values, the extremes of values reported for each determination indicated how erratic and misleading certain laboratory reports are. If a performance of 80 per cent is arbitrarily set as acceptable, only 13 of the 41 participating laboratories qualify. Of these, seven were hospital laboratories previously surveyed, four private commercial laboratories and two hospital laboratories not previously surveyed.

Studies in Nonprotein Nitrogen: Convenient Method for Measuring Urea in Blood. Raymond H. Owings and Emanuel E. Mandel[4] (Pub. Health Service, Atlanta, Ga.) revised the acid hydrolysis method of measuring urea so that it furnishes accurate and reliable results when applied to either whole or cell-free blood.

METHOD.—One ml. of tungstic acid filtrate from whole blood,

(3) Connecticut M. J. 15:667-669, August, 1951.
(4) Proc. Soc. Exper. Biol. & Med. 78:363-366, November, 1951.

plasma or serum and 0.5 ml. of 1 M phosphoric acid are placed in a tube covered with tin foil and autoclaved at a minimum of 20 lb. pressure for 90 minutes. Into the cooled tube, 1 ml. of 0.9 N sodium hydroxide is pipetted, followed by distilled water to the 10 ml. mark and, after mixing, by 2 ml. Gentzkow nesslerizing reagent (2 parts of Folin Nessler's solution, 1 part of 2.5 per cent aqueous nitrogen-free potassium persulfate and 1 part of 1 per cent aqueous potassium gluconate). The mixture must be used within 15 minutes. Both the persulfate and gluconate solutions must be made up fresh weekly and kept refrigerated. The sample is shaken, allowed to stand 15 minutes and read within the next 90 minutes in a spectrophotometer or photoelectric colorimeter at a wavelength between 490 and 510 mμ. A distilled water blank and an ammonium sulfate standard (containing 2 mg. nitrogen/100 ml.) are treated identically except that the standard is not autoclaved. A calibration curve may be prepared by analyzing a series of ammonium sulfate solutions containing 1.5-8 mg. nitrogen/100 ml. Filtrates of blood samples containing more than 80 mg. urea nitrogen/100 ml. must be diluted with distilled water and the results multiplied by the dilution factor. Inclusion of a urea solution of known concentration in the series of unknowns analyzed provides a test for completeness of hydrolysis.

Rapid Method for Estimation of Urea in Biologic Fluids by Means of Reaction between Diacetyl and Urea is described by Samuel Natelson, Mary Lou Scott and Charles Beffa[5] (Rockford, Ill.). The error in the method is less than 3 per cent, and it is the most rapid method suitable for routine use.

REAGENTS.—(1) An acid mixture is prepared by adding 750 ml. of 85 per cent phosphoric acid to 1,000 ml. water, and to this adding 250 ml. concentrated H_2SO_4 (sp. gr. 1.84). (2) Diacetyl in alcohol (5 per cent by volume) is prepared by making up 5 ml. diacetyl (Eastman Kodak Co.) to 100 ml. with 95 per cent ethyl alcohol. If placed in the refrigerator or a dark cool place it keeps indefinitely. (3) Acid diacetyl reagent is prepared fresh daily by making up 1 ml. of 5 per cent diacetyl to 25 ml. with the acid mixture. For diluting unknowns to a range where the standard curve is a straight line, this reagent is diluted 1:1 with water. (4) Tungstic acid reagent is prepared by making up 8.3 ml. concentrated H_2SO_4 to 2 L. with distilled water; 2.2 per cent sodium tungstate is made by dissolving 22 Gm. $Na_2WO_4 \cdot 2H_2O$ to 1,000 ml. with distilled water. Equal parts of the acid and tungstate solutions are mixed on the day of the test. (5) Urea stock standard (1 mg. N/ml.) is prepared by making up 2.144 Gm. dried urea (reagent grade) to 1 L. water, a few drops of chloroform being added as a preservative. (6) Working urea standard (10 μg. N/ml.) is prepared by diluting the stock standard 1:100 with distilled water and adding a few drops of chloroform as a preservative. This should be kept refrigerated. (7) Working urea standard (5 μg. N/ml.) is prepared by diluting the stock standard 1:200.

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(5) Am. J. Clin. Path. 21:275-281, March, 1951.

PROCEDURE.—In the macromethod, 1.9 ml. tungstic acid is added to 0.1 ml. serum or whole blood. The mixture is shaken, allowed to stand for two minutes and centrifuged. A 1 ml. aliquot, 1 ml. urea standard and 1 ml. water as a blank are placed in different tubes. To each tube is added 1 ml. acid diacetyl reagent. After the contents are mixed well, the tubes are heated in a boiling water bath for 10 minutes in the absence of direct light. The tubes are cooled for two minutes in running water and read in a Klett-Summerson colorimeter with a no. 44 filter.

For urine, a 1:100 dilution is made. To 1 ml. of this solution is added 1 ml. acid diacetyl reagent. Then the procedure described for serum is followed. A recovery is run by adding to 0.1 ml. serum, 0.5 ml. (5 μg.) working urea standard solution, and 1.4 ml. tungstic acid, and proceeding as for serum.

In the micromethod, 0.04 ml. whole blood is taken from the fingertip and washed into 1 ml. water; 1 ml. tungstic acid is added. After the proteins are separated by centrifuging, a 1 ml. aliquot is taken. To this is added 1 ml. acid diacetyl reagent. After heating for 10 minutes and cooling as in the macromethod, the solution is transferred to a special 2 ml. cuvet with a 4 cm. light path and read with the no. 44 filter against a blank substituting water for the blood on the Klett-Summerson colorimeter, or diluted to 3.5 ml. and read in the Coleman spectrophotometer at 480 mμ with the 3 ml. cuvets with a 5 cm. light path. The results are compared with a standard curve for the instrument used.

A standard curve is constructed by making serial dilutions of the stock standard to contain 2.5, 5, 10, 20, 30, 50 and 100 μg./ml. To 1 ml. of each solution is added 1 ml. acid diacetyl reagent. The well mixed solutions are heated for 10 minutes in the absence of direct light, cooled to room temperature and read on the Klett-Summerson colorimeter with a no. 44 filter. Concentration equivalent of each solution in milligrams of urea nitrogen/100 ml. is equal to the micrograms/ml. times two. For urine, when no aliquot is taken in the analysis, concentration equivalent is equal to the micrograms/ml. In making calculations for any procedure the standard curve is referred to. It should be checked daily by running 5 μg./ml. and 10 μg./ml. standards along with the unknowns. A straight line drawn between these two points will give the curve which is valid up to a urea nitrogen value of 40 mg./100 ml. If the unknown is out of this range, the sample is diluted with the acid diacetyl reagent which has been diluted 1:1 with water.

When sugar and urea are analyzed simultaneously, this method may be synchronized with the sugar method so that the same filtrate and the same heating time are used.

Blood Levels of Urea Nitrogen, Phenol, Guanidine and Creatinine in Uremia. Norman S. Olsen and John W. Bassett[6] (Washington Univ.) attempted to find a relation between clinical symptoms and chemical changes in uremia. Symptoms

(6) Am. J. Med. 10:52-59, January, 1951.

which could be explained on the basis of either uremia or the accompanying disease were given the value of 1. The remaining signs and symptoms such as those which appear early in uremia (twitching and tremor, reflex changes, psychosis, listlessness, restlessness), in the intermediate phase (diarrhea, hiccup, itching, hemorrhagic tendency) or late or terminally

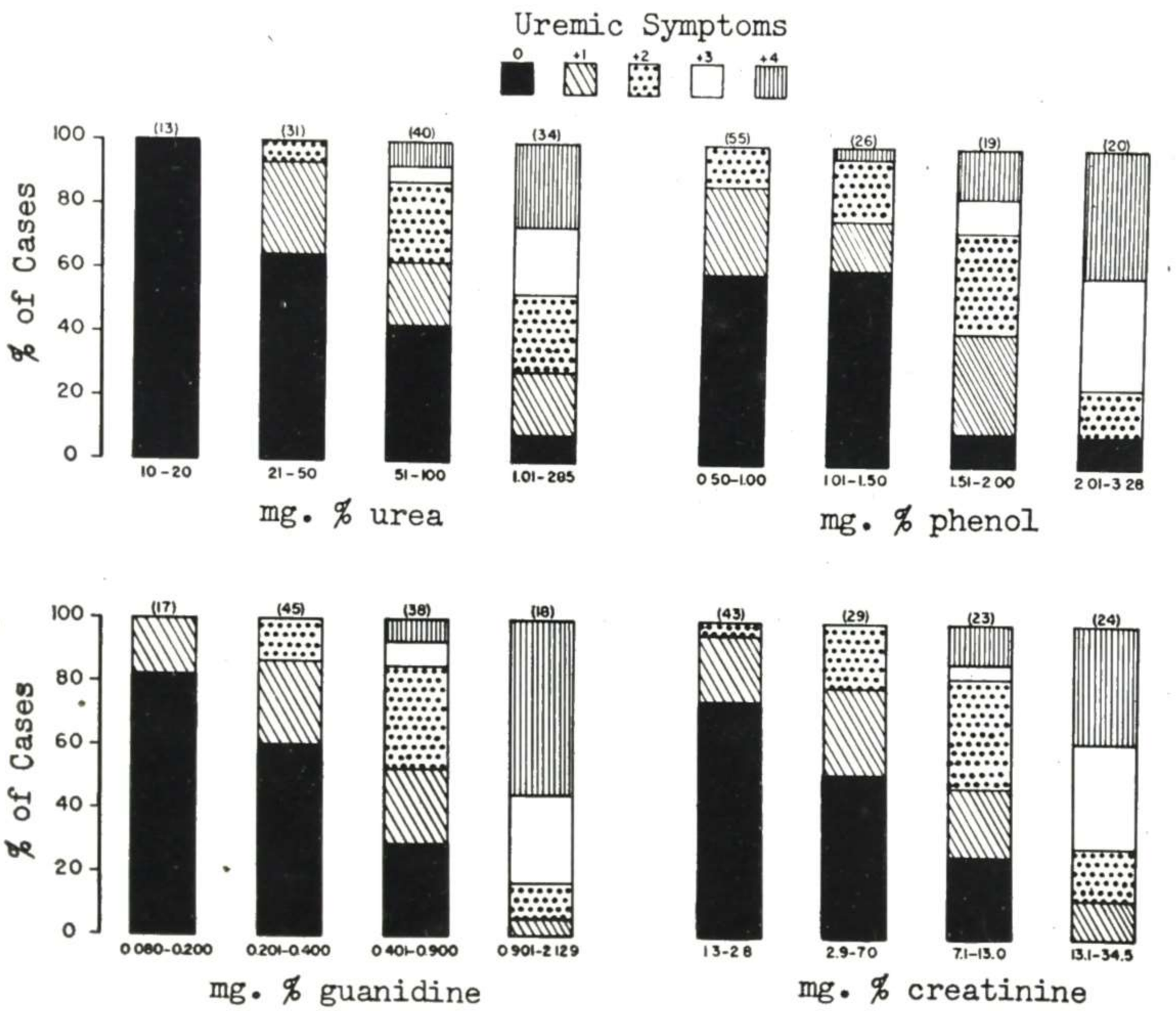


Fig. 102.—Comparison of uremic symptoms with arbitrary levels of blood urea nitrogen, phenol, guanidine and creatinine. Numbers in parentheses on top of bars refer to total number of cases in the group. (Courtesy of Olsen, N. S., and Bassett, J. W.: Am. J. Med. 10:52-59, January, 1951.)

(convulsions, stomatitis, parotitis, frost) were weighted with regard to severity and the weighted and unweighted clinical symptoms evaluated against clinical findings. Average values of blood urea nitrogen, phenol, guanidine and creatinine were determined for 29 normal subjects and 119 hospital patients.

A breakdown of the four chemical variants into four arbitrary levels and a plot of the per cent of patients exhibiting varying degrees of unweighted uremic symptoms are shown in Figure 102. In each group a very rough relation is seen be-

tween the severity of symptoms and the level of each constituent. The relation is not striking, and severe uremic symptoms were noted with comparatively normal levels of phenol, guanidine and creatinine. The breakdown showed still less agreement between clinical and chemical changes when the weighted uremic symptoms were similarly plotted. Although the various chemical levels studied may be increased in uremia, there is no strict correlation with the intensity of symptoms.

However, the definite trend of increasing chemical levels with increasing uremic symptoms cannot be ignored. All four parameters increase somewhat progressively in primary renal disease. In prerenal azotemia and postrenal obstruction the blood urea nitrogen level rises but the guanidine, creatinine and phenol levels are much lower and show a less consistent rise. Comparison of essential and malignant hypertension shows that in both the blood levels of all parameters increase to only moderately high levels with severe uremic symptoms; thus, on a purely chemical basis, they are indistinguishable. Uremia remains a clinical entity without any direct known chemical basis.

Biuret Reaction for Determination of Proteins: An Improved Reagent and Its Application for assay of protein concentrations in small volumes of biologic fluids are described by R. Levin and R. W. Brauer[7] (Louisiana State Univ.).

METHOD.—A Beckman spectrophotometer and 10 mm. Corex glass cells are used to determine optical densities. The reagent, which is stable for several months at room temperature, is prepared as follows: 0.175 Gm. $CuSO_4 \cdot 5H_2O$ dissolved in a few milliliters of water is placed in a 100 ml. volumetric flask, to which a mixture of 30 ml. concentrated NH_4OH, 50 ml. cold distilled water and 20 ml. saturated NaOH are added. For the test 2 ml. of the reagent is added to 3 ml. of a protein solution containing between 0.05 and 1.25 Gm. protein/L. The optical density of the mixture is determined at 540 mμ immediately or up to 10 minutes later. A calibration curve is used to determine protein concentration.

The NH_4OH is necessary to maintain the required Cu++ in solution. The cuprammonium fails to react with proteins until the addition of NaOH results in prompt, quantitative formation of the biuret complex. Applicability of the reagent is limited by development of turbidity in the reaction mixtures after about 30 minutes. The precipitating material represents lipids, previously protein-bound but released by the alkali. Early readings obviate this complication.

(7) J. Lab. & Clin. Med. 38:474-480, September, 1951.

Protein Measurement with Folin Phenol Reagent was studied by Oliver H. Lowry, Nira J. Rosebrough, A. Lewis Farr and Rose J. Randall[8] (Washington Univ.).

METHOD.—Reagents include: A, 2 per cent Na_2CO_3 in 0.10 N NaOH; B, 0.5 per cent $CuSO_4 \cdot 5H_2O$ in 1 per cent sodium or potassium tartrate; C, alkaline copper solution (50 ml. reagent A mixed with 1 ml. reagent B); D, carbonate-copper solution (reagent C without NaOH); E, diluted Folin reagent (Folin-Ciocalteu phenol reagent titrated with NaOH to phenophthalein end point and diluted to 1 N in acid).

Working standards are prepared from human serum diluted 100- to 1,000-fold (700- to 70 μg./ml.). To a protein sample of 5-100 μg. in 0.2 ml. solution or less in a 3-10 ml. test tube, 1 ml. reagent C is added. The substances are mixed well and let stand for at least 10 minutes at room temperature. Reagent E, 0.1 ml., is added rapidly and mixed immediately. After 30 minutes the sample is read in a colorimeter or spectrophotometer.

It is unnecessary to bring all samples and standards to the same volume before adding the alkaline copper reagent if corrections are made for differences in final volume. Critical volumes are those of alkaline copper and Folin reagents. If protein is present in an already very dilute solution (below 25 μg./ml.), 0.5 ml. may be mixed with 0.5 ml. double strength reagent C and treated as before.

Measurement of protein with copper and the Folin reagent has advantages. (1) It is as sensitive as with Nessler's reagent, yet requires no digestion. (2) It is more sensitive than measurement of ultraviolet absorption at 280 mμ, and is more specific and less liable to disturbance by turbidities. (3) It is more sensitive, simpler and easier to adapt for small scale analyses than the ninhydrin reaction and is more sensitive than the biuret reaction.

There are two major disadvantages. The amount of color varies with different proteins, and the color is not strictly proportional to concentration.

The copper-Folin reaction can be used to measure protein during enzyme fractionation, etc.; mixed tissue proteins when absolute values are not needed and very small absolute amounts of protein, highly diluted protein or protein mixed with colored or nitrogen-containing substances. It can also be used to analyze large numbers of similar protein samples.

Precipitation of Cerebrospinal Fluid Globulin by Zinc Sulfate. Alfred M. Donovan, Joseph M. Foley and William C. Moloney[9] (Boston City Hosp.) describe a method.

METHOD.—The reagent is prepared by dissolving 1.44 Gm. zinc

(8) J. Biol. Chem. 193:265-275, November, 1951.
(9) J. Lab. & Clin. Med. 37:374-381, March, 1951.

sulfate, 3.12 Gm., barbital and 1.27 Gm. sodium barbital in 1 liter of distilled water. The resulting clear solution represents an [illegible] concentration of zinc sulfate at pH [illegible]. The reagent will remain stable indefinitely at this pH.

To 1 cc. reagent is [illegible] cc. [illegible] cerebrospinal fluid. The tube is [illegible] and then allowed to stand for four hours. The result is read at the end of four hours as a precipitation reaction of 1+ to 4+ [illegible]. A 1+ reaction shows a slight degree of precipitation in the [illegible]-

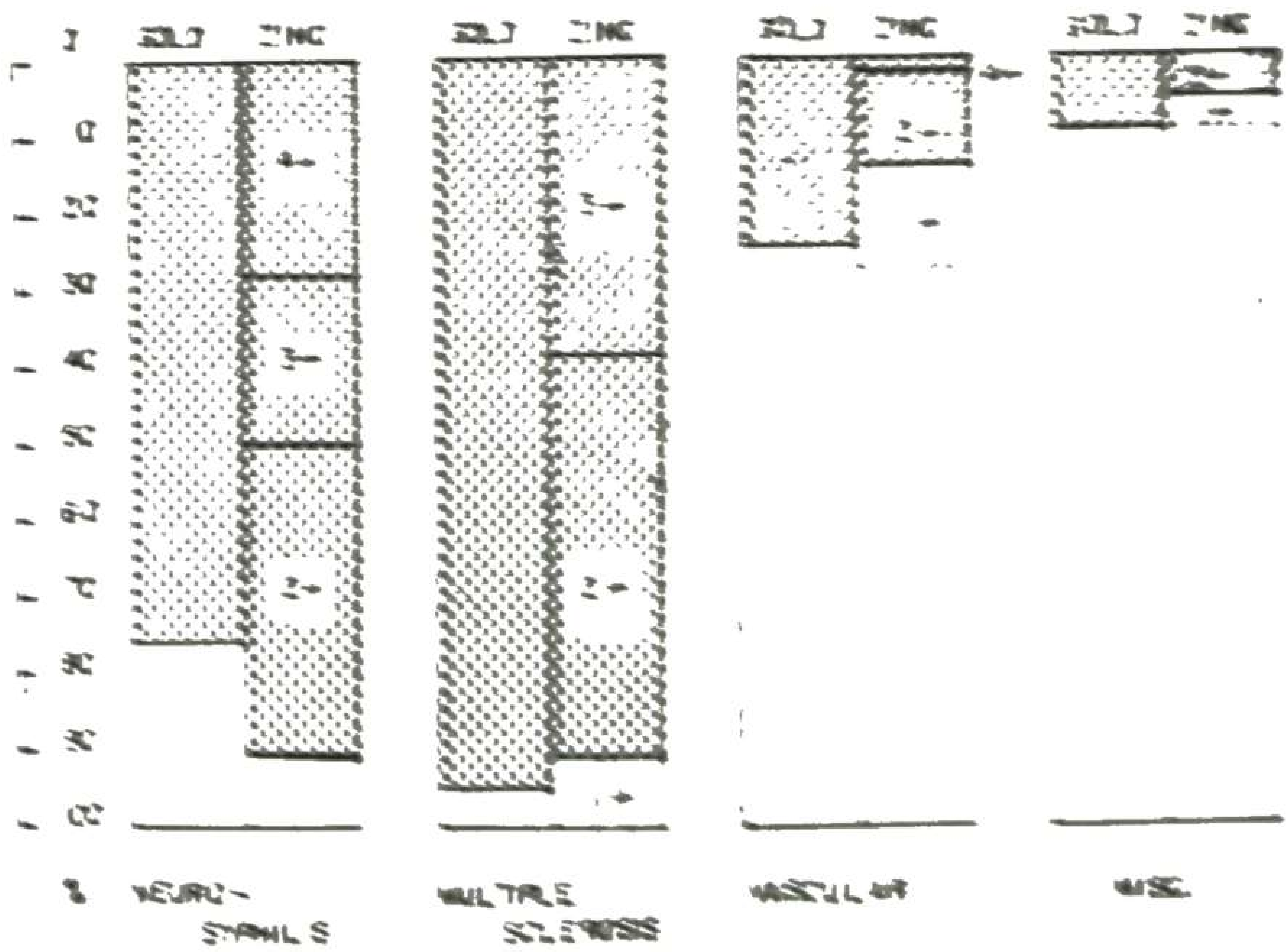


Fig. [illegible]. Comparison of colloidal gold and zinc sulfate precipitation reactions of cerebrospinal fluid in various conditions. [illegible]

cavity of the bottom of the tube; a 2+ reaction is a [illegible] compact mass. 3+ and 4+ reactions are [illegible] greater degrees of precipitation but the former shows slight [illegible] in the supernatant fluid whereas the [illegible]. In general the 1+ reaction is considered a negative result.

Figure 103 shows that there is no [illegible] high order of specificity for the zinc reaction. The reaction occurred in a far higher percentage of cases of multiple sclerosis and neurosyphilis than in a control group of cases of cerebrovascular disease and miscellaneous diagnoses. In no certain case of multiple sclerosis did precipitation fail to occur. Furthermore the variation of the cerebrospinal fluid gamma globulins in multiple sclerosis may represent variation in activity of the disease process. After more effective quantitation, this reaction may be useful in measuring degree of activity of the disease process in multiple sclerosis.

Modified Globin: Method for Preparation from Human Erythrocytes. Max M. Strumia and Albert B. Sample[1] (Bryn Mawr, Pa.) used erythrocytes, which are usually discarded after blood plasma processing, for preparing alkali-modified human globulin suitable for parenteral use.

PROCEDURE.—Extreme precautions must be taken to exclude pyrogens throughout the procedure. To prepare the hemoglobin solution and minimize bacterial contamination, a refrigerated room (4 C.) is utilized. The erythrocytes are filtered through cheesecloth into a 9 L. bottle. After about 5 L. is collected, a 5 ml. sample is removed and analyzed for hemoglobin content. From these results the volume of erythrocytes needed to give a 14 per cent w/v solution of hemoglobin when diluted to 8 L. is calculated. This volume of erythrocytes is measured into a graduated bottle and 10 Gm. sodium citrate dihydrate in 3 L. pyrogen-free distilled water at 4 C. is added. The volume is made to the 8 L. mark with distilled water. After vigorous shaking the solution is stored in the refrigerated room for not more than an hour or two.

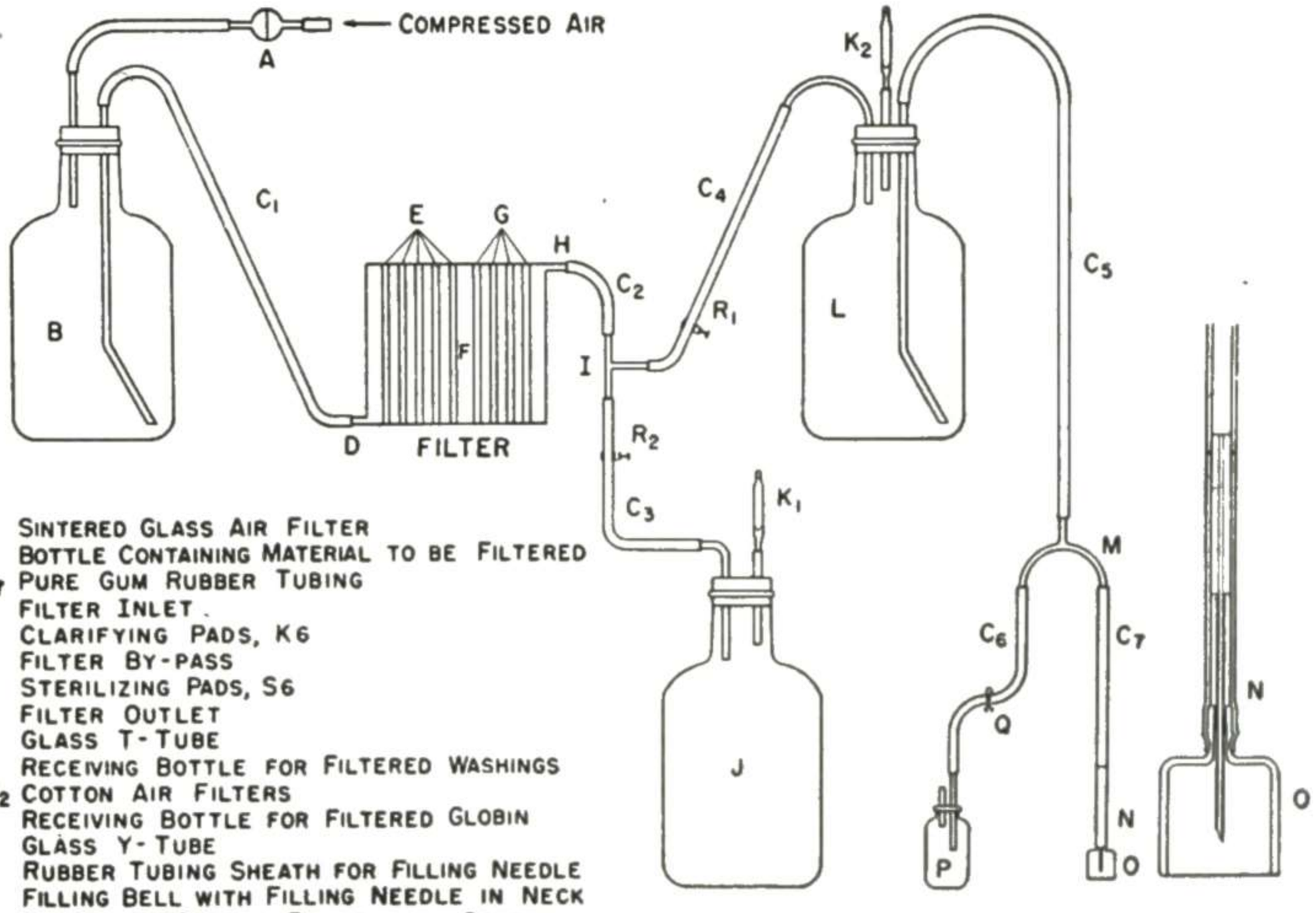
For the globin hydrochloride, 400 ml. of 38 per cent w/w hydrochloric acid is added to 80 L. acetone in a 120 L. glass-lined tank. While the acid acetone is being mixed, the hemoglobin is filtered through a layer of cheesecloth into it at a rate of about 1 L. every five minutes, the stream of hemoglobin solution being directed into the vortex of the acid acetone so that the most rapid possible mixing is obtained. After 30 minutes' stirring, the suspension is run from the tank into a basket centrifuge lined with a canvas bag. The centrifuge is run at about 850 rpm and the suspension added at about the same rate as the waste acetone is removed. The cake is sprayed with acetone until free from hematin, washed with an additional 10 L. acetone and centrifuged at 1,700 rpm for 20 minutes for partial drying of the globin cake. The cake is then pulverized and the moist powder dried in a drum dryer. The powder is stored at 4 C. A 1 Gm. sample is dried at 105 C. for one hour and the dry weight of the powder in the jar calculated.

For alkaline hydrolysis, 95 ml. pyrogen-free water at 37 C. for each 4.55 Gm. dry globin hydrochloride powder to be treated is measured into a 40 L. glass jar. Carbonate-free saturated sodium hydroxide solution equivalent to 1.05 Gm. sodium hydroxide for each 4.55 Gm. globin hydrochloride powder is added. The powder is poured slowly into the vortex created by mixing of the solutions. After all the powder is wet, stirring is continued for 4 minutes and the solution allowed to stand for 15 minutes, when it is again stirred for 4 minutes and allowed to stand for 15 minutes. The solution is drained from the bottom outlet of the jar through four layers of cheesecloth and the filtrate collected in two equal portions in 20 L. bottles which are incubated at 37 C. for exactly 27 hours. The jars are then refrigerated at 4 C. and the solution transferred to a 40 L.

(1) J. Lab. & Clin. Med. 37:959-968, June, 1951.

glass jar with a bottom outlet. During vigorous mixing, 10 per cent w/v hydrochloric acid is added to bring the pH to 7.1, the external electrodes of the pH meter being used directly in the solution.

Dialysis and adsorption are done entirely in a refrigerated room at 4 C. Dialysis bags, filled about three-fourths full of neutralized globin solution and rubber stoppered, are suspended in dialysis jar so that the level of globin solution in the bags is about 7.5 cm. above the level of the dialysis water. The dialysis is allowed to proceed



A Sintered Glass Air Filter
B Bottle Containing Material to be Filtered
C_1-C_7 Pure Gum Rubber Tubing
D Filter Inlet
E Clarifying Pads, K6
F Filter By-pass
G Sterilizing Pads, S6
H Filter Outlet
I Glass T-Tube
J Receiving Bottle for Filtered Washings
K_1-K_2 Cotton Air Filters
L Receiving Bottle for Filtered Globin
M Glass Y-Tube
N Rubber Tubing Sheath for Filling Needle
O Filling Bell with Filling Needle in Neck
P Air Filter Bottle Filled with Cotton
Q Pinch Clamp
R_1-R_2 Screw Clamps

Fig. 104.—Filtration apparatus. (Courtesy of Strumia, M. M., and Sample, A. B.: J. Lab. & Clin. Med. 37:959-968, June, 1951.)

for 20-24 hours with distilled water flowing into the bottom of the jar and overflowing from the top at about 40 L./hour. After this, 30 Gm./L. decalso is added to the solution in the dialysis bags. After vigorous mixing, decalso is allowed to stand suspended for one hour. When decalso has settled, the supernatant is siphoned into a 30 L. bottle and kept at 4 C. (The protein may be adjusted at this point to any convenient concentration by dilution with water. Salt may be added at this point, if desired. If the salt concentration is left low and the material is to be used intravenously, it must be made isotonic by addition of dextrose to make a 5 per cent solution. The pH of this solution after addition of water and/or salt should be adjusted to 7.45 at 25 C. The solution should not be stored for more than an hour before sterilization.)

For sterilization and distribution the Republic filter is set up as described in its operating instructions for double filtration, using K-6 clarifying pads (Fig. 104, *E*) on the inlet side of the by-pass (*F*) and S-6 sterilizing pads (*G*) on the product side. The assem-

bled filter with pads is washed by passing through 9 L. cold pyrogen-free 0.5 per cent w/v sodium carbonate solution and then 9 L. cold 0.9 per cent w/v sodium chloride solution. A positive pressure of not over 7 lb. is used and the air supply filtered through a sintered glass air filter. After thorough cleaning of all parts, the filtration apparatus is set up as in Figure 104. The assembled apparatus is autoclaved for 30 minutes at 120 C. and cooled to room temperature. (The cold room may be used, if necessary, to save time.) The globin solution is filtered and after the receiving bottle *L* is half full, filling of the individual final containers may be started. The filling tube C_5 with its attachments is passed through a small opening into the sterile filling room, where all personnel wear sterile gowns, caps and masks. At the beginning, middle and end of each series of bottles filled, the product is tested for sterility, toxicity and pyrogenicity. The safety of the material for animals is determined by intraperitoneal injections in mice. For tests in human beings a 4 per cent w/v solution of modified globin should be given intravenously at the rate of 3 mg./kg./minute.

If all the safety tests are passed for a lot of globin solution, it may be stored for parenteral use in human beings for more than two years at room temperature or indefinitely if dried from the frozen state.

Modification of Benedict's Method for Measuring Blood Glucose is described by F. William Sunderman and John B. Fuller.[2] It greatly stabilizes the final molybdenum blue solution for color comparison with either a photoelectric or visual colorimeter. Folin-Wu tungstic acid filtrates, the Benedict 1931 copper bisulfite reagent and a modified, smaller sugar tube (Fig. 105) which requires less blood filtrate are used.

The molybdenum blue solution obtained with the Benedict method has a relatively constant transmission at 592 mμ for 23 minutes after addition of the color reagent. If the solution is placed immediately in a boiling water bath for three minutes before cooling, the color becomes relatively stable. To prevent reoxidation due to exposure of the surface of the solution to air, the smaller tube was designed and a marble used as a stopper during the boiling period. Control experiments showed a 6.1 per cent (10 mg./100 ml. blood) greater yield of sugar in the modified sugar tube. Checking indicated that marbles alone do not prevent reoxidation.

PROCEDURE.—One ml. standard glucose solution is transferred to one sugar tube and 1 ml. tungstic acid filtrate of blood to another. One ml. copper bisulfite reagent is added to each tube. A marble is placed over the mouth of each tube and the tubes are placed in a

(2) Am. J. Clin. Path. 21:1077-1084, November, 1951.

boiling water bath for six minutes. The tubes are removed, 1 ml. phosphomolybdic acid color reagent is added and the tubes are shaken thoroughly. They are replaced in a boiling water bath for three minutes, transferred to a cool water bath for one minute and then diluted to the 10 ml. mark. Readings are made in 5-30 minutes.

If a Klett-Summerson instrument is used, the final solution is transferred to matched and calibrated Klett tubes. A Klett no. 54 filter is used, with maximal

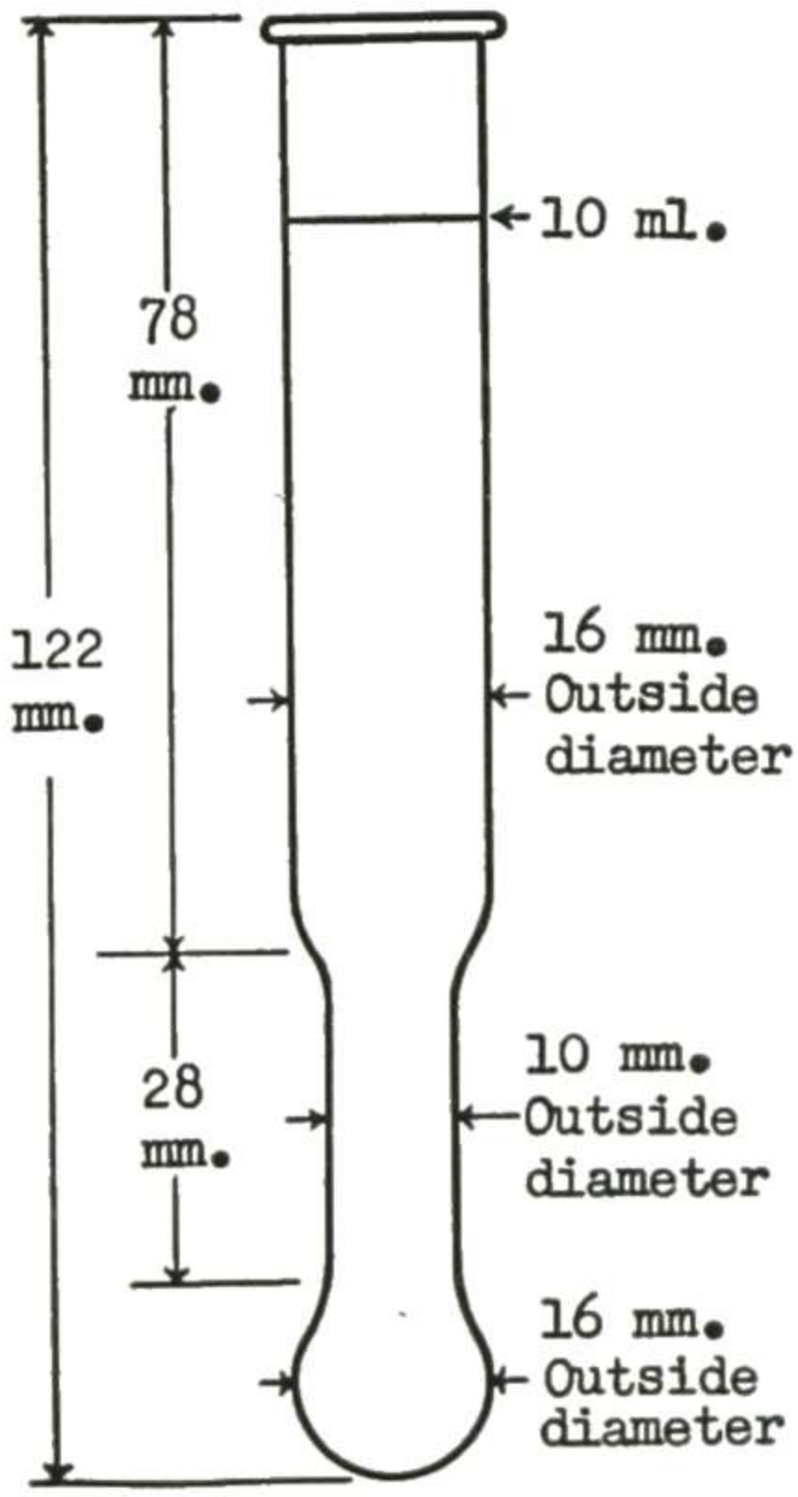


Fig. 105.—Sunderman blood glucose tube. (Courtesy of Sunderman, F. W., and Fuller, J. B.: Am. J. Clin. Path. 21:1077-1084, November, 1951.)

transmission at 450 mμ. Spectroscope readings should be at 590 mμ.

For visual colorimetry (Duboscq's type), Wratten filter G, no. 15, is used over the eyepiece. The unknown is placed in the left cup and the scale reading set at 15.0 mm. The standard is placed in the right cup and adjustment made to a level at which the color matches that of the unknown. Reading of the standard in millimeters multiplied by 10 gives concentration of glucose in milligrams/100 ml.

If the reading indicates a concentration greater than 325 mg./100 ml., the analysis is repeated with 0.5 ml. blood filtrate and 0.5 ml. water. Final reading of the standard is multiplied by 20. If the concentration is low (below 50 mg./100 ml.), 0.5 ml. standard solution and 0.5 ml. water are placed in the standard tube. Final reading of the standard is multiplied by 5.

Estimation of Alkali Reserve: Method for Routine Hospital Use with Small Amounts of Plasma. H. Lehmann[3] (Tunbridge Wells) describes a modification of the Wootton and King method which in 96 tests gave agreement within ±2 m.Eq./L. with parallel tests run by Van Slyke's titration method. In none of 100 estimations was the difference greater than ±3 m.Eq./L.

METHOD.—Dilutions of N/10 sulfuric acid in freshly distilled water, which produce a pH of 5.5 when mixed with equal amounts of plasma varying in alkali reserve from 16 to 36 m.Eq./L. bicarbonate, are prepared as shown in the table. For each dilution, Dreyer's tubes are filled with 0.2 ml. methyl red indicator and 0.1 ml. acid. The two are mixed by rolling the tubes between the hands. The tubes with the same acid concentrations are stored in airtight

(3) Lancet 1:718-719, Mar. 31, 1951.

screw cap jars in a dark place until needed. After about a week some of the methyl red may become converted into a brownish precipitate, but this does not seriously interfere with the color changes on neutralization. At the time of the test, one tube from each container is placed in a rack with holes labeled 16-36. Plasma is saturated with alveolar air by breathing through it from a 1 ml. graduated pipet which is then used for adding 0.1 ml. plasma to each tube. The contents of tubes is mixed by rolling between the hands. The number of the last tube which retains the last definite tint of red corresponds to the alkali reserve of the plasma. If the red is deep

DILUTIONS OF SULFURIC ACID

PLASMA OR SERUM		STRENGTH OF SULFURIC ACID REQUIRED (ML. N/10 ACID/100 ML.)
m.Eq. Bicarbonate/L.	CO_2-Combining Power (Vol. %)	
16	35	30.3
18	40	34.0
20	44	37.9
22	48	41.7
24	53	45.4
26	57	49.0
28	62	52.9
30	66	56.7
32	70	60.6
34	75	64.2
36	79	68.0

NOTE.—If measurements below 16 m.Eq./L. are required, dilutions of acids corresponding to 28, 24, 20 and 16 m.Eq., with equal amount of freshly distilled water, will produce the requisite test acids for 14, 12, 10 and 8 m.Eq. Similarly, acids corresponding to 38 and 40 m.Eq. can be prepared by diluting 36.0 and 37.9 ml. N/10 sulfuric acid respectively to a final volume of 50 ml.

and the preceding tube is clearly yellow, the value can be interpolated. If one tube is red and the next orange-yellow, the alkali reserve corresponds to the value for the red tube.

In emergencies, when only a little plasma is available, tubes corresponding to 22, 26 and 30 m.Eq. plasma bicarbonate/L. or CO_2-combining power of 48, 57 and 66 vol./100 ml. are run. If 0.1 ml. plasma added to the 22 m.Eq./L. turns yellow, there is no severe acidosis; but if it remains red, acidosis is severe. If plasma added to the 26 m.Eq./L. turns yellow, there is no acidosis; but if it remains red, there is acidosis. If plasma added to the 30 m.Eq./L. turns yellow there is alkalosis; but if it turns red, there is none.

Pathologic Physiology: Potassium; Chemical Anatomy of Water and Electrolyte Balance, with Particular Reference to Pregnancy. According to Max Trumper[3a] (Bala-Cynwyd, Pa.), of the three electrolytes, sodium, potassium and chloride, potassium is the least intimately associated with water but the only one that alters cell permeability in function. Though

(3a) M. Clin. North America 35:893-906, May, 1951.

potassium is primarily an intracellular electrolyte, it moves about in accord with demand of shifting membrane equilibriums, especially in the presence of acid-base imbalance. It moves into cells when nitrogen balance is positive and out when it is negative. It passes from cell to extracellular fluids, including the plasma, when excessive quantities of water and sodium are lost from the body, as in hemorrhage, shock, adrenocortical insufficiency, intestinal obstruction and various gastrointestinal fistulas. Using radioactive potassium, Moore found that potassium in the extra- and intracellular compartments requires 15 hours to reach normal equilibrium. This highly significant fact emphasizes the need for caution in giving potassium salts intravenously. Average daily intake of potassium, 3.5-5 Gm. or 90-125 m.Eq., is more than is needed for growth and maintenance. The plasma or serum content of potassium is 3.5-5 m.Eq./L., equivalent to 14-20 mg./100 ml. Erythrocytes contain about 20 times this amount of potassium. The potassium concentration in saliva and in gastric juice is several times that in the plasma, and loss of these substances may result in serious potassium depletion. The kidney can concentrate potassium about 12 times, and about 90 per cent of the daily intake of potassium is excreted in the urine. Normal kidneys do not conserve potassium as they do sodium. On a potassium-free diet, 40-50 m.Eq. is excreted.

There is a reciprocal relation between potassium in tissue cells and sodium. Cells tend to lose potassium and gain sodium when circulation is impaired, as in dehydration, shock and tissue anoxia. In uncomplicated pregnancy there is no problem of potassium balance as long as the kidneys are not seriously diseased and intake of food is adequate. With persistent vomiting, loss of potassium may not be readily observed from serum levels because of dehydration.

There is little likelihood of potassium deficit as long as the patient can eat a normal diet. Equally rare is the occurrence of potassium poisoning, no matter how much food one eats, provided the fluid intake is ample and the kidneys are not seriously impaired. Even in uremia, serum K levels may be normal, low or high, depending on the clinical picture.

Potassium deficiency seldom persists when the patient is on an adequate diet except with disturbances such as certain adrenal cortical tumors, protracted diarrhea, extensive burns and severe diabetic acidosis. It may appear during recovery

from diabetic coma when large doses of insulin are being given or may develop from frequent injections of desoxycorticosterone acetate and after considerable infusions of sodium chloride. Due largely to the law of concentration effect, an excess of sodium pushes out potassium just as intravenous injections of sodium chloride push out bromide from the blood in bromide poisoning. Saline intravenously thus produces larger losses of potassium than does a similar volume of glucose.

[An unusually good list of references accompanies this review, and the author makes pertinent comments after a number of the references. For example, regarding Hoffman, W. S.: Clinical Physiology of Potassium, J. A. M. A. 144:1157, Dec. 2, 1950, he says: "This report contains a wealth of practical guidance on the use of potassium. Students of medicine, both undergraduate and postgraduate, should study this paper." And regarding Marriott, H. L.: Water and Salt Depletion, Brit. M. J. 1:245, Feb. 15; 285, Mar. 8, and 328, Mar. 15, 1947, he says: "These three articles have been of inestimable assistance in preparing my report on potassium. Unless one appreciates the relationship as presented by Marriott, it is difficult to correlate the basic acience aspects of potassium with clinical problems of fluid balance."—Ed.]

Clinical Interpretation of Common Abnormalities in Serum Concentrations of Certain Electrolytes is discussed by Russell D. Squires and J. Russell Elkinton[4] (Univ. of Pennsylvania). Almost 200 years ago it was observed that the weights of elements which had combined with each other chemically always did so in a fixed ratio or set of ratios. As a result of further investigation it was concluded that two atoms of hydrogen combined with one of oxygen and that the atomic weights of the respective elements had a ratio of 1:15.874. It was later decided arbitrarily that it would be more convenient to set the atomic weight of oxygen at 16. The relation of combining and atomic weights are shown in Table 1. This concept of a reproducible combining weight ratio provided the chemist with a means of predicting how much of one element would combine with another. These combining weights are referred to in chemical parlance as equivalent weights. An equivalent weight may be defined as the weight of an atom, radical or molecule divided by its valence. The equivalent weight divided by 1,000 is called a milliequivalent, a unit of convenient magnitude for expressing concentration in body fluid as milliequivalents/liter. Milligrams per cent can be converted to milliequivalents/liter by means of the formula

$$\frac{\text{mg. per cent} \times 10}{\text{molecular weight} \times \text{valence}} = \text{m.Eq./L.}$$

(4) M. Clin. North America 35:1807-1828, November, 1951.

Table 2 contains factors for converting certain solutes important in acid-base balance from milligrams per cent or volumes per cent to milliequivalents/liter or millimols/liter.

It is stated as part of the theory under consideration that when a compound in solution dissociates to form ions there is present in that solution an equal number of positively and

TABLE 1.—Relation of Combining Weight to Atomic Weight

Element	Combining Weight		Valence	Atomic Weight
	If H = 1	If O = 8		
H	1	1.008	1	1.008
O	7.937	8	2	16
Na	22.814	22.997	1	22.997
K	38.786	39.096	1	39.096
Cl	35.175	35.457	1	35.457
S	15.906	16.033	2	32.066
N	13.896	14.008	1	14.008

negatively charged ions. That such a concept of equivalence (law of electrochemical neutrality) is not apparent when concentration is expressed as milligrams per cent is readily discernible from Figure 106, *A*. However, when expressed as ionic equivalents (*B*) the relation is readily apparent, i.e., sum of the cations equals sum of the anions.

Sodium holds an unusual place among the electrolytes of

TABLE 2.—Factors for Conversion of Milligrams Per Cent or Volumes Per Cent to Millimols or Milliequivalents per Liter

Total CO_2 content vol. % × 0.45 = mM./L. CO_2
Total CO_2 content vol. % × 0.423 = m.Eq./L. HCO_3^-
NaCl Gm. × 17.1 = m.Eq. Cl^- or Na^+
Cl^- or Na^+ in m.Eq. × 0.0585 = Gm. NaCl
$NaHCO_3$ Gm. × 11.9 = m.Eq. Na^+ or HCO_3^-
KCl Gm. × 13.4 = m.Eq. Cl^- or K^+

serum and extracellular fluid in that changes in its concentration measure approximately changes in total electrolyte concentration throughout the body fluids (Table 3). In extracellular fluid sodium constitutes most of the cation of the phase and the cations determine total extracellular electrolyte concentration because of the volatility of bicarbonate ion on the anion side. Also, extracellular concentration of cation or total electrolyte cannot differ greatly from that of intracellular fluid because of the free diffusibility of water between the two phases. To raise the extracellular sodium concentration a given number of milliequivalents/liter, the desired change in

concentration must be multiplied by the estimated volume of total body water. In clinical medicine, elevated serum sodium concentration is almost always due to dehydration or deficit of water in relation to sodium and total electrolyte. A low concentration is much more common and may reflect a sodium deficit, an excess of water or both. Determination of total base

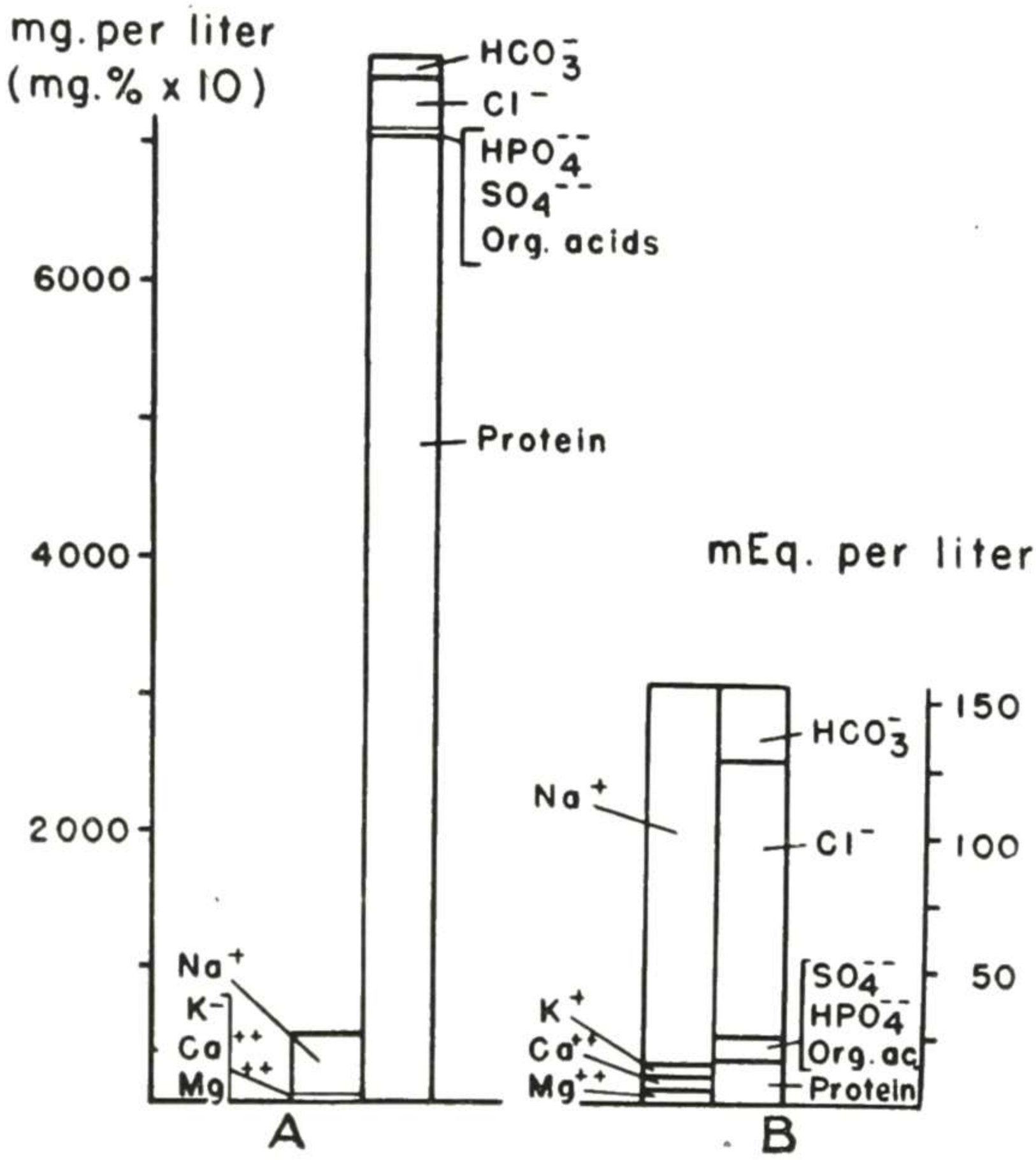


Fig. 106.—Comparison of serum electrolyte concentrations expressed (*A*) as milligrams/liter and (*B*) as milliequivalents/liter. (Courtesy of Squires, R. D., and Elkinton, J. R.: M. Clin. North America 35:1807-1828, November, 1951.)

concentration in the serum is just as useful as that of sodium in the diagnosis of these disturbances.

Serum chloride concentration by itself yields no useful information about the patient. In conjunction with the carbon dioxide content, it tells something of the factors involved in a change in the bicarbonate concentration (metabolic acidosis or alkalosis). In this way it is rationally used and therefore associated with carbon dioxide in Table 4. Carbon dioxide plus chloride plus an assumed value for the other undetermined anions has been used to approximate total acid and

therefore total base concentration. Such a calculation will be greatly in error when any of the undetermined anions are present in abnormal amounts (ketosis, renal insufficiency), and for this reason is of no help in many clinical situations in which such a value is needed. An elevated chloride value may indicate excess of chloride ion, deficit of water or both,

TABLE 3.—CONCENTRATION OF Na^+ IN RELATION TO VOLUME OF BODY WATER

CONCENTRATION	VOL. OF WATER		CLINICAL DIAGNOSIS
	E*	I	
Na+ incr. or upper limits of normal	Decr.	Decr.	Dehydration due to 1. water deprivation in unconscious patient; 2. diabetes insipidus, e.g., after resection of craniopharyngioma in unconscious patient with trauma to supraopticohypophyseal system; 3. excessive sweating, greatly increased by fever (most common in infants and children)
Na+ **decr.**	Decr.	Incr.	1. Addison's disease
	Decr.	?	2. Salt-wasting nephritis
	Decr.	?	3. Excessive loss of bile, e.g., via T tube drainage
	Decr.	?	4. Diarrhea
Na+ **decr.**	Incr.	Incr.	1. Anuria with excessive treatment with salt-free solution, e.g., lower nephron nephrosis
	Incr.	?	2. Low salt syndrome in congestive failure associated with intensive therapy with mercurial diuretics

*Total body water equals extracellular water (E) plus intracellular water (I).

TABLE 4.—CONCENTRATION OF SERUM Cl^- AND SERUM CO_2 IN RELATION TO VOLUME OF BODY WATER

CONCENTRATION	VOL. OF WATER		CLINICAL DIAGNOSIS
	E	I	
Cl− **decr.**	Normal or decr.	Decr.	Vomiting with HCl loss from stomach
CO_2 incr.	Normal	Normal?	Primary CO_2 retention, e.g., pulmonary emphysema
	Usually decr.	?	Potassium deficiency alkalosis
Cl− unchanged or decr.	Normal or decr.	Decr.	Ketosis
CO_2 decr.	Normal or decr.	Decr.	Renal insufficiency with retention of PO_4, SO_4 and organic acids
Cl− incr.	Normal or incr.	?	Treatment of chronic renal disease with large quantities of isotonic NaCl
CO_2 decr.	Incr.	?	Nephrosis, especially with NH_4Cl therapy
	Normal	Normal	Hyperventilation, primary CO_2 deficit

whereas a low serum chloride value may be due to excess of water or deficit of chloride as a result of loss of the ion in gastric fluid or through the kidneys. The carbon dioxide contained in serum or plasma is in the two important physiologic forms of undissociated carbonic acid and dissociated bicarbonate ion. These two substances together with the anionic protein form the main buffer system of the body fluids and

TABLE 5.—CONCENTRATION OF SERUM K (EXTRACELLULAR) IN RELATION TO CALCULATED AMOUNT OF INTRACELLULAR K+ AND VOLUME OF BODY WATER

SERUM CONC. (EXTRACELLULAR)	CALC. CHANGES IN INTRACELLULAR K	VOL. OF WATER		CLINICAL DIAGNOSIS
		E	I	
K+ decr.	Decr.	Decr.	?	Starvation
	Decr.	Incr.	?	Hyperadrenal cortical activity
	Decr.	Decr.	Decr.	Diabetic acidosis (after beginning therapy)
	Decr.	Decr.	Decr.?	Infant diarrhea
K+ decr.	?	?	?	Familial periodic paralysis
K+ incr.	Incr.	Decr.	?	Chronic renal insufficiency (before therapy)
K+ incr.	Decr.	Decr.	Decr.	Diabetic acidosis (before therapy)
K+ incr.	Incr.	Decr.	Incr.?	Adrenal cortical insufficiency; Addison's disease

determine hydrogen ion concentration or pH. In the usual absence of pH determinations the physician has to work with the total carbon dioxide content of serum plus some clinical knowledge of the patient. Any change of total carbon dioxide content from the normal range indicates primarily a change in bicarbonate concentration. Measurement of carbon dioxide capacity or combining power is obsolete and should be replaced determination of total carbon dioxide content.

Potassium is predominantly an intracellular ion. Its concentration in extracellular fluids can give only an indirect clue to concentration and amount of that ion in the intracellular phase. With the exception of the disease familial periodic paralysis, low concentration in extracellular fluid indicates intracellular deficit of the ion (Table 5). High extracellular concentration may indicate intracellular excess or may exist in the presence of a cellular deficit when renal function becomes impaired. High extracellular concentrations may be associated with interference of cardiac function and eventually may lead to asystole.

Electrocardiogram Associated with Low Levels of Serum Potassium. P. M. McAllen[5] (West Middlesex Hosp.) found abnormalities in the ECG associated with low serum potassium level in three cases, with a different type of tracing in each case. Experience with these cases and with cases previously reported suggests that a lowering of serum potassium content may result in changes in almost any part of the ECG and that, contrary to previous reports, prolongation of the Q-T interval is not commonly associated with this condition. Electrocardiographic changes are best seen with potassium levels of about 12 mg./100 ml. A woman, 41, with idiopathic steatorrhea had S-T segment depressions and T-wave inversion. In a woman, 39, with ulcerative colitis, only the T-wave voltage was lowered. In a woman, 45, with ulcerative colitis, a depressed S-T segment was combined with a low T wave and a large U wave. In the first and third patients there was striking difference in the level to which the potassium fell before the ECG became abnormal (18.3 and 11.9 mg./100 ml. respectively), and in the third, when the serum potassium was low, P wave amplitude increased abnormally in contrast to the flattening or disappearance of the P wave known to be associated with high serum potassium levels.

Microdetermination of Chlorides in Serum and Spinal Fluid. Vincent Franco and Bernard Klein[6] (Veterans' Admin. Hosp., Bronx, N. Y.) report a rapid technic which is accurate within ± 2 per cent and does not involve specialized reagents or unusual manipulation.

REAGENTS.—(1) $Ba(OH)_2$, 0.3 N, is prepared from the reagent grade chemical and protected from atmosphere by a soda lime tube. (2) $ZnSO_4$, 5 per cent solution, is used, with solutions adjusted so that the $ZnSO_4$ is exactly neutralized by the $Ba(OH)_2$, with phenolphthalein as indicator. Two parts $Ba(OH)_2$ is diluted in 5 parts chloride-free distilled water. Enough solution of a day's requirement may be prepared and kept out of contact with the atmosphere. (3) NaCl, 1.000 Gm. dried reagent grade, is dissolved in water and diluted to 1 L.; 1 ml. = 1.0 mg. (4) $AgNO_3$, 0.02 N, is prepared by dilution of 0.1 N solution and standardized against NaCl as described by Saifer and Kornblum. (5) Dichlorofluorescein, 0.05 per cent in 70 per cent ethanol is the indicator. Eastman Kodak no. 373 is adequate.

PROCEDURE.—For serum, 7.0 ml. $Ba(OH)_2$ solution is added to 1.0 ml. clear serum followed by 2.0 ml. $ZnSO_4$. The mixture is stoppered, shaken well and centrifuged at 2,500 rpm; 2 ml. aliquots

(5) Brit. Heart J. 13:159-166, April, 1951.
(6) J. Lab. & Clin. Med. 37:950-954, June, 1951.

are transferred to a small Erlenmeyer flask or a 6 ×1 in. test tube. One drop of indicator is added and the solution titrated with 0.02 N $AgNO_3$ until the first pink is seen throughout the solution.

Clear spinal fluid, 0.5 ml., is treated with 3.5 ml. $Ba(OH)_2$ and 1.0 ml. $ZnSO_4$ solution. Two ml. aliquots of centrifugate are titrated as described for the calculation:

$$\text{mM Cl—/L.} = \text{ml. 0.02 N } AgNO_3 \times F$$

$$F = \frac{5000}{58.45} \times a$$

a = ml. 0.02 N $AgNO_3$ used to titrate 1.0 mg. NaCl

Titration may be done in either diffuse daylight or under artificial light. It is possible to reproduce microtitrations of the order of 1.0 ml. to within 0.6 per cent by this method.

Serum Phosphatase Determinations in Diagnosis of Prostatic Cancer: Review of 1,150 Cases. In less than 40 per cent of patients, Reed M. Nesbit and William C. Baum[7] (Univ. of Michigan) found significant elevation in serum acid phosphatase on first admission. Of patients with evidence of metastases on the original examination, 65 per cent had elevated serum titer, whereas of those without metastases, only 20 per cent had significant elevations of serum acid phosphatase. Normal levels in those with recognized extension to lymph nodes or bone may be attributed to failure of the carcinomatous cell to produce the enzyme or to a lag between rise of serum titer and appearance of osteoblastic response to invading tumor.

Almost 86 per cent of patients with metastases had elevated blood alkaline phosphatase levels. This alteration seemed even more closely correlated with evidence of metastases than did the elevated serum acid phosphatase values.

In patients without x-ray evidence of metastases, those with normal serum acid phosphatase levels had far greater opportunity for three year survivals than those with abnormal levels on first admission. This supports the view that an elevated serum acid phosphatase level indicates spread of the neoplasm even though other evidence of metastases is lacking.

Among 122 patients with bony metastases who had normal serum acid phosphatase values, 32.5 per cent survived three years and 60 per cent were benefited by endocrine treatment. This response was more favorable than among 256 patients with elevated serum acid phosphatase values, of whom only 25 per cent survived for three years and 47 per cent were

(7) J. A. M. A. 145:1321-1324, Apr. 28, 1951.

benefited by therapy. A normal serum acid phosphatase level associated with metastases in untreated prostatic cancer does not indicate androgen independence nor is prognosis adversely affected by this observation.

Colorimetric Determination of Phosphatases in Human Serum. Arnold M. Seligman, Howard H. Chauncey, Marvin M. Nachlas, Leon H. Manheimer and Herbert A. Ravin[8] (Boston) describe a simple method which uses stable reagents, is not affected by serum chromogens, provides a high degree of accuracy over a much wider range of phosphatase concentration than prevailing technics and affords correlation of quantitative estimations of phosphatase activity in homogenates and histochemical demonstrations of phosphatases in tissue sections. The mean value for serum acid phosphatase in 62 normal serums was 1 unit/100 ml. The mean value of serum alkaline phosphatase in 89 normal serums was 1.8 units/100 ml. Comparison with other methods for measuring acid and alkaline phosphatase activity is given in the table.

REAGENTS.—(1) For the substrate stock solution of sodium β-naphthyl phosphate, 0.2 mg./ml. distilled water (0.0008 M) is used. It may be stored at 4 C. for a month with no significant spontaneous hydrolysis. (2) Veronal buffer (pH 9.1, 0.1 M) is prepared by mixing 950 ml. of 0.1 M sodium diethyl barbiturate with 50 ml. of 0.1 M hydrochloric acid. (3) Acetate buffer (pH 4.8, 0.2 M) is prepared by mixing 120 ml. of 0.2 M sodium acetate with 80 ml. of 0.2 M acetic acid. (4) Tetrazotized diorthoanisidine (available in powder form containing 20 per cent tetrazotized diorthoanisidine, 5 per cent zinc chloride and 20 per cent aluminum sulfate). The powder, naphthanil diazo blue B, 4 mg./ml., is dissolved in cool water immediately before use. In solution the diazonium compound decomposed extensively on standing at room temperature 20-30 minutes. (5) Sodium carbonate, 1 M. (6) Trichloracetic acid, 40 per cent. (7) Anhydrous ethyl acetate. Just before use the desired amount of stock solution (1) is mixed with an equal volume of either stock buffer solution (2) or buffer solution (3). The appropriately buffered substrate solution is then added to the enzyme preparation.

PROCEDURE.—Serum is obtained by centrifugation of freshly clotted blood, 1 ml. being removed with a pipet and diluted with 19 ml. distilled water. To 1 ml. of this diluted serum in a 20 ml. test tube, is added 5 ml. of appropriately buffered substrate solution. In a separate test tube, buffered substrate solution alone serves as a control for nonenzymatic hydrolysis. The tubes are incubated at 37.5 C. for one hour in determining alkaline phosphatase and for two hours in determining acid phosphatase. In the latter determination, 4 drops

(8) J. Biol. Chem. 190:7-15, May, 1951.

of 1 M sodium carbonate solution are added after the period of incubation, to raise the pH to the optimal level for coupling. To each tube, 1 ml. tetrazotized diorthoanisidine solution is added, and the tubes are agitated vigorously to insure thorough mixing. After three minutes, to each tube is added 1 ml. of 40 per cent trichloracetic

COMPARISON OF METHODS FOR ESTIMATION OF SERUM ACID AND ALKALINE PHOSPHATASE ACTIVITY

Method	Substrate*	Color determinant	Temperature	Time	pH		Unit of activity per hr.	Normal values†	
					Acid	Alkaline		Acid	Alkaline
			°C.	hrs.					
Bodansky (1), 1933	β-Glycerophosphate	PO_4	37	1		8.7	1 mg. P		1.5– 4.0
King and Armstrong (2), 1934	Phenyl phosphate	Phenol	37.5	1	5.0	9.0	1 " phenol	2.4	3.7–13.1
Huggins and Talalay (3), 1945	Phenolphthalein phosphate	Phenolphthalein	38	1	5.5	10.3	0.1 " phenolphthalein	3 –10	3 –15
Bessey *et al.* (4), 1946	*p*-Nitrophenyl phosphate	*p*-Nitrophenol	38	0.5		10.3	1 mM *p*-nitrophenol		2.4– 5.7‡
Hudson *et al.* (5), 1947	" "	"	38	0.5	5.4		" "	1.0– 2.3	
Seligman *et al.*, present paper	β-Naphthyl phosphate	β-Naphthol	37.5	1–2	4.8	9.1	10 mg. β-naphthol	0.7– 1.6	1.0– 3.0

*All substrates used in form of sodium salt.

†Normal values expressed in terms of units/100 ml. serum, except in methods of Bessey *et al.* and Hudson *et al.*, for which they are reported in terms of mM units/L. serum.

‡Values for alkaline phosphatase obtained in 12 normal young children whose Bodansky alkaline phosphatase level was 6-10 units/ml., approximately 2-3 times normal adult levels.

acid and 10 ml. ethyl acetate from a buret. The tubes, shaken vigorously until an even emulsion is obtained, are then centrifuged for 10 minutes at 2,500 rpm, and 5 ml. of the organic layer is transferred with a pipet to a Klett tube. The color density is measured in a photoelectric colorimeter through a green filter (540 mμ). The readings are converted to milligrams of naphthol with a calibration curve prepared from β-naphthol in the presence of serum, according to the procedure described. A calibration curve falling within the range of a colorimeter may be obtained with 0.01-0.08 mg. β-naphthol.

One unit of phosphatase activity is defined as that amount of enzyme which liberates the color equivalent of 10 ml. β-naphthol/hour at 37.5 C. The number of units of alkaline phosphatase/100 ml. serum is obtained by multiplying by 200 the number of milligrams of β-naphthol released in one hour when 0.05 ml. serum is used. The number of units of acid phosphatase/100 ml. serum is obtained by multiplying by 100 the number of milligrams of β-naphthol released in two hours when 0.05 ml. serum is used.

Importance of Serial Determinations of Serum Alkaline Phosphatase in Incomplete Biliary Tract Obstruction. Herman Ulevitch, Edward A. Gall, Paul I. Hoxworth, Leon Schiff and David L. Graller[9] (Univ. of Cincinnati) report serial studies of serum bilirubin and serum alkaline phosphatase concentrations in four patients and a dog with partial biliary tract obstruction. A dissociation between serum bilirubin and alkaline phosphatase values often occurred early in the course. Serum bilirubin elevation was not necessarily accompanied by a rise in serum alkaline phosphatase value. This is possibly attributable to hepatitis related to hydrohepatosis and ascending infection. As the disease progresses repeated determinations may show pronounced rise in the serum alkaline phosphatase with a fall in serum bilirubin concentration. The time in which this occurs varies with the patient. Liver biopsies during this phase may show intralobular alterations as well as severe cholangitis. After surgical correction of an obstructing lesion, both serum bilirubin and alkaline phosphatase values usually return to normal but in some instances serial determinations show persistent elevation in the alkaline phosphatase without increase of serum bilirubin. Histologic examination shows persistent cholangitis or early biliary cirrhosis.

Serum alkaline phosphatase concentration is a more sensitive indicator of incomplete biliary obstruction than the serum bilirubin and should be determined repeatedly even after removal of an obstructing lesion and complete subsidence of jaundice.

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(9) J. Lab. & Clin. Med. 38:693-704, November, 1951.

Test for More Accurate Recognition of Gallbladder and Liver Bile during Diagnostic Biliary Drainage is reported by August A. Hall and John M. Masen[1] (Brooke Gen'l Hosp.). The sequence of bile flow in a normal subject is a small amount of lemon to golden yellow common duct bile followed by yellow-brown gallbladder bile and finally a golden yellow liver bile. Deviations from this color sequence occur in disease and/or pathologic physiologic aberrations of the biliary tract. Exceedingly dark, sometimes jet black, gallbladder bile is seen in gallbladder stasis. The authors' method of determining priodax® in the bile drainage fluid follows.

METHOD.—The biliary drainage fluid containing priodax® is oxidized by boiling with acid permanganate. The organic matter is destroyed and the iodine of priodax® set free and converted to iodate. On addition of an excess of potassium iodide, the iodate oxidizes the iodide, with liberation of free iodine. Addition of starch to this solution results in formation of a blue color, which is measured in a photoelectric colorimeter or spectrophotometer. Its concentration is determined by comparison with a standard priodax® solution submitted to the same procedure.

Thirteen patients with gallbladders were given 6 priodax® tablets 14 hours before biliary drainage. All gallbladders were visualized by cholecystography, and eight showed partial emptying after drainage. The iodine test, performed on all bile fractions, corresponded well in its concentrations to the bile designations made by the examiner. Dye was still present in the liver after 14 hours. In two cholecystectomized patients, given priodax® 14 hours before drainage, iodine was detected in all fractions. In four cholecystectomized patients given priodax® 38 hours before drainage, no iodine was detected in any bile fractions. In nine patients with gallbladders given priodax® 38 hours before drainage, no iodine was detected in any fraction designated grossly as liver bile. One false negative reaction was encountered with designated gallbladder bile.

In most instances, gross designation of drained bile by a trained observer is clinically accurate and valid. The iodine test is not advocated as a routine test during diagnostic biliary drainage. It is subject to the same disadvantages that obtain with cholecystography but not to the same extent.

Hyperbilirubinemia: Rapid Screening Test for Its Detection is described by Charles H. Wabnitz[2] (Veterans' Admin. Hosp., Louisville, Ky.). The test, which can be done on

(1) Ann. Int. Med. 35:812-819, October, 1951.
(2) J. Lab. & Clin. Med. 37:477-480, March, 1951.

drop of serum in one minute, gives negative results when serum bilirubin concentration is less than 1.2 mg./100 ml.

METHOD.—Fouchet's reagent is prepared by diluting 10 ml. of a 10 per cent aqueous solution of ferric chloride to 100 ml. with 25 per cent trichloracetic acid in a volumetric flask. After thorough mixing the reagent is filtered through Whatman no. 42 filter paper. When kept in a dark brown bottle at room temperature, the reagent is stable for at least six months. It may be dispensed from a dropper bottle.

Sheets of Schleicher and Schuell no. 470 analytical filter paper are cut into pieces 50 × 36 mm. and immersed completely in 35 per cent aqueous solution of barium chloride in a 100 ml. beaker at room temperature for five minutes. They are then removed separately with forceps, the excess solution is allowed to drain off and papers are individually laid horizontally across two pieces of glass tubing placed parallel about 1 in. apart. After five minutes in this position the papers are turned over, again placed across the tubing and allowed to air-dry at room temperature overnight. Strips, 50 × 6 mm., cut from the dried, impregnated papers, are ready for immediate use. They are stable indefinitely if kept in an airtight container.

For the test, 1 drop (about 0.05 ml.) of serum is placed on the center of a piece of barium-chloride-impregnated paper. After 30 seconds, 1 drop of the reagent is placed directly on the drop of serum adsorbed on the center of the paper strip. Thirty seconds later the test is read for color. A 60 watt, frosted electric light bulb in a reading lamp placed about 12 in. directly above the paper strip provides satisfactory illumination. A positive reaction is indicated by blue-green color on the strip in the area where serum and reagent were added. Even the faintest appearance of blue-green color should be read as positive. This color fades out after several minutes except when high bilirubin concentration is encountered. The test should be performed as soon as possible after the blood is drawn, preferably within two hours.

Of the commonly used drugs tested, only the salicylates may yield false-positive colors. Pink, red or brown may indicate presence of certain other drugs. No false positive paper strip tests were encountered during a two year study comprising more than 700 tests.

Simple Bedside Test for Liver Function. C. R. Woolf[3] (Univ. of Cape Town) utilizes a test described originally by Mallen *et al.*

PROCEDURE.—The only reagent is strong Lugol's solution prepared by mixing 20 Gm. iodine with 40 Gm. potassium iodide in a mortar and dissolving in distilled water to make 300 ml. The test is done by mixing 1 drop of the patient's serum with 1 drop of iodine solution on a glass slide. The result is read usually within one to

(3) South African M. J. 25:789-792, Nov. 3, 1951.

two minutes. In a negative reaction the mixture remains transparent, the serum shows only a change of color owing to its mixture with the iodine solution. In a very strongly positive reaction the serum immediately forms a heavy amorphous dark brown to black precipitate. In a strongly positive reaction the serum shows a heavy granular precipitate. A moderately positive reaction is characterized by a definitely granular precipitate, lighter than the preceding one. In a weak positive a very fine precipitate occurs. If there is any difficulty in distinguishing a weak positive from a negative, the test may be compared with a mixture of a drop of iodine solution and a drop of water.

Mallen showed that the test depends on a precipitate of globulins. Many of the tests used to determine liver function depend on the presence of abnormal serum proteins either quantitatively or qualitatively. In this sense Mallen's test is similar to tests of colloidal gold, thymol turbidity, thymol flocculation and the albumin-globulin ratio.

In a series of 235 tests reported by Woolf, analysis indicates the unreliability of the usual liver function tests and suggests that the iodine test is the most dependable. If the iodine test is negative and one of the other tests disagrees, the iodine test is the more likely to be acceptable.

Clinical Determination of Protein-Bound Iodine. S. B. Barker, M. J. Humphrey and M. H. Soley[4] (State Univ. of Iowa) emphasize that extreme care is required in the quantitative measurement of 0.05 μg. protein-bound iodine/cc. plasma and in avoiding contamination. Several investigators have noted that this test is more reliable than determination of basal metabolic rate. Normal range is 3.5-8 μg./100 cc.

Solutions.—All solutions should be made with double-distilled water. If there is still too much iodine in the water to give a satisfactory blank, distillation should be carried out with alkali and permanganate. All reagents should be of the highest purity obtainable. The iodine content of each must be judged from the blank values rather than from estimates shown on the labels. (1) Zinc sulfate solution is prepared by dissolving 100 Gm. $ZnSO_4 \cdot 7H_2O$ in 1 L. water. (2) Sodium hydroxide solution, 0.5 N, is prepared by adding 20 Gm. NaOH to 1 L. water. When 10 cc. $ZnSO_4$ solution is diluted with 50-70 cc. water and titrated with NaOH solution, 10.8-11.2 cc. NaOH should be required to produce a faint pink color with phenolphthalein. (3) Hydrochloric acid, 2 N, is used. (4) Sulfuric acid, 7 N, is used. (5) Iodine standards are prepared by dissolving 118.1 mg. NaI in water and making to 1 L. This concentrated stock solution contains 100.0 μg. I/cc. A more dilute stock is obtained by diluting 2 cc. of this solution to 1 L. To prepare

(4) J. Clin. Invest. 30:55-62, January, 1951.

the standard solution used in each determination, 10 cc. of the dilute stock is further diluted to 50 cc. All of these solutions keep indefinitely at refrigerator temperatures, but it is preferable to make up the most dilute standard fresh every week or two. (6) Ceric ammonium sulfate solution, 0.02 N, is made by dissolving 12.65 Gm. in 500 cc. water plus 230 cc. of 7 N H_2SO_4. When the solution is clear, it is made to 1 L. with water. (7) Sodium arsenite solution, 0.1 N, is prepared by dissolving 4.95 Gm. As_2O_3 in 25 cc. of 4 per cent NaOH. Solution is hastened by warming. This is diluted with about 300 cc. water, and dilute H_2SO_4 is added until the solution is

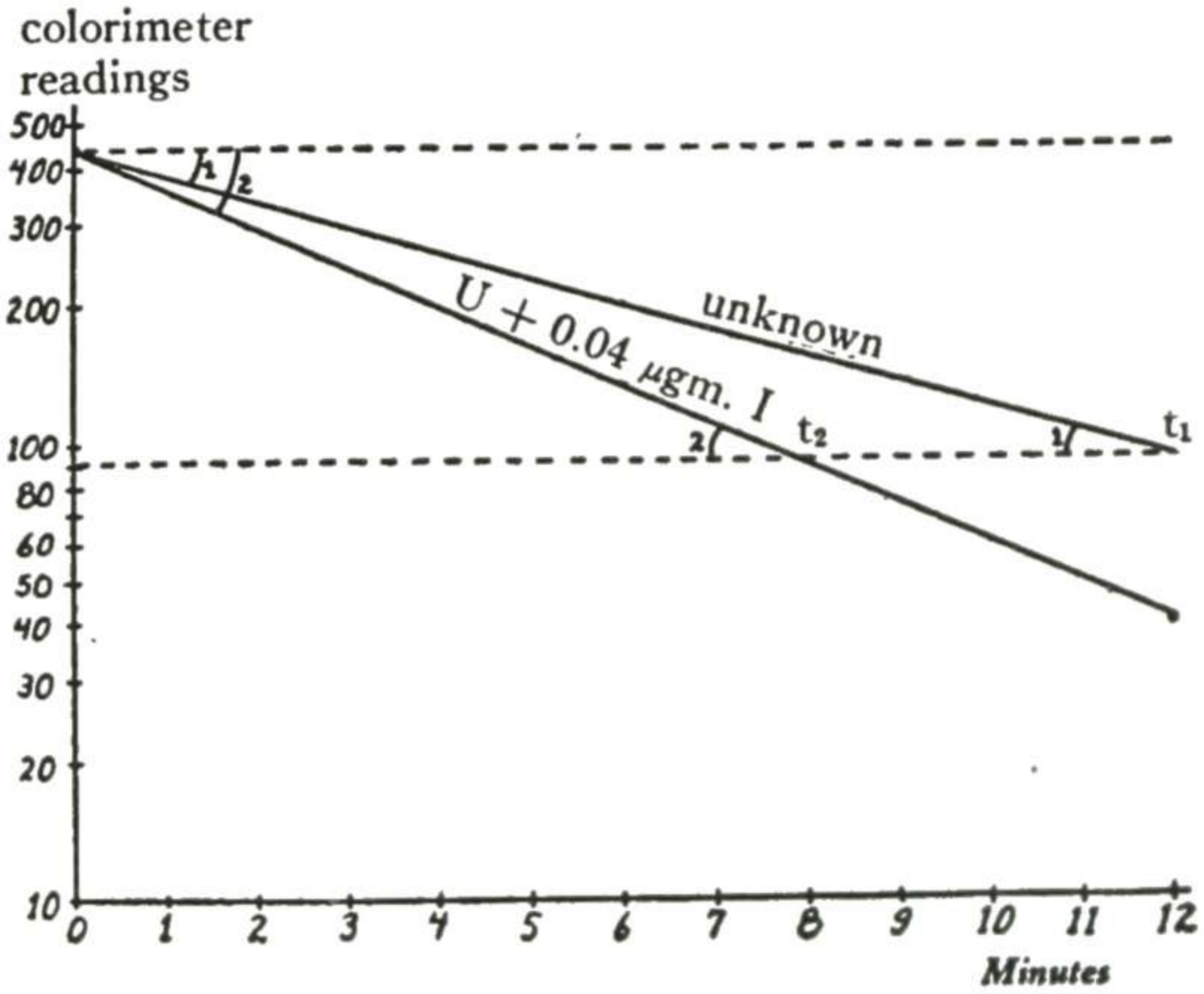

Fig. 107.—Method of plotting results for calculation of protein-bound iodine. (Courtesy of Barker, S. B., *et al.*: J. Clin. Invest. 30:55-62, January, 1951.)

slightly acid to litmus paper. It is then made to 1 L. If sodium arsenite itself is used, 6.50 Gm. is dissolved in distilled water and diluted to 1.0 L. No acid or alkali is required with this salt.

PROCEDURE.—About 10 cc. blood is centrifuged and the plasma or serum withdrawn. Duplicate aliquots of 1 cc. are pipetted into 15 × 125 mm. Pyrex tubes and diluted with 7 cc. distilled water; 1 cc. of 10 per cent $ZnSO_4$ solution is added. The contents are mixed with a glass stirring rod and 1 cc. of 0.5 N NaOH added. The solutions are thoroughly mixed. Any material adhering to the rod is removed by rubbing it with a rotary movement against the inside wall of the tube. The tube and contents are centrifuged for 10 minutes and the supernatant fluid poured off; 10 cc. distilled water is added. The protein is resuspended with the same stirring rod, with care to avoid dividing the precipitate so finely that it cannot easily be centrifuged down. Centrifugation is again carried out and the supernatant fluid discarded. This is repeated two more times. Then 0.8 cc. of 4.0 N Na_2CO_3 is added and thoroughly stirred into the precipitate with the same stirring rod. To remove material clinging to the rod the procedure described previously is carried out and fol-

lowed by adding 0.2 cc. of 4.0 N Na_2CO_3 dropwise down the rod. The tube is then placed in an oven set at 85-95 C. and left in for 12-18 hours to drive off water. After thorough drying, ashing is carried out by placing the tube in a muffle furnace for 2½ hours at 600 ± 25 C. The tube is then removed and allowed to cool to room temperature. With caution, 2 cc. HCl is added to avoid excessive effervescence. The unreacted ash is mixed with the acid and 2 cc. of 7 N H_2SO_4 and 3 cc. water are added. The contents of the tube are stirred until the reaction is finished, transferred to a clean tube and briefly centrifuged to pack the insoluble material.

For each tube a pair of colorimeter tubes is taken. To one is added 1.0 cc. distilled water which contains 0.04 μg. I and to the other 1.0 cc. water. A 3.0 cc. aliquot of the supernatant fluid in the sample tube is pipetted into each colorimeter tube and 0.5 cc. As_2O_3 solution added to each. The contents should be mixed by some technic which avoids loss of solution and contamination. The colorimeter tubes are then placed in a well stirred constant temperature bath maintained at 39 ± 0.1 C. for 10 minutes to come to temperature. The ceric ammonium sulfate solution should also be warmed in the bath. The actual determination of iodide is accomplished by measuring iodide catalysis of the rate of decolorization of yellow ceric ammonium sulfate by arsenous acid. With great accuracy, 1.0 cc. ceric solution is added to each tube with adequate time allowance for reading the color change in the previous tube. Mixing must be rapid and thorough.

Klett-Summerson readings (blue filter no. 42 is used) are plotted on semilog paper against the time in minutes. The colorimeter setting at zero time is obtained for any given batch of reagents by quickly reading a reagent blank immediately after addition of ceric sulfate. The lines describing the two reaction rates are drawn using the points for 0, 6 and 12 minutes as shown in Figure 107, and then the horizontal line indicating times required to reach equal amounts of decolorization. The amount of iodide in the colorimeter tube is calculated from the following equation, obtained from similar triangles:

$$\mu g.\ I \text{ in colorimeter tube} = \frac{0.04 \times t_2}{t_1 - t_2}$$

where t_1 is time in minutes at intercept of the rate of decolorization in tube containing no extra iodide with the designated horizontal and t_2 is time in minutes at intercept of the rate of decolorization in tube containing added iodide with the designated horizontal. The variable amounts of iodide in the reagents used must be determined by blank analyses ($ZnSO_4$ solution first, NaOH second, etc.). The blank value calculated by the same formula shown previously has been 0.015-0.017 μg. I/tube.

To express the values in terms of μg./100 cc. plasma, the complete calculation is:

$$\text{plasma PBI } \mu g.\ I/100 \text{ cc.} = 100 \times \frac{7}{3} \text{ (I in final aliquot — blank I)}$$

If so much iodide is present in the sample that decolorization is

nearly complete in the second tube with 0.04 μg. added I, the determination may be repeated using less than the 1 cc. plasma usually called for and adjusting computation as necessary.

Scrupulous care must be exercised to ensure clean glassware. If elemental iodine is being used in any chemical determination or histologic procedure in the same laboratory, irregular and unpredictabel contamination is almost inevitable. A patient who has been administered any form of organic iodide may have elevated protein-bound iodine values as long as six months to three years later in the absence of any genuine metabolic disorder.

Plasma Protein-Bound Iodine Determinations. William N. Harsha[5] (Oak Ridge, Tenn.) describes a technic which is reproducible with great accuracy by unskilled technicians and seems suitable as a routine clinical laboratory procedure. The reaction is quantitative at 0.005-0.1 μg. iodine. Plasma protein-bound iodine levels were 3-10 μg./100 ml. in 22 normal persons, 12-46 μg. in 18 thyrotoxic patients and 0.2-3 μg. in 20 hypothyroid patients.

REAGENTS.—(1) Iodine-free arsenous acid is made by dissolving 3.71 Gm. AS_2O_3 in 50 ml. iodine-free 1 N NaOH, adding 200 ml. redistilled water and neutralizing with 70 per cent (by weight) arsenic and nitrogen-free sulfuric acid solutions. Then, 54 ml. of 70 per cent acid is added in excess and the volume made up to 500 ml. To this solution is added 3.125 Gm. iodine-free NaCl. (2) Ceric sulfate solution is made by dissolving with stirring 12 Gm. ceric ammonium sulfate in 500 ml. of 3.5-N iodine-free H_2SO_4 solution. The ceric and arsenite solutions should not react appreciably when mixed for several hours.

PROCEDURE.—A heparinized fasting venous blood sample, drawn in a chemically clean syringe, is placed in a chemically clean test tube. All glassware must be chemically cleaned, preferably in iodine-free solution of KOH. Plasma is separated from whole blood by centrifugation and 10 ml. is placed in a round bottom test tube. The protein is precipitated accurately according to the Somogyi method with ZOH. The precipitate is washed twice with ZOH, the precipitate being separated each time by centrifugation. The protein precipitate is dissolved by 5 ml. of 2 per cent NaOH and the test tube, stopped with cotton, is autoclaved for 30 minutes at 20 lb. pressure at 115 C. This solution is filtered into a spectrophotometer tube, the filter paper being washed with a small amount of 2 per cent NaOH. The filtrate is neutralized to pH 7 with sulfuric acid, and 0.4 ml. arsenous acid reagent is added and the tube incubated at 37 C. until the solution is equilibrated. The ceric $(NH_4)_2SO_4$ solution is warmed to 37 C. and 0.5 ml. added to the incubated tube, which is shaken. After the tube is incubated for exactly 15 minutes the absorption at 480 mμ is read in a spectrophotometer. The test is standardized against various solutions of KI at 0.008-0.10 μg iodine. A blank determination is always made.

(5) Am. J. M. Sc. 221:626-627, June, 1951.

Determination of Serum Protein-Bound Iodine as Routine Clinical Procedure. Bernard L. Hallman, Philip K. Bondy and Mary Ann Hagewood[6] (Emory Univ.) report observations based on 2,157 determinations of protein-bound iodine (1,446 patients) made by the method of Barker and Humphrey. Until recently the only reliable method for diagnosis of thyroid disease was determination of the basal metabolic rate. The inadequacies of this method are well known. The chief disadvantage is that it is often difficult to obtain completely basal conditions. Protein-bound iodine is not affected by factors which interfere with the basal metabolic rate. In addition, blood can be drawn at any time of the day without regard to the patient's general physical condition. Furthermore, determination of protein-bound iodine does not require that the patient be present. In certain circumstances the metabolism may be unrelated to the concentration of circulating thyroid hormone. In patients with clearcut thyrotoxicosis or myxedema, the protein-bound iodine level like the basal metabolic rate is merely a confirmatory finding. If a good basal metabolism rating can be obtained, a definite relation will be found between the height of the protein-bound iodine and the basal metabolic values. In cases in which an accurate basal metabolic rate cannot be obtained, determination of protein-bound iodine may be solely responsible for establishing the diagnosis.

A serious defect in the determination of protein-bound iodine is the inability to distinguish between the iodine of thyroid hormone and that of radiopaque dyes or inorganic iodides circulating in the blood. The test therefore should not be carried out on patients who have recently received therapeutic doses of iodides or who have had x-ray studies with radiopaque dyes. Gallbladder dyes may cause abnormally high values for as long as six months, renal excretory dyes for as long as four weeks, radiopaque substance used for bronchograms for about a week and inorganic iodides for one to four months. Mercury, chloride and fluoride are without effect on the results, but large amounts of bromine may have a minimal influence on the method.

After institution of antithyroid medication, the protein-bound iodine level falls more rapidly than the basal metabolic rate, thus giving an early indication of the adequacy of the dosage used.

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(6) A.M.A. Arch. Int. Med. 87:817-824, June, 1951.

Method for Determination of Small Doses of I^{131} in Urine, utilizing the gamma scintillation counter, is described by W. E. Goodwin and W. D. Harris[7] (Veterans' Admin. Center, Los Angeles). After a tracer dose of 1 μc. I^{131} is administered, two specimens of urine (0-6 hour and 6-24 hour periods) are collected and measured. Fluid intake should be restricted to 1,000 ml. daily to limit excretion. The total volume is shaken well and 250 ml. is put into a pint sized cardboard container.

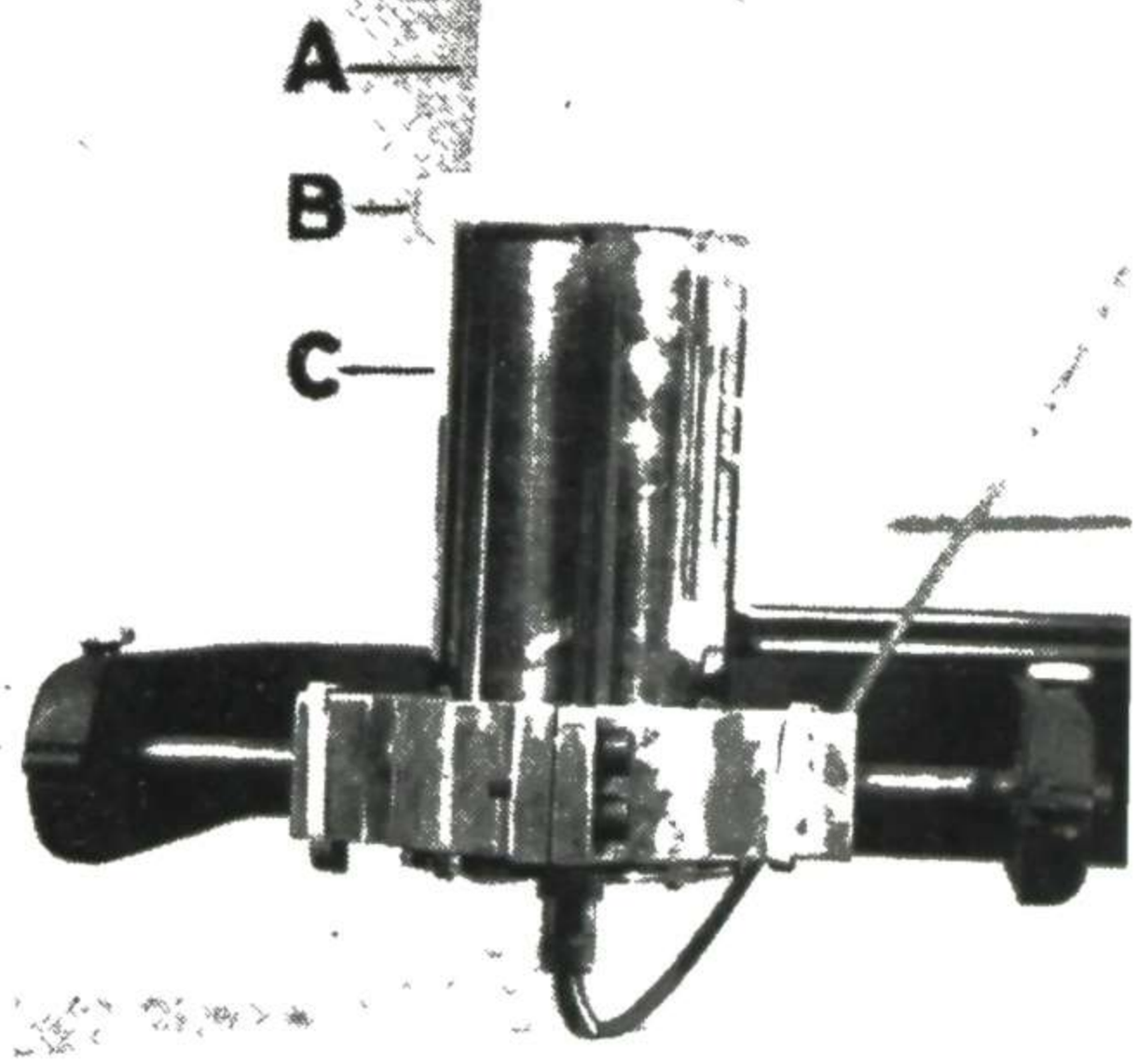


Fig. 108.—*A*, cardboard container. *B*, Lucite holder. *C*, scintillation tube. (Courtesy of Goodwin, W. E., and Harris, W. D.: J. Lab. & Clin. Med. 38:470-473, September, 1951.)

This is placed in the Lucite holder on top of the scintillation tube (Fig. 108). The number of counts of this volume is recorded. Microcuries of I^{131} in the total urine specimen equal $U/S \times V$, where U represents counts per second of the patient's urine sample (250 ml.); S, counts per second of 1 μc. I^{131} in sample, and V, volume of urine (ml.) divided by 250.

Data obtained from 36 studies on 35 euthyroid controls, 13 studies on 11 hyperthyroid patients and 15 studies on 13 hypothyroid patients were comparable to results with other "standard" methods.

Inhibition by Thiamine Hydrochloride on Retinal Responses Caused by Nicotine. Austin I. Fink[8] (State Univ. College of Medicine at New York) mapped angioscotomas before and after five subjects smoked one cigaret with inhala-

(7) J. Lab. & Clin. Med. 38:470-473, September, 1951.
(8) Am. J. Ophth. 34 (pt. 2):139-142, May, 1951.

tion. A second cigaret was smoked 20 minutes later after subcutaneous injection of 100 mg. thiamine.

Maximal effects following a cigaret alone were noted in an average of 14 minutes. Increase in scotoma units averaged 28.

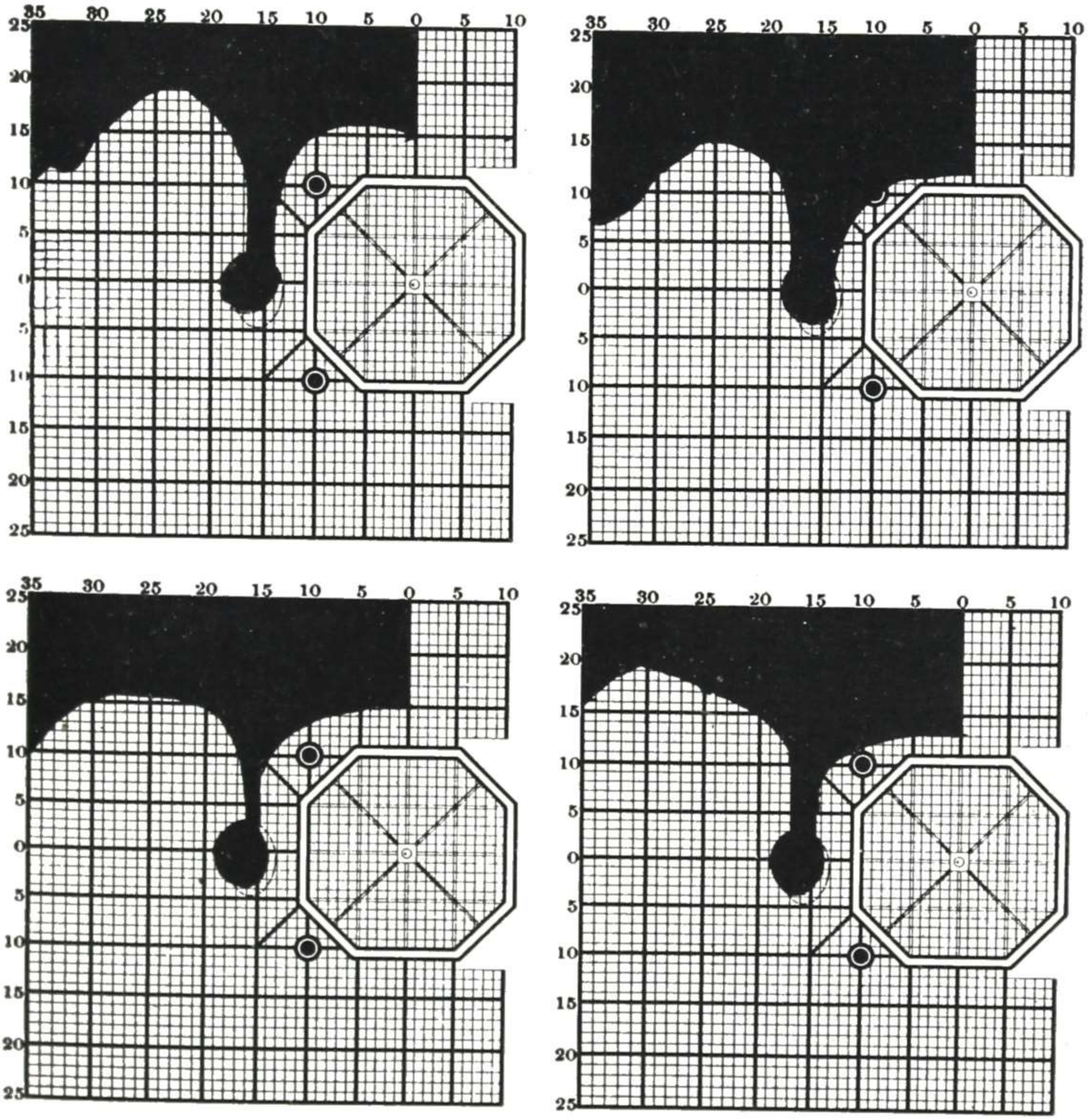


Fig. 109 (top).—Effect of tobacco without thiamine. Increase from 140 to 188 scotoma units (25.5 per cent.)

Fig. 110 (bottom).—Effect of tobacco with thiamine. Decrease from 150 to 143 scotoma units (4.6 per cent).

(Courtesy of Fink, A. I.: Am. J. Ophth. 34 (pt. 2):139-142, May, 1951.)

The effect was lost in an average of 59 minutes (Fig. 109). With thiamine plus cigaret, maximal effects were noted in an average of 18 minutes; increase in scotoma units averaged 2, and the effect was lost in 47 minutes (Fig. 110). In each subject, alteration of angioscotoma was definitely decreased when smoking of a cigaret was preceded by injection of thiamine.

Inhibition by thiamine on the nicotine molecule has been

attributed to competitive antagonism between structurally similar molecular rings. Thiamine acts as a moderator of overstimulated sympathetic synapses, the point at which stimulation by nicotine takes place.

Use of Infra-red Radiation in Colorimetry: Rapid Estimation of Alcohol in Body Fluids Using Test Tube Still is described by Frederick R. Weedon, Jack H. Gustafson and Jean D. Rolfe[9] (Jamestown, N. Y.).

METHOD.—The reaction assembly (Fig. 111) is prepared by pipet-

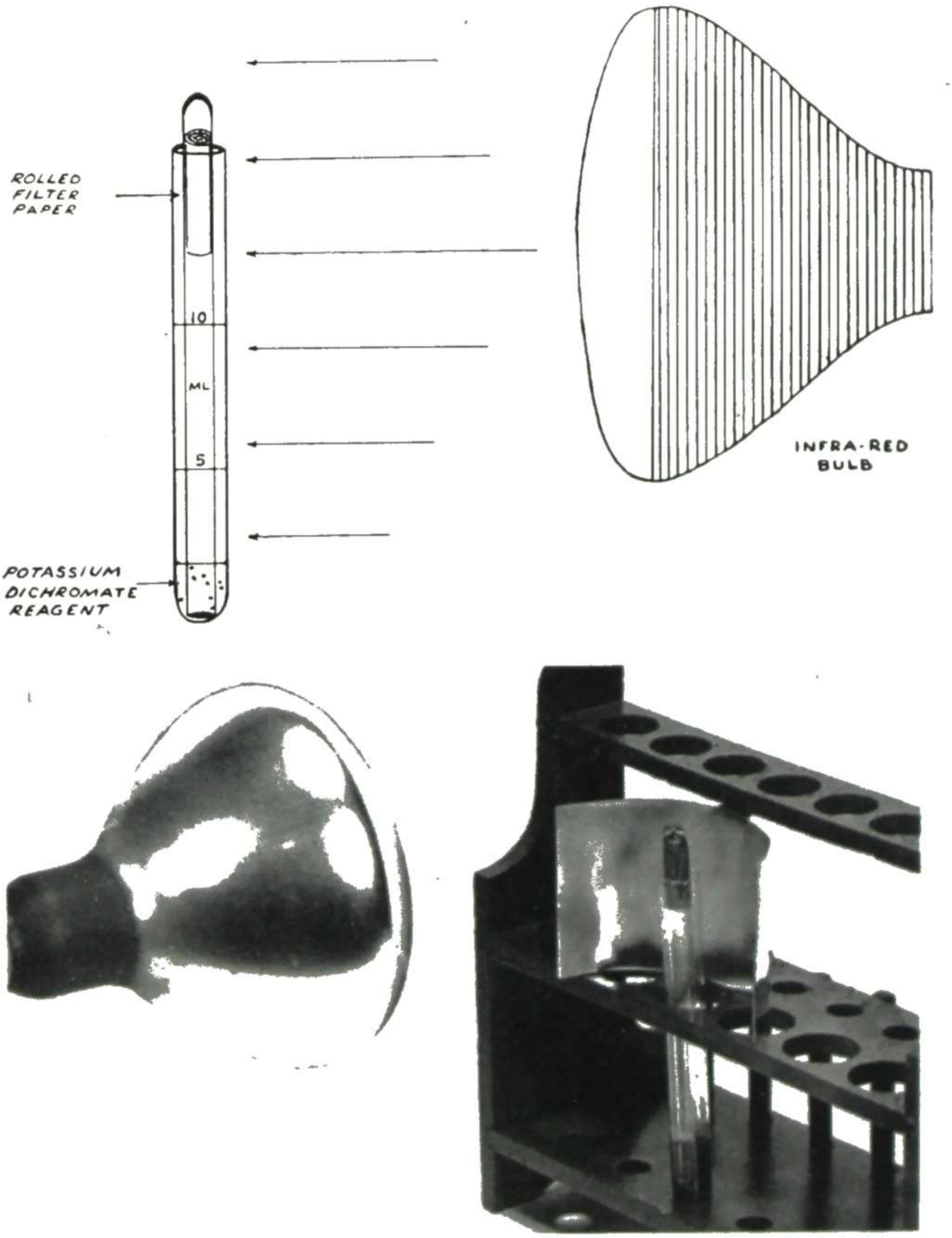


Fig. 111.—Test assembly. (Courtesy of Weedon, F. R., *et al.*: New York J. Med. 51:2744-2745, Dec. 1, 1951.)

(9) New York J. Med. 51:2744-2745, Dec. 1, 1951.

ting a 0.2 ml. sample into a 13 cm. length of 8 mm. bore glass tubing which has been closed by fusing at one end. A 10 × 2.5 cm. piece of tightly rolled filter paper is inserted to within 4 mm. of the bottom of the tube so as to absorb the sample. The tubing is then placed open end down in a colorimeter tube containing 1 ml. standard 0.6393 per cent potassium dichromate reagent in 50 per cent by volume H_2SO_4. The assembly is then placed in a test tube rack and the infra-red bulb adjusted so that it is exactly 11.5 cm. from the glass tube. The tube is heated for 10 minutes, after which it is turned 180 degrees and heated for an additional 10 minutes. The assembly is allowed to cool for five minutes. The inner tube is removed and washed with distilled water, with care that the water does not reach the roll of filter paper. The partially reduced potassium dichromate solution and washings are diluted to 10 ml. with distilled water. The colorimeter tube is stoppered with a paraffin-treated cork and the contents mixed by inversion. The Klett-Summerson photoelectric colorimeter is set at 230; no. 42 blue filter (400-465 mμ) and a reagent blank containing 1 ml. potassium dichromate are used. The tube is placed in the colorimeter and readings are taken. Percentage of alcohol is estimated by referring to a standard curve which has been prepared by using known amounts of alcohol in distilled water.

Specificity of Desiccation Method for Determining Alcohol in Biologic Fluids. According to H. Ward Smith[1] (Univ. of Toronto) the simplicity, reliability and specificity of this method makes it suitable for routine medicolegal work.

Apparatus.—Fifty ml. Erlenmeyer flasks are used. A glass cup (1.2 ml. capacity) and stem are sealed to the solid glass stopper, which is held in place with a rubber band (Fig. 112). An all glass automatic microburet equipped with reservoir is necessary for storage and delivery of the oxidizing solution. It should be fitted with a capillary tip to control rate of delivery of the oxidizing solution (1 ml. in 20 seconds).

Reagents.—(1) Distilled water is redistilled after addition of 1 Gm. NaOH and 0.5 Gm. $KMnO_4$/L. (2) Oxidizing solution is prepared by adding 400 ml. of 24 N H_2SO_4 to 200 ml. redistilled water containing 0.9 Gm. potassium dichromate. This solution is heated for one hour at 80 C. and then cooled. After acid concentration is adjusted to 15 N, the solution is stored in the automatic buret. (3) Ferrous ammonium sulfate is prepared by mixing 2.5 Gm. $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ and 50 ml. concentrated H_2SO_4/L. distilled water. The exact alcohol equivalent of this solution is determined by analyses of known concentrations of alcohol. (4) Orthophenanthroline ferrous complex B. D. H. is prepared by diluting 1 ml. to 50 ml. with distilled water. (5) Diphenylcarbazide B. D. H. is prepared by diluting 0.5 ml. to 50 ml. with freshly prepared 95 per cent ethanol.

Procedure.—All glassware must be cleaned immediately before use in concentrated chromate cleaning solution. It should be rinsed

(1) J. Lab. & Clin. Med. 38:762-766, November, 1951.

thoroughly with distilled water and finally with redistilled water. Pipets may be suction dried.

Three ml. oxidizing solution is added to each flask, a half sheet of 7 cm. filter paper (no. 42 Whatman trimmed to 2 cm. width) is rolled around the stem of the cup and 0.5 ml. solution to be analyzed (redistilled water is used as a blank) is pipetted into the cup. The filter paper is then slipped down into the cup (it is held in place by the side of the cup and provides a large evaporating surface for the sample). The stopper and cup are quickly placed in the flask and held in place with a rubber band. The flasks and contents are heated in an oven at 60 C. for 90 minutes for an 0.5 ml. sample or 150 minutes for a 1.0 ml. sample. Alternatively, the flask may be left over-night (12-16 hours) at room temperature (20-24 C.).

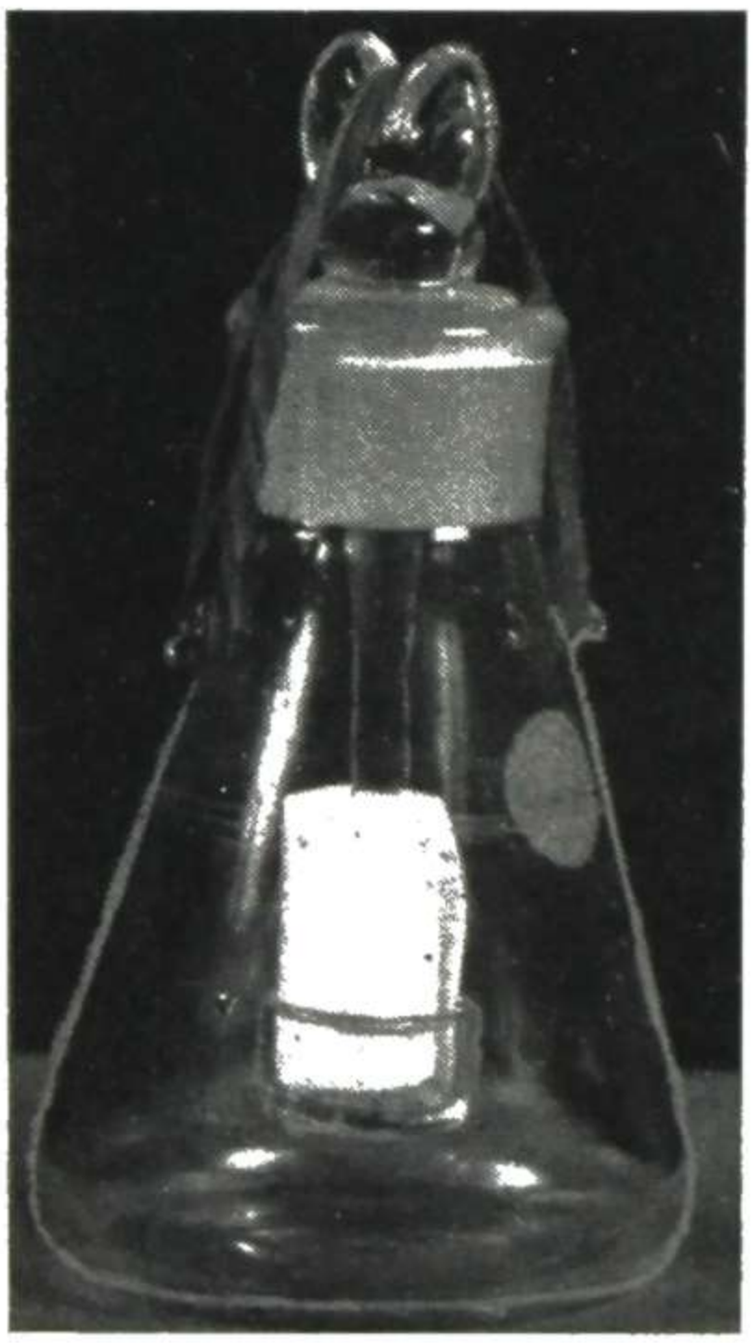


Fig. 112.—Desiccation flask with filter paper in place. (Courtesy of Smith, H. W.: J. Lab. & Clin. Med. 38:762-766, November, 1951.)

The flasks are then chilled for 10 minutes in a refrigerator, stopper and sample cup are removed and 20 ml. redistilled water is added. The dichromate remaining in the flask can either be determined by titration or colorimetrically.

For titration, standardized ferrous ammonium sulfate is added to the flask until a faint blue color appears; 5 drops of orthophenanthroline is added and the titration continued until a pink color becomes permanent. Alcohol concentration equals the number of milliliters to titrate the blank minus the number of milliliters to titrate the sample multiplied by the alcohol equivalent per milliliter of ferrous ammonium sulfate solution used.

For colorimetric determination, contents of the flask are diluted to 50 ml. with distilled water; 2 ml. is pipetted (pipets are rinsed several times with the solution to be measured) into a 100 ml. volumetric flask containing 0.4 ml. of 24 N H_2SO_4 and about 50 ml. distilled water. Then 1 ml. diphenylcarbazide solution is added and contents are diluted to 100 ml. and mixed. About 10 ml. this solution is placed in matched colorimeter tubes and read in the Evelyn colorimeter (filter 540 is used). The galvanometer is set to 100; a solution containing 0.4 ml. of 24 N H_2SO_4 and 1 ml. diphenylcarbazide in 100 ml. water is used. Concentration of alcohol in the sample (parts/100 for 0.5 ml. sample) equals 0.234 ("L" blank minus "L" sample) where "L" equals 2—log galvanometer reading.

Method for Detecting Cortisone in Body Fluids is described by C. L. Cope[2] (Postgraduate Med. School of Lon-

(2) Brit. M. J. 1:271-272, Feb. 10, 1951.

don). Preliminary results showed activity in normal urine extracts and increased excretion in late pregnancy, during administration of ACTH and in adrenal cortical carcinoma. Such activity was absent in most cases of Addison's disease and in three of hypopituitarism.

METHOD.—A 24 or 48 hour collection of urine, without preservative added, is acidified with concentrated H_2SO_4 to the extreme range of thymol blue. After standing at room temperature overnight

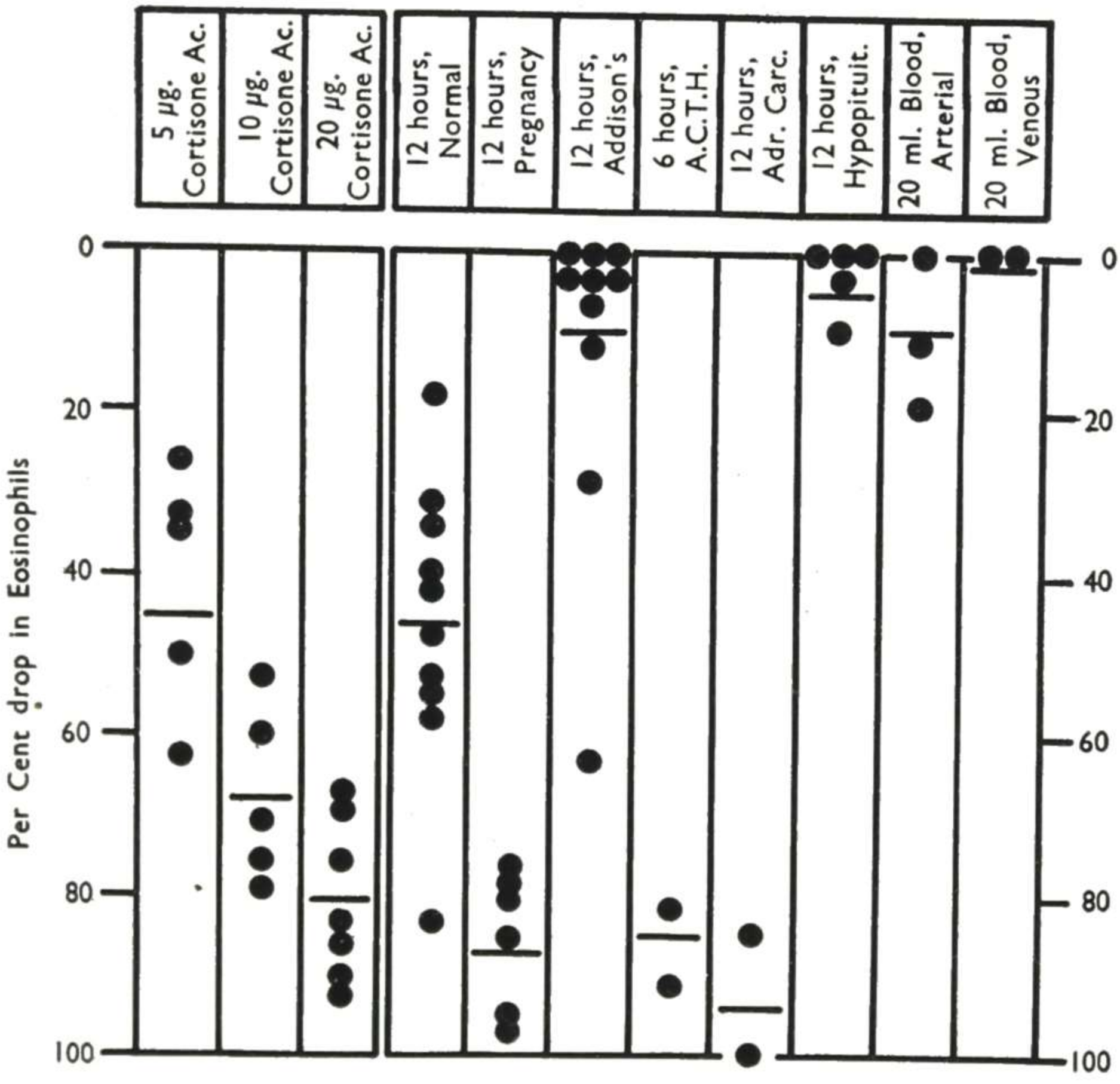


Fig. 113.—Eosinophil drop in mouse after injection of cortisone, urine extracts and blood extracts. (Courtesy of Cope, C. L.: Brit. M. J. 1:271-272, Feb. 10, 1951.)

it is extracted by shaking in separating funnels with four successive quantities of chloroform, each being equal to 15 per cent of the urine being extracted. The resultant chloroform emulsions are centrifuged clear of aqueous phase in a large centrifuge. The clear chloroform phase is evaporated under reduced pressure to about 30 ml., temperature of the water bath being kept below 50 C. throughout the distillation. The concentrated chloroform extract is transferred quantitatively to a small separating funnel and washed three times with N/soda (6 ml.) and then three times with water (6 ml.), each washing being back-extracted with chloroform which is added to the extract. The washed chloroform solution is evaporated to dryness under reduced pressure at a temperature below 50 C. Then

0.2 ml. propylene glycol is added. The tube is gently warmed to about 45 C. and rotated slowly until the extract is fully dissolved. Normal saline solution, 0.8 ml., is added and the tube shaken to give a homogeneous mixture.

Bilaterally adrenalectomized mice maintained on 1 per cent saline solution and full diet for at least four days are used for the test. The same animals may be used for several tests on different days. Blood is drawn from the tail vein to the 0.5 mark on a white cell pipet. It is diluted to the top mark with Randolph's diluting fluid and eosinophils are counted in duplicate in the Fuchs-Rosenthal chamber. Randolph's diluting fluid consists of 0.1 per cent phloxine and 0.1 per cent methylene blue, each dissolved separately in 50 per cent propylene glycol. The two solutions are mixed in the proportion of 2 parts of phloxine solution to 1 of methylene blue immediately before use. The count is performed after about 15 minutes are allowed for the cells to stain. The equivalent of 12 hours' urine extract in 20 per cent propylene glycol is injected subcutaneously and the animals returned to their cages. Volume of the injection should not exceed 0.5 ml. Further eosinophil counts are done four and six hours after injection.

The significant response is the maximal drop in eosinophils (Fig. 113), expressed as a percentage of the initial value. The drop may be greatest at either four or six hours. In the adrenalectomized mouse the tendency of cortisone-like substances to lower the eosinophil count is apparently a much more potent stimulus than the tendency of minor trauma or other factors to produce a rise.

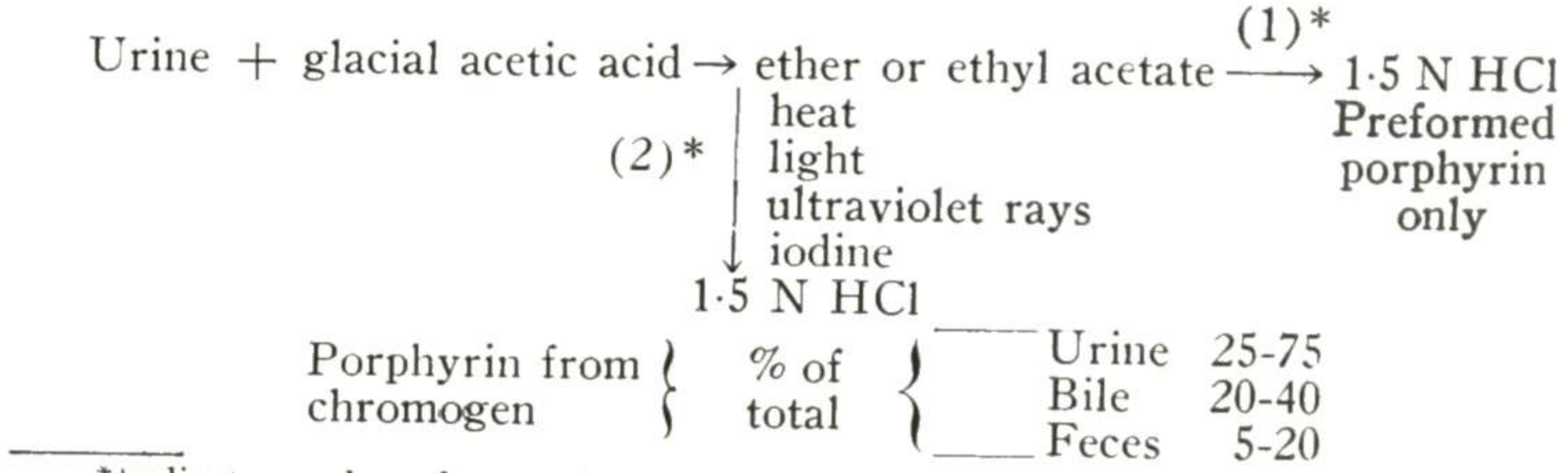
Some Recent Studies of Porphyrin Metabolism and Porphyria are reported by C. J. Watson[3] (Univ. of Minnesota). Earlier studies of prophyrins confused artificial hematoporphyrins with those that occurred in nature because of the relatively crude spectroscopes available. The slight differences in absorption spectrums, especially between hemato- and coproporphyrin, could not be distinguished. Crystallization and physical criteria helped to clarify this confusion. Coproporphyrin, first found in feces, is ether soluble, whereas uroporphyrin, first isolated in urine, is insoluble in ether. The most widely represented porphyrin, that of the hemoglobin molecule and of other iron porphyrin enzymes, protoporphyrin, is ether soluble but is easily separated from coproporphyrin by being extracted from ether only by more concentrated HCl, by exhibiting an insoluble sodium salt and by chloroform solubility.

(3) Lancet 1:539-542, Mar. 10, 1951.

CLASSIFICATION AND MAIN FEATURES OF PORPHYRIA

Type and incidence	Clinical	Chemical
1. PHOTOSENSITIVE (" congenital ") Rare	Somewhat more common in males Onset early in life; either prenatal or postnatal Hydroa æstivale ; eventual scarring and mutilation of exposed skin ; erythrodontia Splenomegaly and hæmolytic anæmia not infrequently ; splenectomy may relieve and cause porphyria to become latent, with disappearance of photosensitivity	Absence of Ehrlich-reacting porphobilinogen in urine Presence of large amounts of uroporphyrin and coproporphyrin I, both excreted in part as colourless, Ehrlich-negative chromogens (in urine urocopro-; in fæces vice versa) Slight or negative zinc complex formation by these porphyrins Uroporphyrin I methyl ester M.P. 284°C
2. INTERMITTENT ACUTE (" acute " or " toxic ") Relatively common	Somewhat more common in females Abdominal pain of various types Peripheral neuropathy with pain in extremities Varying neuropsychiatric disturbances : weakness, paralyses, hysterical behaviour or outspoken psychoses Pigmentation of skin in some cases	Porphobilinogen usually present in urine at least during attacks (Ehrlich, $CHCl_3$-insoluble aldehyde) Non-Ehrlich-reacting chromogen → (−H_2) Waldenström uro-type porphyrin (zinc complex) ; methyl ester M.P. 258–260°C Porphobilinogen (colourless) → (?) Porphobilin (dark brown), non-porphyrin, probable dipyrryl-methene, or polymer thereof ; coproporphyrin III usually excessive
3. MIXED (" chronic ") Relatively uncommon	Differing sex-incidence not clearly defined ; probably somewhat more common in males Late appearance of photosensitivity (so-called " cutanea tardive " type). Relatively benign course Liver disease or functional impairment frequent Attacks of abdominal pain ; neuropsychiatric disturbances infrequent	Porphobilinogen inconstant. Varying appearance of Waldenström uro-type porphyrin or uroporphyrin I, with various mixtures of coproporphyrins I and III

The relation of coproporphyrin precursor to porphyrin from chromogen is as follows:



*Indicates order of procedures.

Classification of porphyria, an inborn error of metabolism, is shown in the table.

Method for Determination of Gentisic Acid in Serum. P. S. Gerald and B. M. Kagan[4] (Michael Reese Hosp.) describe a method of determining the gentisic acid content of serum with a standard deviation of 0.62 mg./100 ml. for concentrations in the range of 1-15 mg./100 ml. serum.

METHOD.—Iron reagent is prepared by adding 50 Gm. $FeCl_2 \cdot 4H_2O$ (Merck) and 20 ml. $FeCl_3 \cdot 6H_2O$ (1 Gm./100 ml. of 0.01 N HCl) to a liter volumetric flask and diluting quantitatively to 1 L. with 0.01 N HCl. If the mixture is turbid, it should be filtered.

To precipitate the proteins, 1 ml. serum and 8 ml. distilled water are mixed; 0.5 ml. of 2/3 N H_2SO_4 is added and mixed, and 0.5 ml. sodium tungstate (9.75 Gm./100 ml.) is then added. Thorough mixing is carried out. This is important if a clear supernatant is to result. After mixing or at any time up to 15 minutes later, the solution is centrifuged for about 15 minutes at 3,000 rpm. Five ml. of iron reagent is added to 5 ml. of the clear centrifugate in a cuvet. After these substances are mixed by inversion, the color is read within one minute at 595 mμ against a blank of 5 ml. distilled water plus 5 ml. iron reagent.

To eliminate the error due to incomplete recovery, the instrument is standardized by adding increments of a known solution of gentisic acid to normal serum and developing the color as described. The graph made by plotting optical density against the concentration of gentisic acid yields a straight line. Since the iron reagent is only moderately stable, it must be standardized within 24 hours of the time of using. The method is not applicable to the serum of patients receiving salicylates since these drugs give a color reaction with $FeCl_3$.

Flame Spectrophotometry: Sodium and Potassium in Blood and Urine. B. Frankenberg, V. Hospadaruk and A. H. Neufeld[5] (Queen Mary Veterans' Hosp., Montreal) detail the

(4) J. Biol. Chem. 189:467-472, April, 1951.
(5) Canad. M. A. J. 65:388-389, October, 1951.

procedures established for estimating sodium and potassium contents of blood and urine.

METHOD.—For stock solutions the amounts per 100 cc. are: 1.0166 Gm. NaCl (400 mg. per cent K), 0.2861 Gm. KCl (150 mg. per cent K) and 0.341 Gm. $(NH_4)_2HPO_4$ (80.0 mg. per cent P). For working solutions the dilutions are: NaCl, 10:100; KCl, 2:100; $(NH_4)_2HPO_4$, 5:100; 27.4 mg. $CaCO_4$/100 cc.; 70.1 mg. $MgCl_26H_2O$/100 cc., and 2.0 Gm. urea/100 cc. From these solutions, working standards are developed. A single series of working standards is used for sodium assay in both serum and urine. Potassium assay requires three series of standards for serum and two for urine. Serum is diluted 1:50 for potassium and 1:100 for sodium estimation. If cloudy, the serum must be centrifuged and, if necessary, also filtered. Urine is diluted 0.1:50 for potassium and 1:100 for sodium estimation. These dilutions are approximate since urine concentrations vary considerably. Therefore, preliminary readings must be taken and correct dilutions deduced from the initial results. If the urine is turbid, it should be well mixed and an aliquot removed and gently heated until all crystals are dissolved. Other liquids can be analyzed similarly. However, if the composition differs conspicuously from that of serum, special standards must be made, approximating the composition of the unknown.

The authors indicate in detail the procedure applicable to the DU Beckman spectrophotometer with a standard flame attachment utilizing propane, oxygen and air pressures. However, with slight modifications, it is equally applicable to other types of instruments.

Preliminary readings of the unknowns are taken, to determine which standards are suitable for comparison. Sodium readings are always taken first, since the amount of sodium in the sample later determines which of the three series of potassium standards should be used for potassium analysis.

Paper Chromatography of Blood Plasmas in Multiple Sclerosis. Roy L. Swank, A. E. Franklin and J. H. Quastel[6] (McGill Univ.) state that of 81 chromatograms from 26 patients, 31 tests on 17 patients showed distinct abnormalities. In some, abnormalities took the form of absence from the protein pattern of a large number of central fractions and of some upper fractions.

Significance of changes observed in plasma proteins of patients with multiple sclerosis is not known. Abnormalities in the plasma protein chromatograms do not seem specific for multiple sclerosis, since patterns have been abnormal in multiple myeloma and liver cirrhosis. Although it has been

(6) Proc. Soc. Exper. Biol. & Med. 76:183-189, January, 1951.

shown that sedimentation rates of patients with multiple sclerosis are often abnormally high there is no clear correlation between such changes and the abnormal chromatograms of these patients.

Correlation of these changes to activity of the disease is not possible. Apparently alterations may be concomitant to or immediately follow onset of exacerbation. Changes in protein patterns usually revert to normal during remissions.

Photochromatographic Analysis of Amino Acids in Body Fluids: Method and Some Applications are described by Anthony A. Albanese, Reginald A. Higgons, Betty Vestal and Loukia Stephanson[7] (New York City). The procedures are based on the ascending principle. For most purposes the one-dimensional technic provides a rapid means of obtaining satisfactory results. When identification of unusual components is being attempted, the two-dimensional procedure should be used.

PROCEDURE.—*One-Dimensional Technic.*—The components of the apparatus are shown in Figure 114. The outer structure is a rectangular museum jar (*A*) made airtight by laying a piece of wax paper across the top of the jar. The ground surface of the edge of the cover is liberally coated with melted paraffin; just before this solidifies, the cover is pressed firmly into place on top of the jar. After the paraffin has solidified, the paper and cover are both lifted from the jar and the paper is gently peeled from the paraffin to leave an accurately fitting impression in the paraffin corresponding to the top of the jar. The rack which supports the paper strips is constructed of strips of pine 1× ¾ in. joined by Duco cement (*A*). Wire brads nailed through the wooden frame form the suspension points. The frame is supported at the required distance from the bottom of the jar by 7 mm. Pyrex glass rods which pass through tightly fitting holes in the end sections of the wooden frame. Two pony glasses, placed diagonally opposite at the bottom of the jar, complete the apparatus. The strips are dried without removal from the rack in the drying pan, constructed as shown in *C*. The sampling table is assembled from sections of ¼ in. plate glass (*B*). The samples are measured with Sahli-type hemoglobinometer pipets of varying capacity. The strips of paper, 1.8 ×29 cm., are cut from squares of filter paper (Carl Schleicher and Schuell Co. no. 507).

Pure phenol, saturated with water, provides the most convenient and reliable partitioning agent for the amino acids. To 1 lb. phenol crystals, Merck reagent grade, is added 500 ml. water and the mixture is allowed to stand about a week with intermittent shaking before separation. The phenol-water and water-phenol fractions are collected in separate glass-stoppered bottles. The bottom of the museum jar is then covered with a layer, about 1 cm. deep, of the

(7) J. Lab. & Clin. Med. 37:885-893, June, 1951.

phenol-water fraction. The two pony glasses, filled within 1 cm. of the top with water-phenol fraction, are set at opposite corners of the bottom of the jar. Before use, the partitioning chamber should be allowed to come to phase equilibrium, which requires 48-72 hours. A well sealed chamber can be used continuously, with occasional replenishing of solutions, for at least 12 months. In setting up an analysis, identifying notations are printed clearly within 2 cm. of

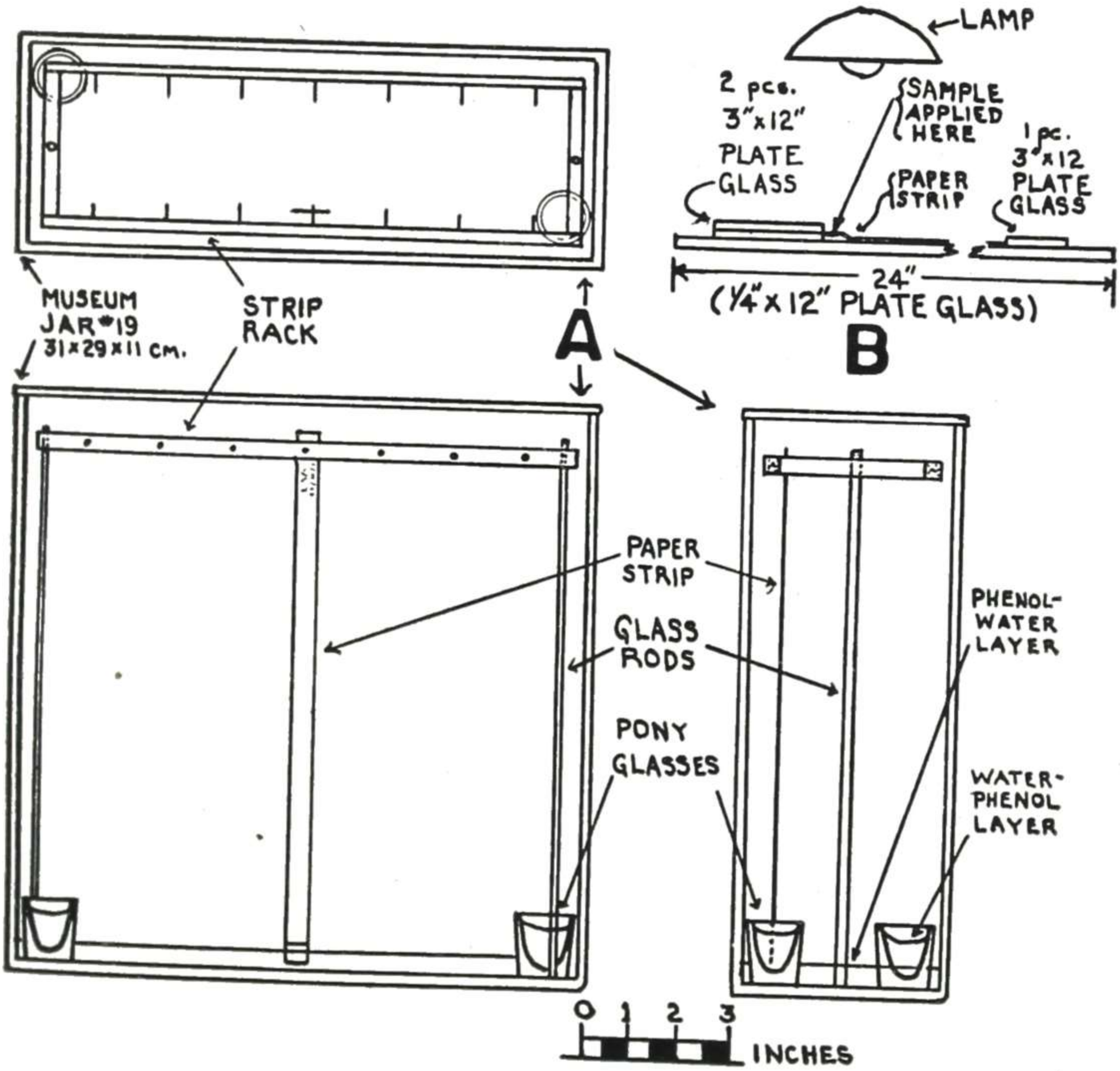


Fig. 114.—Apparatus for chromatographic analysis: *A*, projection views of partitioning chamber and supporting rack for paper strips; *B*, side view of sampling table. (Courtesy of Albanese, A. A., *et al.*: J. Lab. & Clin. Med. 37:885-893, June, 1951.)

the top and a line is drawn across the strips at 2 cm. from the bottom. The strips are placed on the sampling table and held in place by sandwiching between plate-glass sections (*B*). The samples are applied by drawing the pipet over the pencil line, three or four applications being required for a 20 cu. mm. aliquot. To avoid diffuse bands, the sample should be applied in the thinnest possible line and the paper allowed to dry completely between applications. Drying can be hastened considerably by placing a student's gooseneck lamp, lighted, over the area.

When the sampling process has been completed, the strips are hung on a rack in the jar so that the sample line is about 1 cm. above the level of the phenol-water layer. The jar is then covered with a paraffined glass top. The chamber should be placed in a draftless area, preferably an enclosed cabinet. With suitable samples and temperature conditions (22-25 C.) adequate separation is achieved in about 18-20 hours. The solvent should be allowed to rise about 22 cm. above the fluid layer. At the end of the run the rack, laden with the strips, is transferred to the drying pan located in an exhaust hood. The level attained by the solvent is marked with a

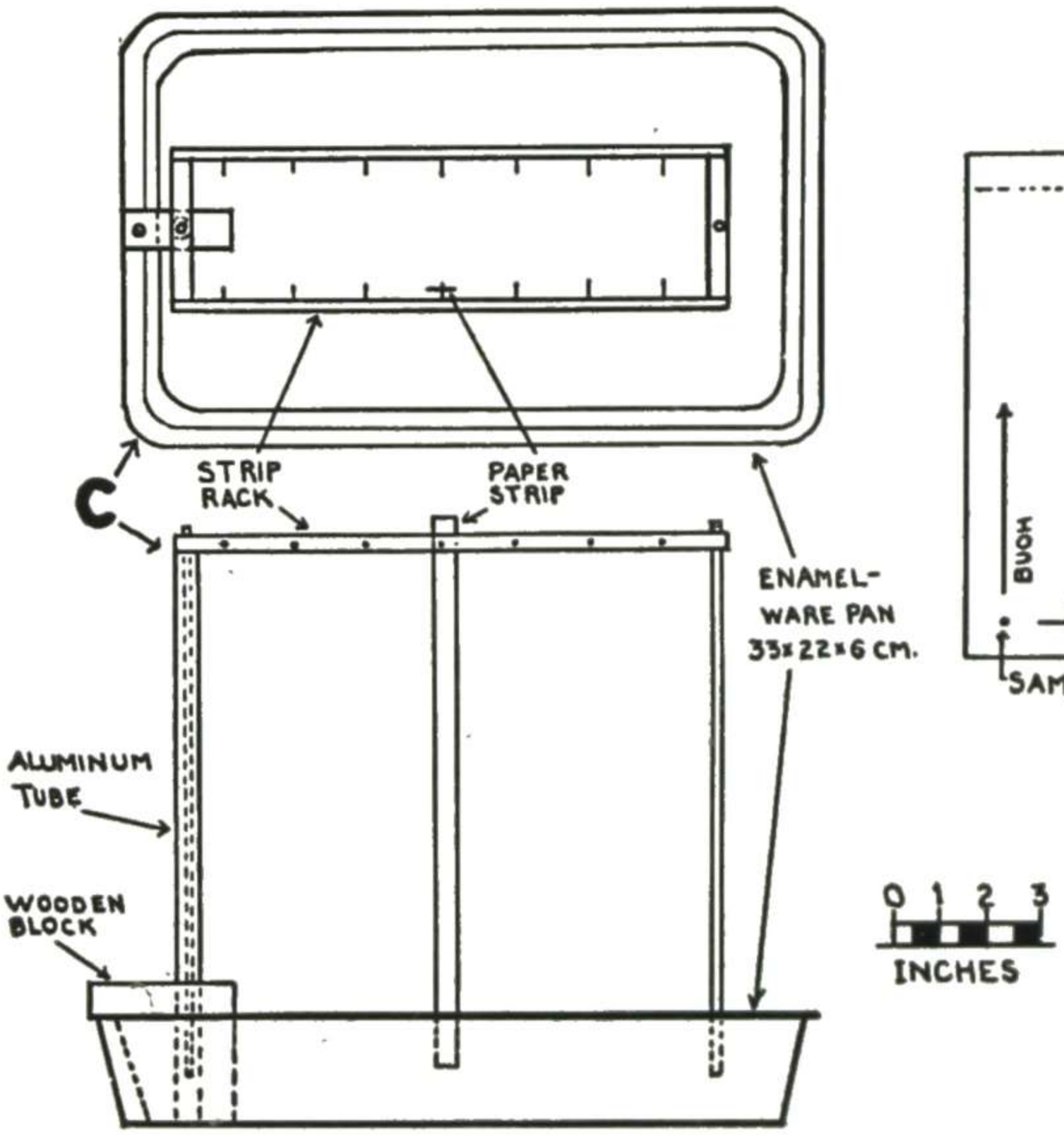


Fig. 114 (cont.).—Apparatus for chromatographic analysis: *C*, side and top view of drying pan with supporting rack in position; *D*, cut-out details for filter paper to be used for two-dimensional analysis. (Courtesy of Albanese, A. A., *et al.*: J. Lab. & Clin. Med. 37:885-893, June, 1951.)

pencil line and the strips are allowed to dry thoroughly. The strips are developed by painting with a 5 per cent solution of ninhydrin (Pfanstiehl) in normal butyl alcohol which has been prepared one hour before use. Complete color development is attained on standing about six to eight hours at room temperature. For record purposes, because of color fading, photographic reproductions of the chromatograms should be made immediately.

Satisfactory photochromatograms of hydrolysates derived by acid or enzymatic hydrolysis can be obtained by the use of 10 or 20 cu. mm. of solution containing about 0.5 mg. N/cc. In analyzing normal urine the size of sample depends on the total amino N content of the specimen, which can be determined rapidly by the copper method.

The following amounts of urine should be used: infants, 10 cu. mm.; older children, 20 cu. mm.; adults, 20 cu. mm.; elderly persons, 30 cu. mm. In estimating the amino acid content of urines containing proteins, especially those from nephritic or nephrotic patients, 3 ml.

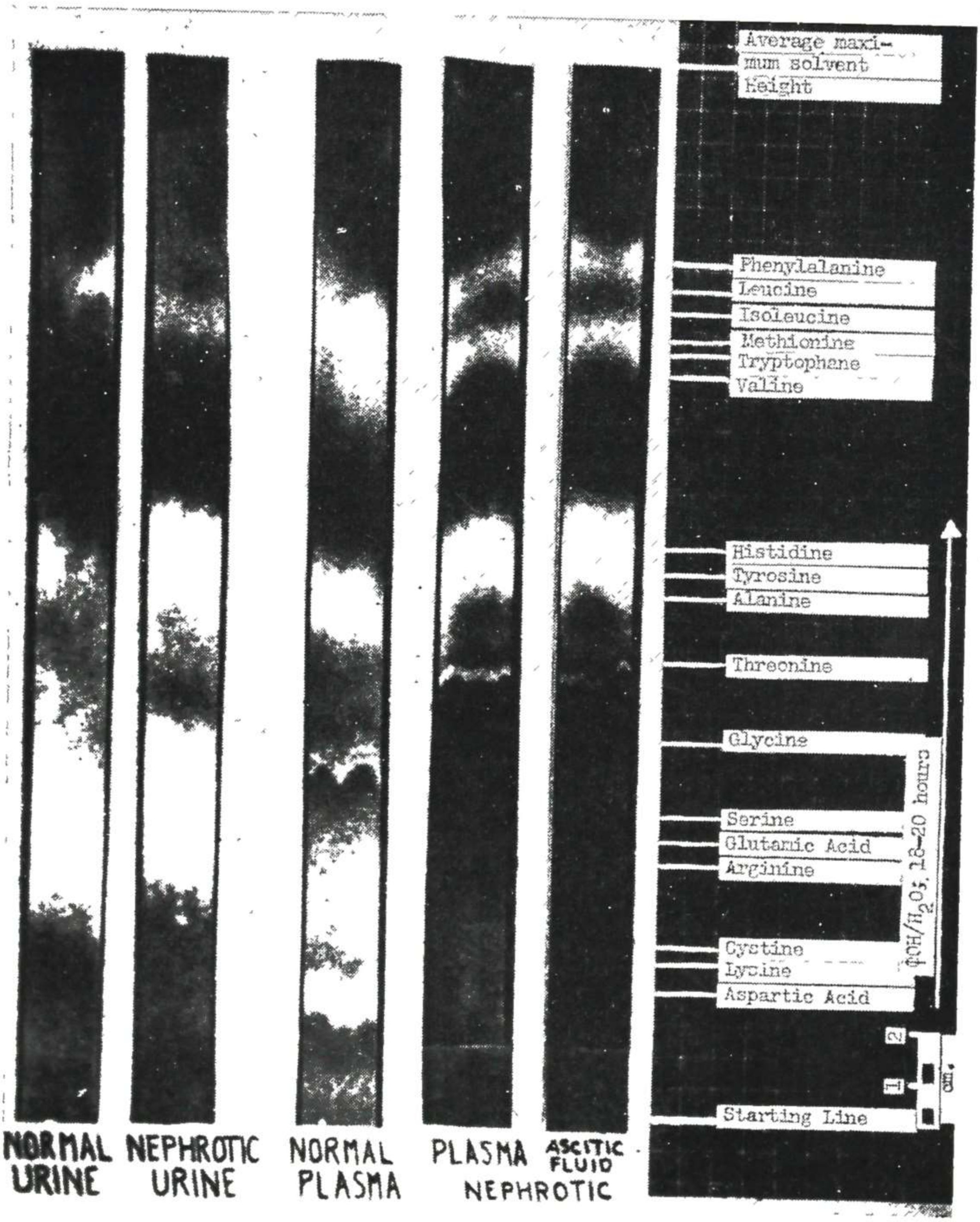


Fig. 115.—Amino acids in body fluids of normal and nephrotic boy. (Courtesy of Albanese, A. A., *et al.*: J. Lab. & Clin. Med. 37:885-893, June, 1951.)

urine is placed in a 15 ml. centrifuge tube and 3 ml. acetone (U.S.P.) added with mixing. After five minutes the conglomerate is centrifuged at 2,500 rpm for 10 minutes in a clinical centrifuge with an angle head. Amino acid determinations of ascitic fluids are performed with samples prepared in the same manner. In determining

the distribution of free amino acids in blood, 0.5 ml. plasma, serum or whole blood is added to 0.5 ml. acetone in a 15 ml. centrifuge tube with vigorous shaking. After 10 minutes the mixture is centrifuged and 50 cu. mm. aliquots of the supernate applied to duplicate paper strips. The rest of the protein-free supernatant fluid can be used for sodium or potassium determinations.

Two-Dimensional Technic.—Except for differences in the par-

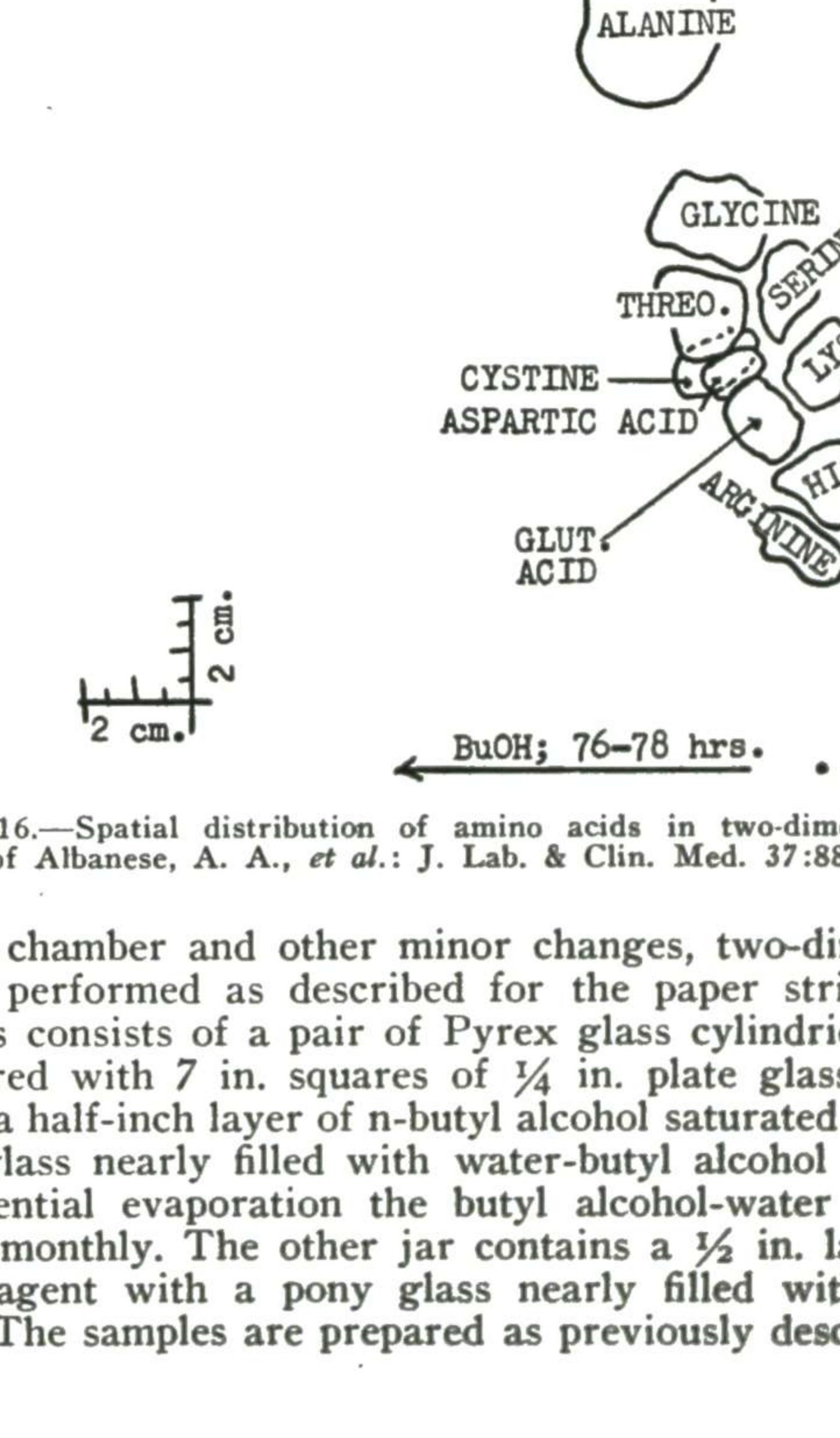


Fig. 116.—Spatial distribution of amino acids in two-dimensional separation. (Courtesy of Albanese, A. A., *et al.*: J. Lab. & Clin. Med. 37:885-893, June, 1951.)

titioning chamber and other minor changes, two-dimensional analyses are performed as described for the paper strip method. The apparatus consists of a pair of Pyrex glass cylindrical jars, 6 × 18 in., covered with 7 in. squares of ¼ in. plate glass. One of these contains a half-inch layer of n-butyl alcohol saturated with water a pony glass nearly filled with water-butyl alcohol phase. Beca of differential evaporation the butyl alcohol-water layer must be replaced monthly. The other jar contains a ½ in. layer of ph water reagent with a pony glass nearly filled with water- reagent. The samples are prepared as previously described in

the amounts used for the one-dimensional strips applied at the lower left-hand corner, 2 cm. from each edge, of 29 cm. squares. A 2 cm. strip, as indicated by the area *A* (Fig. 114, *D*) is cut out and a cylinder formed by clipping the tab to the upper left-hand corner of the sheet. Care must be exercised that the two edges of the paper remain at last 1 cm. apart. The cylinder is placed upright in the partitioning chamber containing the butyl-alcohol and water solvents. After 72-78 hours the cylinder is removed from the jars and hung to dry in an exhaust hood. Drying can be extended overnight or week ends. The cylinder is unfolded, the tab cut off and a second one formed by cutting out area *B* (Fig. 114, *D*). A new cylinder is reformed by clipping the new tab to the lower right-hand corner of the sheet. This is allowed to stand in the phenol-water chamber for about 20 hours. At the end of this time the phenol-soaked paper is hung in the hood and aired thoroughly. The cylinder is unrolled on a piece of glass, 30 × 30 cm., painted with generous amounts of ninhydrin reagent and returned to dry in the hood. For purposes of permanent record the paper is cut 8½ × 11 in. and printed by direct contact with a piece of Scona Reflex paper of the same size. The paper is placed over the chromatogram, emulsion side down, and a printing frame back strapped into place. Exposure can be made from any light source. The exposed paper is processed in the same solutions and manner as used for x-ray films. For interpretation, negative copies are more satisfactory and useful than positive.

The Rf value is defined as the distance of movement of the solute divided by the distance of movement of the solvent measured in centimeters. The values were obtained by using 10 cu. mm. of solutions containing 0.5 mg. amino acid/cc.: phenylalanine and isoleucine, 0.85-0.80; methionine, tryptophane and valine, 0.77-0.75; histidine, tyrosine and alanine, 0.59-0.55; threonine and glycine, 0.47-0.41; serine, glutamic acid and arginine, 0.34-0.30, cystine and lysine, 0.21; aspartic acid 0.18. Rf values vary in practice with concentration of total N content of the sample and distribution of amino acids. These variations always involve a shift of the Rf spectrum upward or downward as a whole group, never a spatial change in sequence of any individual amino acid. After some experience the correction for these shifts presents no serious problems. Any scale such as is affixed to Figure 115 must be regarded as a relative rather than an absolute standard. The positional characteristics of the amino acids in the two-dimensional chromatogram were determined by use of 2-10 μg. quantities (Fig. 116). Overlapping of areas will occur if the amino acid exceeds the usual concentrations.

Studies of Urinary 17-Ketosteroid Excretion by Means of New Microchromatographic Fractionation Procedure. A. S. Zygmuntowicz, M. Wood, E. Christo and N. B. Talbot[8] (Boston) describe a method by which 0.3 mg. samples of neutral urinary 17-ketosteroids can be separated into 40 fractions.

METHOD.—Urine is collected without preservative. It is boiled

(8) J. Clin. Endocrinol. 11:578-596, June, 1951.

with 15 vol. per cent of HCl for 10 minutes under a reflux condenser, cooled rapidly, transferred to a separatory funnel and extracted four times with 15 vol. per cent of carbon tetrachloride. The combined extract is evaporated to a convenient volume and washed four times with 25 ml. lots of 1 N NaOH and four times with 25 ml. lots of water. All washed extracts are evaporated to dryness, residual traces of solvent are removed with methanol, and the nonvolatile residue (crude, neutral 17-ketosteroid fraction) is separated into ketonic and nonketonic fractions.

To 20 mg. or less of dry crude, neutral 17-ketosteroid material are added 200 mg. Girard's reagent P and 0.5 ml. glacial acetic acid. The flask containing this mixture is stoppered with aluminum foil, heated in a boiling water bath for three minutes and then chilled in an ice bath. The contents are transferred quantitatively to a separatory funnel with 40 ml. ice-cold distilled water. After 3 ml. of 10 per cent NaOH solution is added, the mixture is extracted three times with 20 ml. portions of chloroform. The chloroform extracts are combined and backwashed once with 60 ml. distilled water. This chloroform extract is discarded. One ml. of concentrated H_2SO_4 is added to the 60 ml. water backwash, which is then combined with the aqueous phase remaining after extraction of the nonketonic fraction. After addition of 20 ml. chloroform, the mixture is shaken and left standing at room temperature for two hours. The resultant acid-hydrolyzed aqueous mixture is extracted with the chloroform present and three additional 20 ml. lots of chloroform. The chloroform extracts containing the ketonic material are combined, washed once with 10 ml. of 0.1 N NaOH and three times with 10 ml. lots of water. These washings are discarded. The washed ketonic chloroform extract is evaporated to approximate dryness, and residual traces of solvent are removed with methanol. All residues are dried over anhydrous $CaCl_2$ in a vacuum desiccator for at least 48 hours. The dried residues are dissolved in benzene and stored in bottles. The amount of benzene used depends on the amount of steroid present. An attempt is made to add such an amount that the resultant solution will contain about 0.5 mg. 17-ketosteroid/ml. The exact concentration is determined by colorimetric assay.

An accurately measured 0.1 ml. or 0.2 ml. aliquot of benzene solution of 17-ketosteroids is transferred to a colorimeter tube and the benzene evaporated. The dry residue is dissolved in 0.1 ml. absolute ethanol (U. S. Industrial Chemicals, Inc.) to which are added 0.1 ml. of absolute ethanolic m-dinitrobenzene solution and 0.1 ml. ethanolic KOH solution. A blank solution containing no steroid is similarly prepared. The tubes are rotated gently, then immersed in a darkened constant water bath for 80 minutes at 25 C. ±0.05. The contents of each tube are diluted with 10 ml. of 95 per cent ethanol and mixed by inverting the tube once. Colorimetric readings are made with green and blue filters, having maximal transmission at 540 and 420 μ respectively, in the Evelyn photoelectric colorimeter after adjusting the galvanometer to 100 with the blank in place. The green filter reading is referred to a calibration curve or table with crystalline pure androsterone, isoandrosterone or dehydroi

androsterone. The resultant value is called the uncorrected 17-ketosteroid value and indicates approximately the quantity present in the sample analyzed. This crude value is corrected for errors of overestimation due to interfering chromogens by application of a color correction equation. The corrected assay value is used in determining the concentration of 17-ketosteroids in the benzene solution to be

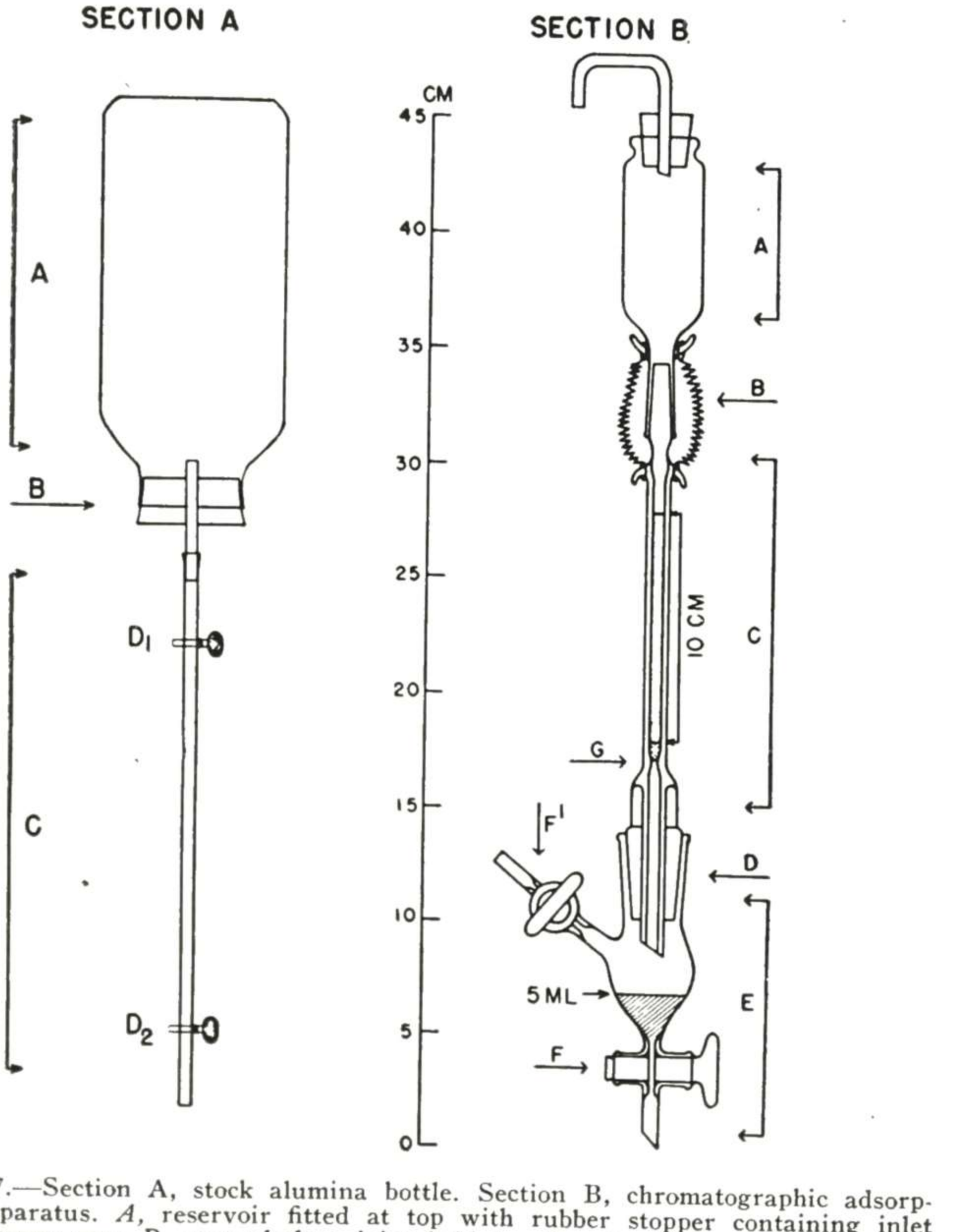


Fig. 117.—Section A, stock alumina bottle. Section B, chromatographic adsorption microapparatus. *A*, reservoir fitted at top with rubber stopper containing inlet tube for nitrogen gas; *B*, ground-glass joint held in place by two springs; *C*, glass column with inside diameter of 4 mm.; *D*, ground-glass joint; *E*, funnel fitted with two ground-glass stopcocks, *F* and *F'*. *F'* acts as air vent and may be used to stop flow of solvent when pressure is released at *A*. Apparatus made by Macalaster Bicknell Company. (Courtesy of Zygmuntowicz, A. S., *et al.*: J. Clin. Endocrinol. 11:578-596, June, 1951.)

fractionated and hence in determining what volume of this solution should be placed in the chromatographic column.

The foregoing procedure may be modified to facilitate determination of 17-ketosteroids in each of the 41 fractions obtained by elution of the column. Equal volumes of the reagents are combined and

mixed immediately before use. To a blank tube and to each tube containing dry residue, 0.3 ml. of the mixture of reagents is added. The tubes are rotated and incubated as described previously, after which the contents are diluted with 10 ml. of 95 per cent ethanol, mixed and read. The eluates are usually assayed in three lots of 14 each with a freshly prepared reagent mixture for each lot. The galvanometer readings obtained are referred to the calibration curve previously mentioned.

For chromatographic fractionation, anhydrous aluminum oxide is used. Alumina must be kept free from contamination with acid fumes

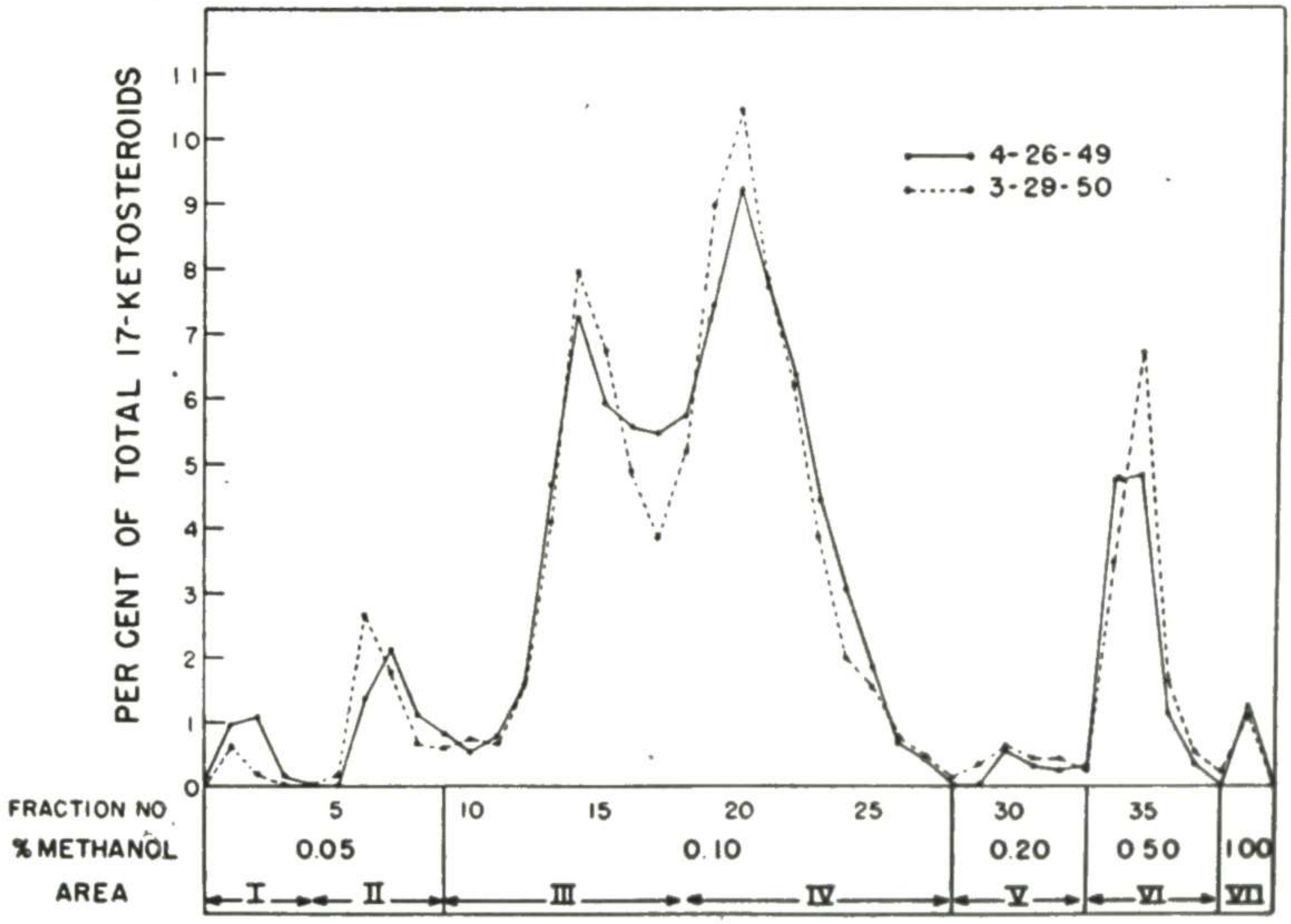


Fig. 118.—Duplicate chromatographic separations of neutral urinary 17-ketosteroids on equal aliquots of single, normal female urine extract. Each point indicates per cent of total 17-ketosteroids eluted which was recovered in individual eluate fraction. Areas *I-VII* are arbitrarily intended to conform approximately to areas subtended by curve peaks. (Courtesy of Zygmuntowicz, A. S., *et al.*: J. Clin. Endocrinol. 11:578-596, June, 1951.)

and moisture. This is done by replacing the cap of the container (Fig. 117, section A, *A*) with a large rubber stopper (*B*) in which is inserted a glass tube 5 cm. long and 5 mm. wide. A 25 cm. length of thin-walled, small-bore Tygon tubing (*C*) is attached to the glass tube. The tubing is clamped off with two screw clamps (D_1 and D_2) placed about 15 cm. apart. The stopper and connecting parts are sealed in place with paraffin, thus making it possible to withdraw small portions of alumina without contaminating the remainder.

To carry out fractionation, a plug of alkali-free glass wool inserted at the constriction (*G*) of the column of the mic apparatus in Figure 117, section B. The Tygon tubing of the s bottle of alumina is attached to the upper end of the chroma

graphic tube (*C*). Clamp D_2 is opened, and alumina is allowed to run into the glass tube *C* until a column exactly 10 cm. long has accumulated. The clamp is closed and glass tube *C* disconnected and attached to funnel *E*, which has a 20 ml. capacity and is calibrated at 5 ml. After a 50 ml. glass reservoir (*A*) is secured to the column by wire springs, 5 ml. thiophene-free benzene is percolated through the column under nitrogen pressure (dry, 99.7 per cent pure) until the upper level of the solvent is within 2-3 cm. of the top layer of alumina, which must always be kept covered with solvent. The benzene which has accumulated in funnel *E* is discarded. The surface of the alumina is now smoothed by removing reservoir *A*, stirring the surface slightly with the tip of a finely drawn-out dry, glass rod and tapping the column once or twice. Reservoir *A* is replaced and enough benzene added to bring the fluid level to the top of the ground-glass joint *B*.

An aliquot of the previously assayed benzene solution containing 0.3 mg. urinary 17-ketosteroids is diluted to 5 ml. with benzene in a dry 10 ml. test tube. The solution is transferred quantitatively to reservoir *A* with the aid of an additional 1 ml. benzene. The solution, totaling 7 ml., is percolated through the column of alumina, slowly under pressure, the percolate being collected directly into the first of a series of 41 photoelectric-colorimeter tubes. When the surface of the initial 17-ketosteroid-containing aliquot of benzene has fallen to a point about 1 cm. above the surface of the alumina, pressure is discontinued and solvent mixture a (0.05 per cent methanol in benzene) is added to the reservoir; pressure is then resumed. Each 5 ml. eluate is collected in a photoelectric-colorimeter tube when the level of the calibration mark of funnel *E* is reached. The process is repeated with solvent mixtures b (0.10 per cent methanol in benzene), c (0.20 per cent methanol in benzene) and d (0.50 per cent methanol in benzene) and finally with absolute methanol (acetone-free), until elution is complete. The total amount of each solvent percolated through the column and the total number of fractions collected are shown in the table.

TOTAL AMOUNT OF EACH SOLVENT PERCOLATED THROUGH COLUMN; TOTAL NUMBER OF FRACTIONS COLLECTED

SOLVENT	No. 5 ML. ELUATE FRACTIONS	TOTAL ML. ELUATE
Benzene (containing 17-ketosteroids)	(1)	7
Mixture a	9	45
Mixture b	19	95
Mixture c	5	25
Mixture d	5	25
Absolute methanol	2	10

Each eluate fraction is evaporated to dryness. The nonvolatile residue, which tends to creep up the sides of the test tube, is rinsed down with about 1 ml. absolute, acetone-free methanol and dried with suction.

The 17-ketosteroid content of each eluted residue, expressed as milligrams per fraction, is measured by colorimetric reaction as described. The total recovery is determined by adding the values of

the 41 eluates. Each fraction is then recalculated as "per cent of total 17-ketosteroids" recovered.

The chromatographic fractionation curves obtained for normal adults (Fig. 118) are of quite constant character. Each of the peaks represents groups of 17-ketosteroids with different physicochemical properties. There is a slight but seemingly definite sex difference in these curves. Abnormal curves have been obtained for patients with adrenal cortex disease and for endocrinologically normal patients with trauma, infection or cancer. This suggests that adrenocortical hormone metabolism may undergo qualitative changes not only in adrenal cortex involvement but also in response to physiologic stress.

Rapid CO_2 Determination with Beckman O_2 Analyzer is described by Vivian G. Behrmann and Frank W. Hartman[9] (Henry Ford Hosp.) and shown diagrammatically in Figure 119. In step *A* the gas is pressed from its butyl bag at an op-

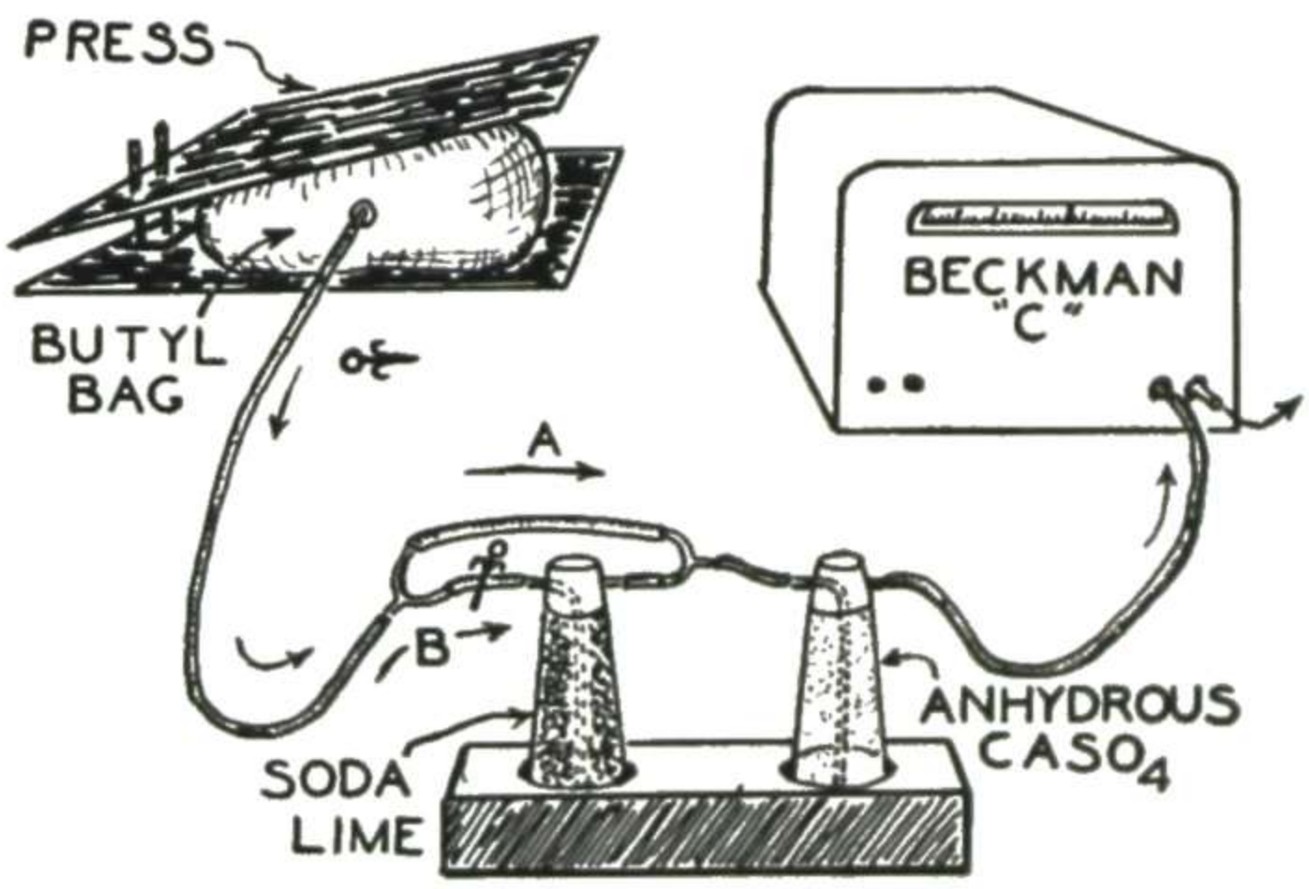


Fig. 119.—Method of determining CO_2 with Beckman O_2 analyzer. (Courtesy of Behrmann, V. G., and Hartman, F. W.: Proc. Soc. Exper. Biol. & Med. 78:412-416, November, 1951.)

timal flow rate of 200 cc. minute for 2½ minutes through anhydrous calcium sulfate to the Beckman analyzer (C model). The reading of O_2 tension is in millimeters of mercury partial pressure, which is readily converted to O_2 percentage. Without delay, O_2 percentage after CO_2 removal (step *B*) is similarly determined by diverting the gas through soda lime and anhydrous calcium sulfate into the analysis chamber. The procedure is facilitated by a continuous barometric record (Baroscribe), 2½ minute timer, small lamp and magni-

(9) Proc. Soc. Exper. Biol. & Med. 78:412-416, November, 1951.

focuser (binocular loupe which magnifies 3×) to increase accuracy of scale reading.

With this method, both CO_2 and O_2 analysis may be completed within 5 minutes as compared to 15-20 minutes with the Haldane method. Of 300 duplicate CO_2 analyses made by this method, 95 per cent agreed within 0.10 per cent with duplicate Haldane data.

Why Do Gastric Analysis?—*Theoretical Considerations.*—J. L. A. Roth and H. L. Bockus[1] (Univ. of Pennsylvania) state that the acidity of gastric contents is the net result of (1) the total volume and rate of parietal secretion, reflecting the interplay of stimulating and inhibiting mechanisms, and (2) evacuation or alteration of parietal secretion.

Uses of Various Procedures.—The fractional gastric analysis of Rehfuss, with some form of carbohydrate as a test meal, is the commonest procedure in clinical study of gastric function. It provides a crude but complete measure of the stomach's work, not only its secretory capacity but also its motor functions. Examination of the fasting gastric residuum may supply useful information. Recovery of gross food from the stomach after a 12 hour fast is almost always due to delay in stomach emptying and often to obstruction at or near the pylorus. If free acid but no bile or food particles are present, a fasting volume in excess of 50 ml. suggests hypersecretion. The odor of gastric contents should be noted. Normally it is pungent and penetrating, but it is important to recognize the odor due to a reflux of colonic content, for this may provide the first indication of a gastrojejunocolic fistula. A similar odor may be detected in the presence of advanced ulcerative and necrotic carcinoma of the stomach. If the fluid in the fasting residuum is colored by gross blood, then carcinoma, ulcer or severe erosive gastritis should be suspected and the tube removed at once. Microscopic examination of the fasting residuum may reveal parasites, tubercle bacilli, cholesterin crystals and calcium bilirubinate pigment or malignant cells in tissue fragments. After complete evacuation of the fasting residuum, postprandial samples should be aspirated every 15 minutes for at least 2 hours. The stomach may exhibit average acidity after a carbohydrate meal but show a hyperacid response to alcohol or meat extract meals, so that a normal

(1) Gastroenterology 18:546-565, August, 1951.

and a hypersecretory stomach cannot be differentiated. If an initial hyperacidity is found after the Ewald meal, histamine injection will produce little if any increase in free acid concentration. Starches are not secretagogues. The stimulating action of these test meals is due to psychic secretion and distention with the release of gastrin. The magnitude of the response is probably conditioned by the level of the vagus activity. In a patient known to have duodenal ulcers, the level of acidity in response to the Ewald meal in a fractional gastric analysis may serve as a guide in therapy or prognosis. Partial neutralization of gastric contents is all that needs to be achieved by the strictest medical regimen. The need for antacids and atropine is indicated in the higher grades of hyperacidity. Complicated ulcer is associated with high grade hyperacidity oftener than is the uncomplicated duodenal ulcer. Probably the more profound the hyperacidity, the greater the chance of development of a gastrojejunal ulcer after partial gastric resection. On this basis, more extensive resection, complementary vagotomy or stricter postoperative management may be indicated.

If no free acid is found in fractional gastric analysis with an Ewald test meal, the procedure should be repeated with histamine before true achlorhydria is diagnosed. The potency of the histamine preparation should be determined by use on other patients in order to make certain that the particular lot of histamine is not inactive or has not lost potency by deterioration. The adequacy of the dose of histamine base is inferred from recognizable physiologic effects such as facial flush, headache or slight fall in blood pressure, since the threshold dose for producing these effects is greater than that for stimulating the parietal cells. The maximal dose of histamine tolerated without untoward effects should be used; 0.1 mg. histamine base or 0.275 mg. histamine acid phosphate/10 kg. body weight is a satisfactory empiric dose for this purpose. Fractional sampling of gastric contents every 15 minutes should be continued for a minimal period of 2 hours to detect the slight and transient appearance of hydrochloric acid and any delayed response. Demonstration of true achlorhydria provides crucial information when the diagnosis concerns primary pernicious anemia, stomach carcinoma and a pseudoulcer syndrome or invalidates a postvagotomy negative insulin test.

The insulin test has a limited use in evaluating the completeness of the vagotomy, and its reliability in prognosis of clinical results remains to be demonstrated. The sustained gastric secretory response to caffeine may aid in diagnosis of duodenal ulcer disease. The caffeine gastric analysis may be used to greatest advantage (1) when the patient has a characteristic ulcer history but careful x-ray studies do not demonstrate a crater or deformity of duodenal cap or (2) when the patient presents the differential diagnosis of asymptomatic upper intestinal bleeding with negative x-ray examination of the esophagus, stomach, duodenum or small intestine.

Simple Measure of Gastric Secretion in Man: Comparison of 1 Hour Basal Secretion, Histamine Secretion and 12 Hour Nocturnal Gastric Secretion. Erwin Levin, Joseph G. Kirsner and Walter L. Palmer[2] (Univ. of Chicago) describe a simple and reliable procedure for characterizing quantitatively the gastric secretory response in man. Evidence indicates that this response is greater in patients with duodenal ulcer than in normal persons; the response in patients with gastric ulcer is the same or often lower than normal. Various test meals are time consuming, require special personnel and may (histamine test) be accompanied by undesirable side effects. The fasting 12 hour gastric secretion test is not practical for routine use.

Data on the one hour morning basal secretion and histamine response were obtained for 560 normal persons (319 males), 222 duodenal ulcer patients (174 males) and 50 patients with benign gastric ulcer (40 males). Fasting gastric secretion was measured at 15 minute intervals for 1 hour (8:30-9:30 a.m.) and compared with the secretion for 1 hour after administration of histamine (0.01 mg. histamine base/kg. body weight) and continuous 12 hour night secretion (8:30 p.m. to 8:30 a.m.).

The data demonstrate that the procedure using morning basal secretion parallels the other two procedures whenever acid is present in the basal secretion and that this analysis may yield information of diagnostic value. In every instance average basal secretion was significantly higher in patients with duodenal ulcer and lowest in patients with gastric ulcer. These differences were maintained in comparisons by age and sex.

Simple determination of the fractionated one hour morn-

(2) Gastroenterology 19:88-98, September, 1951.

ing basal secretion yields the necessary information relative to the secretory response of the stomach for routine clinical purposes. The procedure is reliable and not time consuming and does not require special personnel. As with other secretory tests, the diagnostic value is modified whenever acid is present throughout the entire period, even though average secretion of the three groups differs significantly. However, when acid is absent for one or more periods, the presence of an active duodenal ulcer is unlikely. The only practical indication for administration of histamine, caffeine or alcohol is in individual cases in which it is necessary to establish the presence of true achlorhydria.

Twenty-Four Hour Gastric Analysis in Patients with Histamine Achlorhydria, as described by James and Pickering, was used by G. Watkinson and A. H. James[3] in the study of 22 patients. Results cast doubt on the reliability of histamine meals in assessment of achlorhydria, even when repeated on several occasions. Among 10 patients with micro- or macrocytic anemia, acidity never exceeded pH 4.2, which suggested that all had true achlorhydria. Achlorhydria was associated with peptic ulcer in 12 patients, in 10 of whom the 24 hour test showed that pH 3.0 was exceeded for periods of 4-18 hours.

This evidence indicates that the Rehfuss histamine meal overestimates the incidence of achlorhydria, particularly in cases associated with peptic ulcer. It was concluded that large chronic gastric ulcers may develop in patients in whom the gastric contents are acid only for short periods during the day.

↓ See also 1949 YEAR BOOK, pages 190-191, for an article by A. H. James and G. W. Pickering on the role of gastric acidity in the pathogenesis of peptic ulcer.—Ed.

Determination of Gastric Acidity without Intubation: Clinical Evaluation of Quininium Exchange Indicator Compound. According to Harry L. Segal[4] (Univ. of Rochester) the quininium cations of the quininium exchange indicator compound are displaced when subjected to the action of the hydrogen cations in dilute hydrochloric acid. By noting the amount and time of appearance of the quininium cations in the urine after oral administration of the compound to 325 persons, three groups were detected. (1) Subjects in whom the quininium cations appear in the urine during the first

(3) Clin. Sc. 10:255-266, May, 1951.
(4) M. Clin. North America 35:593-602, March, 1951.

and second hours after administration have free gastric hydrochloric acid. (2) Subjects in whom the cations do not occur in either the first or second hour excretion secrete no free hydrochloric acid. (3) Presence or absence of free hydrochloric acid in the small group in whom the quininium cations are excreted for the first time in the urine of the second hour may be determined by the amount of quininium cations excreted in the second hour urine. The test can be applied in clinical diagnosis and as a screening technic to select achlorhydric persons in the gastric cancer age group for further investigative studies.

METHOD.—The patient should eat nothing after midnight and save the first urine on arising. At 8:30 a.m., 50 ml. of 7 per cent alcohol is taken, followed by a glass of water. Breakfast is not eaten. At 9 a.m. he urinates, if possible. After taking 2 Gm. quininium cation indicator exchange compound and half a glass of water, he lies on his back or left side for half an hour. At 10 and 11 a.m., specimens of urine are taken, the bladder being emptied completely each time. If it is necessary to urinate before the scheduled time, this urine is saved and added to that passed at the designated time. The urine samples should be kept in a cool place and returned to the clinic.

Urine is examined for quinine by measuring and placing the total urine excreted in a separatory funnel. After alkalinizing with 2 per cent sodium hydroxide, 20 ml. of 95 per cent alcohol and 70 ml. ether are added; 30 ml. ether is added for each additional 100 ml. urine. The specimen is shaken well and separated. This is repeated three times, using 50 ml. ether instead of 70 ml. and adding 30 ml. ether each time for every 100 ml. additional urine. All the quinine in the urine should now be present in the ether. The ether mixtures are combined and washed with alcoholic potassium hydroxide solution (N/10 potassium hydroxide in 20 per cent alcohol solution). This usually requires two washings, after which exactly 15 ml. of N/10 sulfuric acid is added to the ether mixture and shaken well. Sulfuric acid solution is examined for quinine by observing the intensity of the fluorescence when the solution is viewed in ultraviolet light (Hanovia ultraviolet lamp type 16103) in comparing it with standard concentrations (faint trace = ± 0.005 mg. quinine; trace = ± 0.012 mg.; 1 + = ± 0.040 mg.; 2 + = ± 0.076 mg.; 3 + = ± 0.145 mg., and 4 + = ± 0.180 mg. quinine). Exact quantitative estimation can be made with the Lumetron photofluorometer no. 402EF with primary filter B1 and photofluorometer test tubes with outside diameter 22 mm. The readings obtained with a photofluorometer are translated into milligrams by appropriate tables.

Chloride-Testing Paper for Clinical Use by Untrained Persons in the rapid estimation of chlorides in urine or other body fluids is described by John Devine[5] (Royal Melbourne Hosp.).

(5) M. J. Australia 2:264-266, Ang. 25, 1951.

Fantus' method (1936) for quantitative estimation of chlorides in urine requires too much skill and is too tedious to be used for examination of every specimen of urine passed by a patient.

The paper indicator method consists of immediately and completely wetting a piece of indicator paper and noting the time taken for it to change from brown to white. The paper has been selected for testing urine of patients receiving intravenous therapy and is adjusted so that a change occurs in five to six seconds if the urinary chloride content is 0.3 per cent. Therefore, if the change occurs immediately or before five to six seconds, there is over 0.3 per cent of sodium chloride present in the specimen being tested. A color change within six seconds indicates adequate urinary chloride excretion.

Figures for the time of color change of Chlorest Paper A were noted: 0.3 per cent sodium chloride in the solution changes the color in 6 seconds; 0.2 per cent, in 13-15 seconds, and 0.1 per cent in 48 seconds. Temperature variations within the usual range cause no clinically significant difference in the result.

The time of color change of the indicator paper is dependent on a number of factors. Rate of reaction should be predictable. The reagent (water-soluble silver chromate) must be in solution. The time taken for the reagents in the paper to be dissolved depends on the character, capillarity, density, thickness and size of the papers; humidity (presolution); temperature of the solution, and presence of inhibitors or substances other than chlorides which might affect the indicator reagent. With reagents, paper and temperature kept constant, the time-rate of reaction should bear a relation to the concentration of the solution and so measure such a concentration.

Simple One Tablet Test for Urinary Chloride in Low Sodium Diets is described by W. B. Looney, C. D. McGrath and W. A. Thomas[6] (Presbyterian Hosp., Chicago). This test may be used by patients on low salt diets to follow their salt balance. Results are valid in patients with normal kidney and cardiac function and in those with compensated renal or cardiac damage. They are not valid when there is uncompensated cardiac or renal disease or clinical evidence of edema.

METHOD.—Tablets are prepared which contain silver nitrate and potassium chromate. If when 1 tablet is added to 5 drops of urine and

(6) J. Lab. & Clin. Med. 38:275-280, August, 1951.

15 of distilled water the color changes from yellow to reddish brown, urinary sodium chloride value is 0-60 mg. per cent. If 2 tablets are necessary to bring about a color change, it is 60-120 mg. A total of 5 tablets may be added (color change may be from a slight pink to a reddish brown). The solution should be examined carefully after each tablet has been added to see if the tablet has disintegrated and if there is a color change.

Orthostatic Albuminuria: Time-Saving Method of Diagnosis is described by Alan Watson.[7] The condition can almost always be diagnosed within an hour of discovery of albuminuria without need for the patient to return with a specimen after a night's rest. A patient found to have albuminuria should be told to empty the bladder completely and drink about ½ pt. water and then should sit quite still. Further urine tests should be made after 15, 30, 45 and 60 minutes' rest. Of 62 patients with albuminuria in the original test only 1 had albuminuria after 60 minutes' rest but produced a clear specimen after a night in bed. Three patients could not obtain a full hour of rest but each produced a clear specimen after a night in bed. Shortest rest period resulting in a clear urine was 25 minutes. Density of albumin in the original specimen had no relation to length of rest required to pass an albumin-free urine. There is no evidence that people with albuminuria due to nephritis pass albumin-free specimens after rest. Incidence of orthostatic albuminuria and apparent monthly variation of incidence can be explained if the figures depend on time the patient has been standing about before the urine is collected.

Urinary Diastase in Mumps. Martin M. Nothman[8] (Tufts College) determined the urinary diastase level several times in 27 children and three adults with mumps. The test was also performed on 10 patients with swelling of the face due to conditions other than mumps. The Somogyi method was used. Normal range is 80-350 units.

All patients with mumps showed striking increase, the levels often being well over 1,000 units and the highest being 7,700. The rise begins on the first or second day after swelling of the parotid gland and may last five or six days or even longer. There was no rise when facial swelling was due to conditions other than mumps.

When mumps started in the submaxillary gland, urinary diastase level was elevated even though the swelling remained

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(7) Lancet 1:1196-1197, June 2, 1951.
(8) New England J. Med. 244:13-15, Jan. 4, 1951.

limited to these glands. When involvement of the parotid gland was unilateral, it fell with decrease in size of the gland and rose again when the opposite side became enlarged. Apparently the salivary glands are the source of the excess diastase in the urine.

Chemical Tests for Blood in Urine. Chemical tests for hematuria are still used in the absence of centrifuge and microscope, but little information is available on their sensitivity. Harold Caplan and George Discombe[9] (London) report on the absolute and relative sensitivity of the orthotolidin hydrochloride, benzidine, Kastle-Meyer or reduced phenolphthalein, aminopyrine and guaiac chemical tests, direct spectroscopy and microscopy under standard conditions.

Urine samples were prepared from blood (hemoglobin 14.3 Gm./100 ml.; red cells 5,000,000/cu.mm.) diluted with urine obtained from the same subject. Dilutions were made with fresh urine, urine which had stood for 24 hours to allow oxidation of ascorbic acid and the same two urines to which had been added 2 mg. ascorbic acid/100 ml. This was done because an excess of ascorbic acid inhibits the benzidine and orthotolidin hydrochloride tests and 2 mg./100 ml. represents an average normal concentration.

No single test proved ideal under all conditions. Microscopy of the centrifugal deposit is at least 500 times more sensitive than the guaiac test, and if the Addis procedure is used the ratio reaches 2,500. For domiciliary work and as a ward test, the orthotolidin hydrochloride test is convenient, extremely sensitive and cheap. It is 10 times more sensitive than any other chemical test and rarely gives false positive results. The other tests are less sensitive. The Kastle-Meyer test is reliable, but the caustic reagent makes it unsuitable for domiciliary practice. The guaiac test is expensive, insensitive and not reliable; it should be abandoned. Should a test of comparable sensitivity be needed, the aminopyrine test is suitable.

In the laboratory it is preferable to examine a centrifuged deposit. If information on the degree and persistence of hematuria alone is needed, it is justifiable to examine a 2.5 cm. layer of urine with the hand spectroscope. If the specimen is positive, hemoglobin equivalent to about 2,500,000 red cells/ml. is present; if it is negative, the deposit from 12.5 ml.

(9) Brit. M. J. 2:774-775, Sept. 29, 1951.

urine should be suspended in 0.5 ml. and examined in a Fuchs-Rosenthal chamber.

Evaluating Trichloroethylene Exposures by Urinalyses for Trichloroacetic Acid. Axel Ahlmark and Sven Forssman[1] (Stockholm) state that trichloroethylene is used as a solvent in the metal, motor, dry cleaning and rubber industries. Excretion of trichloroacetic acid, a product of trichloroethylene metabolism, is less dependent on recency of exposure or minor daily variations of exposure than is that of other products of metabolism. The urinary content of this product remains for some time at a measurable level and gives a good picture of the average exposure over a long period. Symptoms of trichloroethylene exposure include abnormal fatigue, increased need of sleep, gastric symptoms such as tympanitis, nausea, mouth dryness, intolerance of alcoholic liquors, increased psychic irritability, impaired capacity for concentration, headache, painful sensations in the cardiac region and dyspnea.

Examination of 122 workers exposed to trichloroethylene disclosed that excretion of trichloroacetic acid of less than 20 mg./L. urine usually was not associated with a definite trichloroethylene effect. Values over 20 mg./L. urine were associated with an increasing percentage of cases directly proportional to the increasing excretion. With values of 40-75 mg./L., such effects occur in about half the cases and are very common with excretion exceeding 100 ml. No relation could be shown between the effects of trichloroethylene and the age or sex of the exposed workers.

Detection of Occult Blood in Feces, Including Observations on Ingestion of Iron and Whole Blood. The advent of cancer detection clinics makes a simple, rapid screening method to exclude malignancy of the gastrointestinal tract important. Ann Peranio and Maurice Bruger[2] (New York City) compared the sensitivity of four occult blood indicators (orthotolidin, benzidine, phenolphthalein and guaiac) and investigated the effect of ingestion of iron and whole blood in both stool and urine.

METHOD.—A homogeneous specimen of feces about the size of a large pea was added to 4 ml. water. After the mixture was heated and cooled, 1 ml. was used for the determinations. Measurements were exact and constant to insure comparable results. The orthotolidin test (hematest® tablet method) was performed as outlined by

(1) A.M.A. Arch. Indust. Hyg. 3:386-398, April, 1951.
(2) J. Lab. & Clin. Med. 38:433-445, September, 1951.

the manufacturer. The result was read in two minutes. The benzidine test was performed by mixing 1 ml. stool emulsion with 1.5 ml. glacial acetic acid containing a pinch of benzidine and adding dropwise with shaking 5 drops of 3 per cent hydrogen peroxide. The result was read in two minutes. For the guaiac test, 1 ml. emulsion was mixed with 2 ml. glacial acetic acid, and 2 ml. ether and 2 ml. alcoholic guaiac solution (1 Gm. in 60 ml. of 85 per cent ethyl alcohol) were added. Five drops of 3 per cent hydrogen peroxide were added dropwise with shaking and the result was read immediately. The phenolphthalein test of Meyer was performed as described by Gettler and Kaye.

A meat- and fish-free diet for 72 hours is essential for the orthotolidin, benzidine and phenolphthalein test but not for the guaiac test.

Ingestion of ferrous sulfate caused no false positive reactions except possibly with the orthotolidin test, but other substances unrelated to blood may produce color interference or false positive reactions. Mild gum bleeding is not sufficient to produce positive reactions. The following amounts of ingested whole blood will produce positive reactions with the four indicators: orthotolidin, 1 ml.; benzidine, 3.5 ml.; phenolphthalein, 3.5 ml., and guaiac, 20 ml. Normal subjects on unrestricted diets will show faintly positive reactions to guaiac after ingestion of 2-3 ml. blood.

The orthotolidin test is the most sensitive but is misleading because of common false positive reactions. The benzidine test, second in sensitivity, gives frequent false positive reactions if the quantity of benzidine used is not restricted. Phenolphthalein is third in sensitivity and guaiac is least sensitive.

BACTERIOLOGY

Culture Flask for Estimation of Growth of Microorganisms by Optical Methods is described by Frederick G. Sherman and Barbara M. Grant[3] (Brown Univ.). With this flask, periodic measurements of the light absorbed by the cell suspension and medium can be made directly on the entire culture with the Evelyn photoelectric colorimeter without the removal of samples. The flask (Fig. 120) is constructed by sealing absorption tubes selected for optical uniformity, such as those used with the Evelyn photoelectric colorimeter, to 125 ml. Erlenmeyer flasks blown from Kimble's standard flint glass stock (made by L. G. Nestor Co., Millville, N. J.). The

(3) J. Lab. & Clin. Med. 37:325-326, February, 1951.

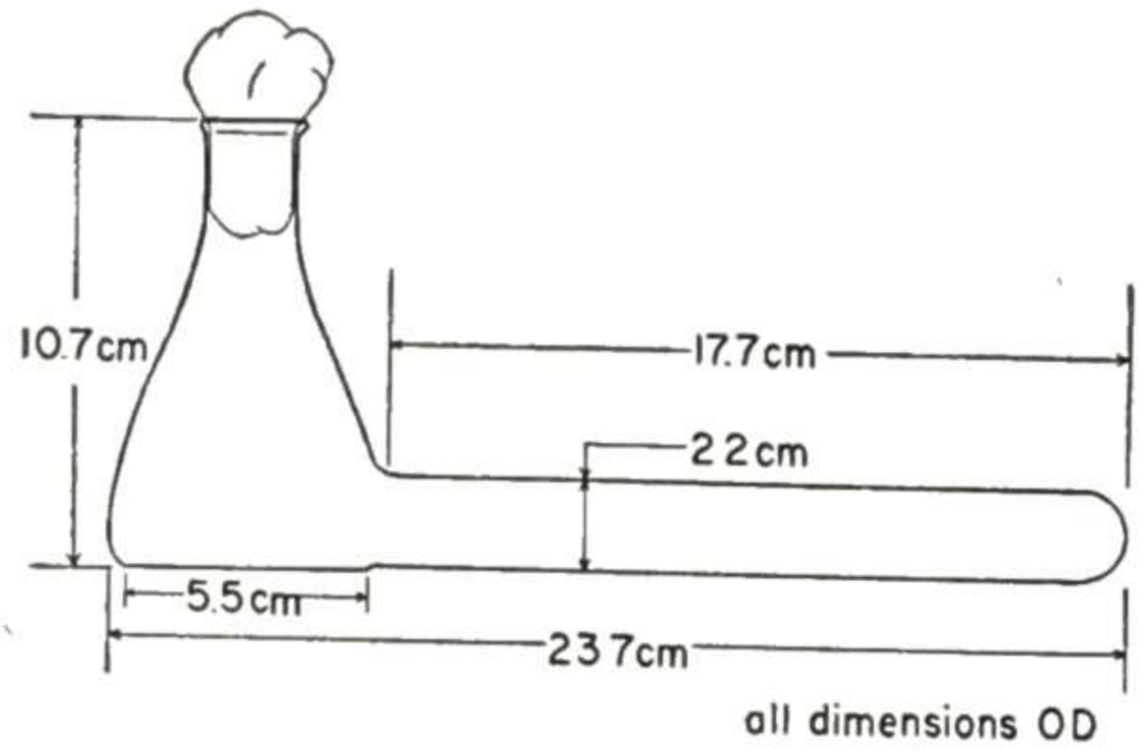


Fig. 120.—Culture flask for turbidimetric estimation of growth of microorganisms. (Courtesy of Sherman, F. G., and Grant, B. M.: J. Lab. & Clin. Med. 37:325-326, February, 1951.)

flasks are clamped to the shaking rack of a Warburg constant temperature bath with the absorption tube in a horizontal plane, perpendicular to the direction of shaking. The flasks should be mounted parallel to one another to ensure the same degree of aeration in each.

Simple Single Cell Technic for Genetic Studies of Bacteria is described by M. R. Zelle[4] (Cornell Univ.). The method is an extension of the usual technic and makes possible critical genetic studies through isolation of several single cell cultures of known relationships descending from one original cell.

Method.—An agar film is prepared by spreading about 0.05 ml. melted 2 per cent agar with a heated 0.1 ml. graduated pipet over the central portion of a 22 × 50 mm. no. 1 cover slip. The cover slip is inverted over a moist chamber about 10 mm. deep which is closed at one end and contains strips of wet blotting paper to prevent drying. A regular pattern of reference holes is punched in the agar film with a sterile micropipet 10-30 μ in outside diameter (Fig. 121). The holes are spaced about two-thirds the width of the field of a 44 × objective apart, thus permitting easy location of cells at given positions. The relationships among the progeny cells are recorded. As many as 150 related single cell cultures may be obtained from one initial cell. The time at which each cell is separated and placed in its position is recorded.

By means of a sterile micropipet with a lumen 10-15 μ diameter, a few cells are deposited near the center of the pattern of reference holes. This is accomplished by allowing the pipet to fill by capillarity from a liquid suspension or culture of bacteria and then touching it lightly to the surface of the agar film. Since any excess moisture in the microdrop is quickly absorbed by the agar, the cells all lie in a horizontal plane with no brownian movement. After deposition of this drop, several individual cells are teased into various central positions by means of a sterile microneedle about 3-5 μ in diameter

(4) J. Bact. 61:345-349, March, 1951.

with a smooth tip. This same needle is used in separating the daughter cells after division and moving them to their predetermined positions. The cells may be moved quite easily by appropriate movements of the microscope stage after touching the needle lightly to the agar nearby. After several individual cells have been put in position, the moist chamber is incubated in a Petri dish at the appropriate temperature. After the first division of the individually posi-

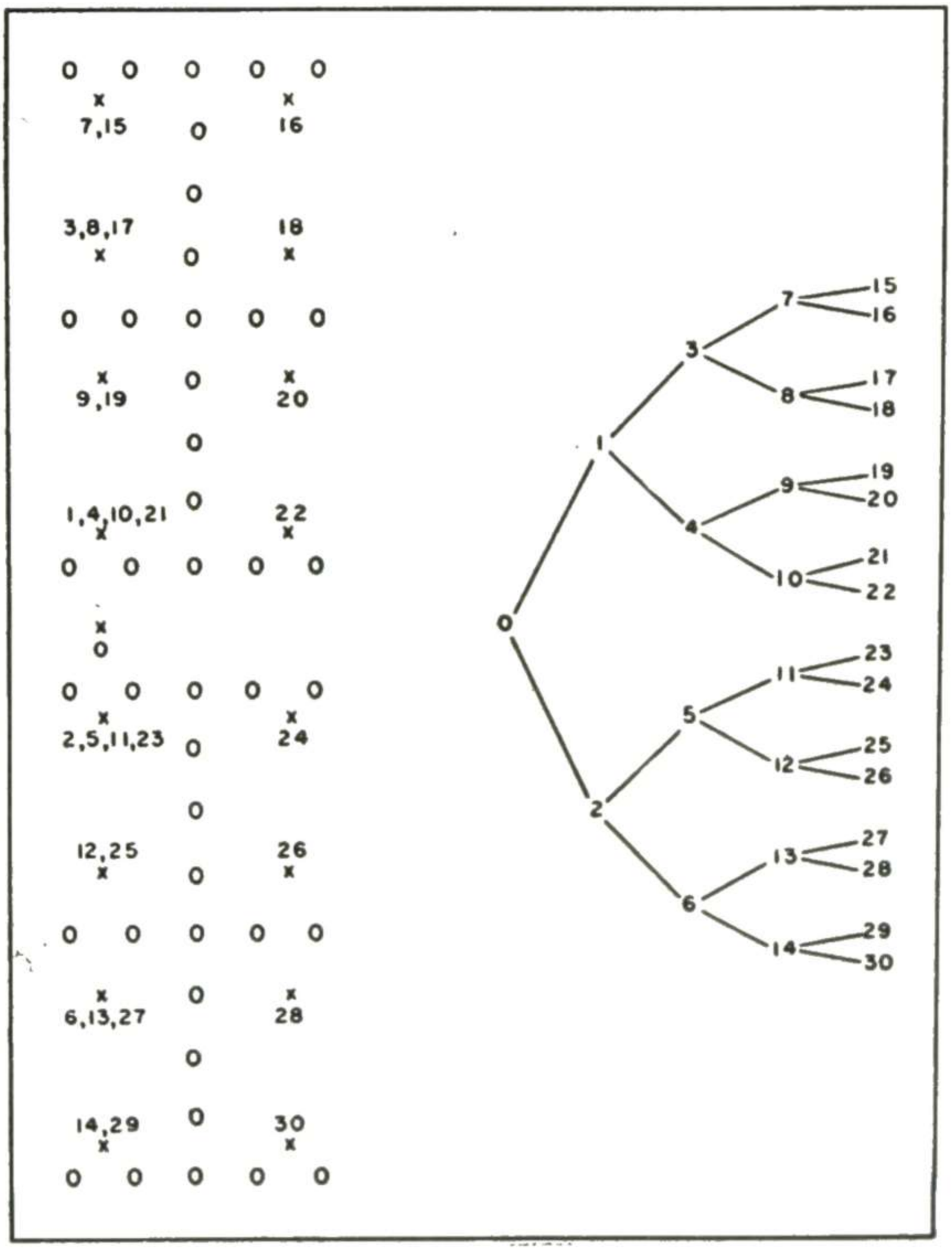


Fig. 121.—Pattern for reference holes (*O*) with number of cells that will be placed at predetermined locations (*X*). Pedigree on right shows relationships of cells. (Courtesy of Zelle, M. R.: J. Bact. 61:345-349, March, 1951.)

tioned cells, one or more may be selected as the initial cell or cells and the two daughter cells of each moved to predetermined locations in the pattern. The excess cells are then picked up with another sterile micropipet by touching the pipet to the agar near the cells. Capillarity will cause the cells to flow into the pipet. Thus the only cells remaining on the agar film are those whose subsequent progeny will continue to be separated in the manner described. After the desired number of progeny cells have been derived from each initial cell, the moist chamber is incubated and each cell allowed to develop

into a microcolony. Each microcolony is then transferred to 0.5 ml. liquid medium and incubated further.

Since most of the actual moving of the cells is done with a microscope stage, any binocular microscope with a good, preferably rotating, mechanical stage may be used. An Abbe 1.40 NA condenser with the top lens removed and 15 × eyepieces are used. The greater working distance of an achromatic, 4 mm., 0.65 NA objective is convenient. A parfocal, 16 mm. objective is used to center micro-

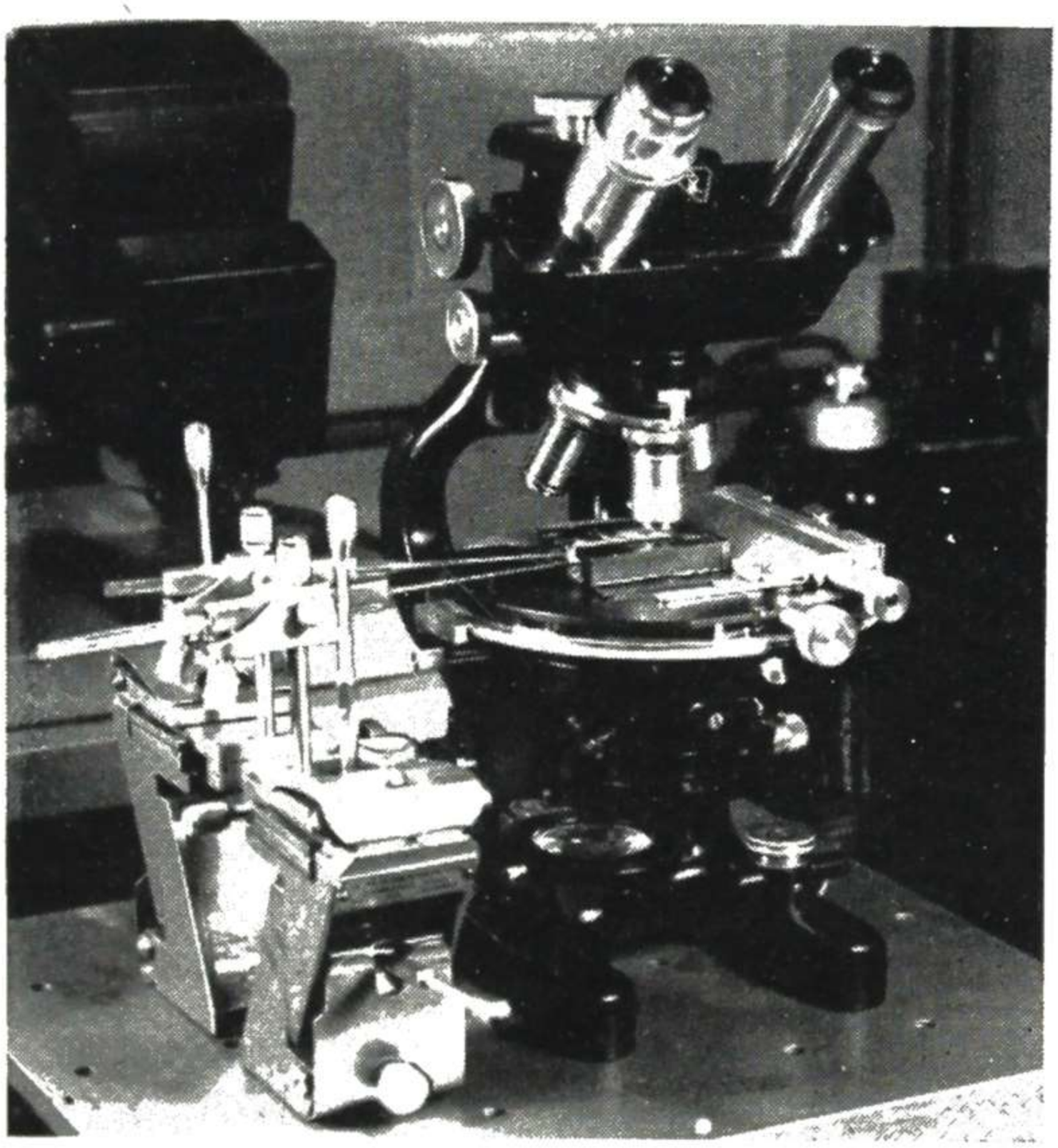


Fig. 122.—Microscope and micromanipulator arrangement. (Courtesy of Zelle, M. R.: J. Bact. 61:345-349, March, 1951.)

pipets in the field and for most operations other than actual separation of the sister cells. Figure 122 shows the micromanipulator and microscope arrangement. The Chambers and Emerson designs of micromanipulators are highly adequate. Pipets are made by first drawing 4 or 5 mm. Pyrex or soft glass tubing into a capillary and then drawing the capillary portion to a fine point in the flame of a microburner having a blunt 26 gauge hypodermic needle for a tip. Solid glass rod is used for making the microneedles. The larger pipets used for transferring microcolonies are most easily made by drawing the capillary to a fine tip and then breaking it after nicking with the edge of a fragment of a carborundum glass-cutting wheel.

New Method for Staining Bacterial Capsules. With the simple procedure described by Ove Möller[5] (Malmö Gen'l Hosp.), capsules can be demonstrated on pneumococcus (Fig.

(5) Acta path. et microbiol. scandinav. 28:127-131, 1951.

123), klebsiella (Fig. 124), escherichia, gaffkya and sarcina, as well as on some organisms previously regarded as uncapsulated, Serratia marcescens and Candida albicans.

METHOD.—A clean slide is flamed and cooled. A small portion of the culture is mixed on the slide with a loopful of solution which contains 0.2 ml. normal serum, 0.15 ml. of 20 per cent glucose and 0.65 ml. normal saline solution. After being allowed to dry in the air, the smear is fixed with a reagent containing 20 ml. of 40 per cent Formalin, 9 gr. lead acetate and 280 ml. distilled water. The fixative is allowed to flow in over the preparation on the slide, which is held on the slant. After it has been allowed to act for 15 seconds, the fixative is poured off. The preparation is then dried between filter

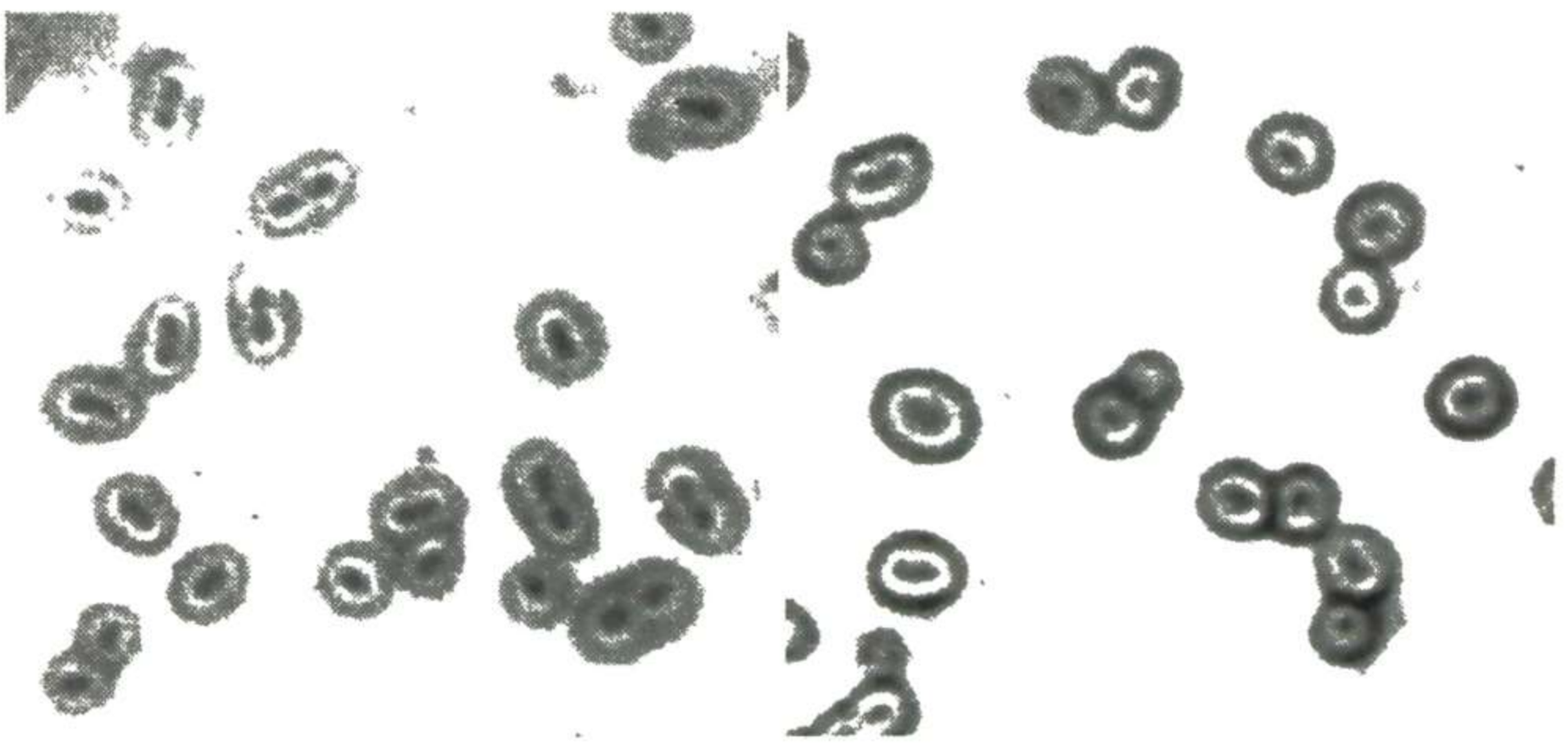


Fig. 123.—Pneumococcus. Fig. 124.—Klebsiella.
(Courtesy of Möller, O.: Acta path. et microbiol. scandinav. 28:127-131, 1951.)

papers. An ample amount of stain (10 ml. of 5 per cent alcoholic crystal violet solution mixed with 90 ml. distilled water) is poured on the preparation and allowed to act for one to three minutes. The preparation is rinsed immediately in saturated copper sulfate solution, usually for not more than 5-10 seconds. It is carefully dried between filter papers and is then ready for microscopic examination. The fixative should be kept in a closed vessel.

Bacterial bodies stain dark bluish violet, whereas the color of the capsules is any of various shades of fairly light violet. In some cases the actual capsule may not be stained deeply but the border of the capsule is nearly always distinct.

Studies on Unusual Streptococci Isolated from Human Urine are reported by Odd Gardborg and L. O. Borgen[6] (Oslo Mun. Hosp.). The characteristic growth in broth was a coherent, loose-meshed, slightly slimy sediment, which on shaking made the broth appear evenly cloudy. Microscopic examination of broth cultures revealed gram-positive cocci and coccobacilli in extremely long chains (Fig. 125). On 10 per

(6) Acta path. et microbiol. scandinav. 28:116-126, 1951.

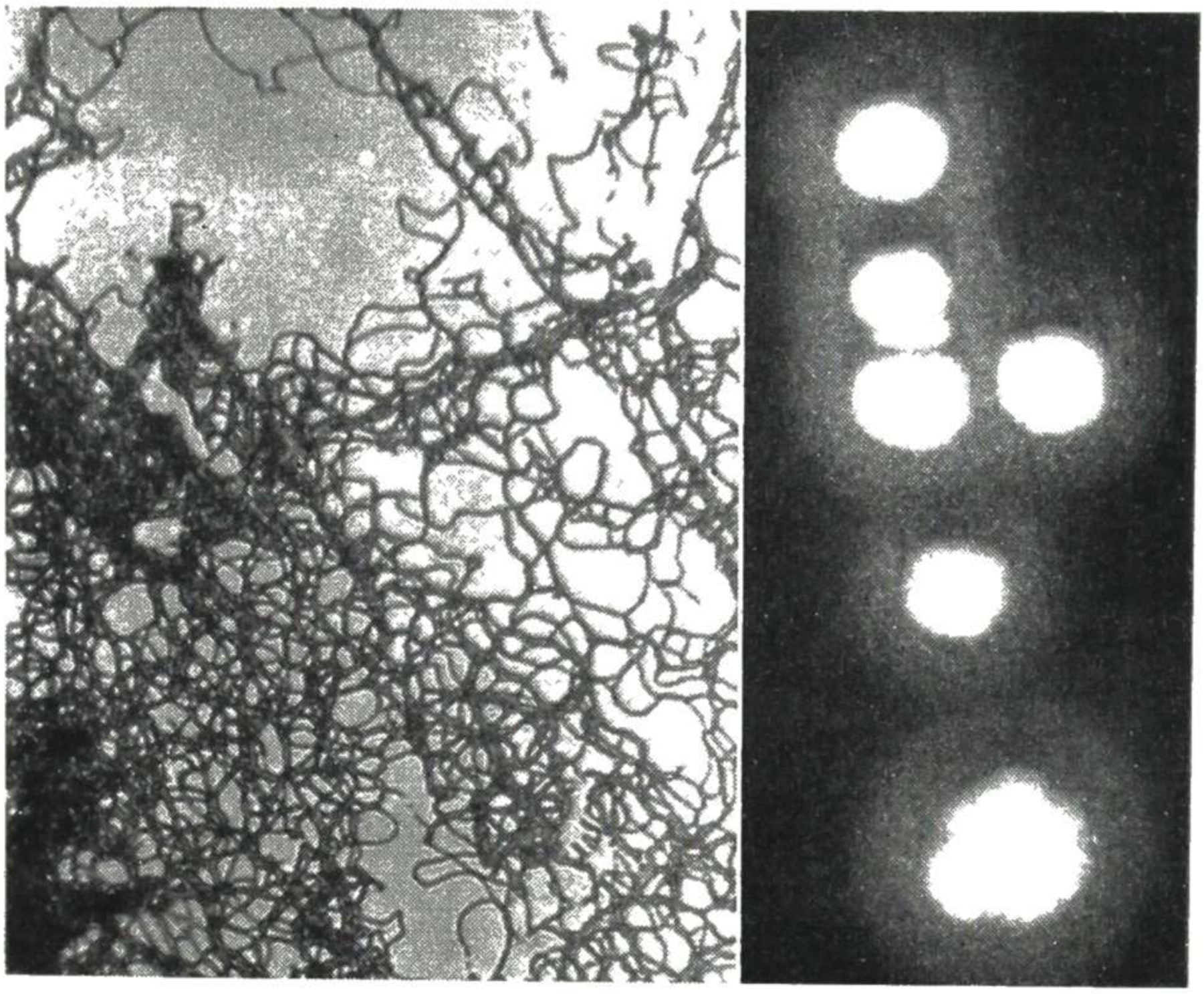


Fig. 125 (left).—Broth cultures stained by Gram's technic; × 700.
Fig. 126 (right).—Colonies on 10 per cent blood-agar; reduced from about × 15 (Courtesy of Gardborg, O., and Borgen, L. O.: Acta path. et microbiol. scandinav. 28:116-126, 1951.)

Results of Fermentation Tests

Carbohydrates	Strains												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Starch	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	—	—	—	—	—	—	—	+	—	—	—	—	—
Sorbitol	—	—	—	—	—	—	—	—	—	—	—	—	—
Glycerol	—	—	—	—	—	—	—	—	—	—	—	—	—
Mannitol	—	—	—	—	—	—	—	—	—	—	—	—	—
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+
Saccharose	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	+	—	—	+	—	+	—	—	—	—	+	—	—
Salicin	+	+	—	—	+	—	—	+	—	—	—	+	—
Inulin	—	—	—	—	—	—	—	—	—	—	—	—	—
Esculin	—	—	—	—	—	—	—	—	—	—	—	—	—
Xylose	—	—	—	—	—	—	—	—	—	—	—	—	—
Dulcitol	—	—	—	—	—	—	—	—	—	—	—	—	—
Levulose	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	—	—	—	—	—	—	—	—	—	—	—	—	—
Dextrin	+	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	—	—	—	—	—	—	—	—	—	—	—	—	—
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+

cent human blood-agar there was good growth, with formation of a striking green zone around both surface and deep colonies (Fig. 126) after 24 hours' incubation at 37 C. All strains were facultative anaerobes. None produced soluble hemolysin. Results of fermentation tests are shown in the table. No strain produced mucoid colonies on saccharose agar or increased the viscosity of saccharose broth. All were hippurate negative and none produced ammonia from peptone. None showed liquefaction of gelatin, coagulated ox serum or coagulated human plasma, was soluble in bile or produced nitrite from nitrate. All acidified and coagulated litmus milk. With regard to catalase, addition of hydrogen peroxide to five day old agar slant cultures gave negative results for all strains. No strain was sensitive to streptomycin but all failed to grow on penicillin medium. When injected intraperitoneally into mice, five strains were pathogenic and caused death in one to four days. Pure cultures of the organisms were obtained from the heart blood and peritoneal exudate of the animals.

It seems proper that this organism should be considered a new and distinct species within the viridans group. It may be a mutant of one of the already recognized viridans species, but determination of this requires further extensive investigation.

Investigation of Cultivation of Pleuropneumonia-Like Organisms from Man. Harry E. Morton, Paul F. Smith and Paul R. Leberman[7] (Univ. of Pennsylvania) found that the following basal medium adequately supports growth of pleuropneumonia-like organisms, is easily prepared and is clear. To an infusion prepared from 50 Gm. bacto®-beef heart for infusion and 1,000 ml. distilled water are added 1 per cent bacto®-peptone, 0.5 per cent sodium chloride and 1.5 per cent agar. The pH is adjusted before sterilization to 7.8 colorimetrically with phenol red. When this medium was used with 25 per cent ascitic fluid, pleuropneumonia-like organisms were isolated from the genitourinary tract of 20 per cent of 80 males and 44.5 per cent of 18 females.

Other peptones found desirable for promoting growth of these organisms are bacteriologic peptone (Parke, Davis & Company), bacto®-tryptose and bacto®-yeast extract. Rabbit serum in a concentration of 20 per cent is a satisfactory supplement which may be used in place of ascitic fluid. Other

(7) Am. J. Syph. 35:361-369, July, 1951.

serums in concentrations greater than 10 per cent cause inhibition of growth. There appears to be no difference in the amount of growth obtained by incubation in aerobic or anaerobic conditions.

Culture Medium for Detecting and Confirming Escherichia Coli in 10 Hours is described by George H. Chapman[8] (New York City). Because E. coli does not reduce triphenyltetrazolium chloride, whereas other coliforms rarely fail to do so, the culture medium is made by adding 40 mg. of this agent to 1 L. tergitol-7 agar just before pouring. Inoculation is made on the surface with a glass spreader. On this medium, surface colonies of E. coli produce greenish yellow colonies surrounded by a yellow halo. Other coliforms produce dark red colonies. If the medium is warmed before inoculation and then rapidly raised to 37 C., colonies can be recognized in 6 hours and final reading made in 10 hours.

Metastatic Suppurative Arthritis with Subcutaneous Emphysema Caused by Escherichia Coli. Joseph M. Miller and Ralph L. Engle, Jr.[9] (Fort Belvoir, Va.) report a case.

Woman, 55, was hospitalized because of fever, nausea, vomiting, malaise and generalized myalgia for five days. There was moderate tenderness over the bilateral costovertebral angle, but examination of the joints was negative. White cell count was 10,500, with 75 per cent neutrophils. A sickle cell preparation was strikingly positive within two hours. Blood culture was sterile. On the 4th hospital day, she had a shaking chill, diffuse abdominal pain, temperature of 107 F., crepitant râles at the right lung base, diffusely tender abdomen without spasm and tenderness in the calves and over the tibiae. White cell count was 5,500. Blood culture at the height of fever was negative. Urine culture showed E. coli. On the 10th day, she had another shaking chill and, in addition to pain in the right knee which had been present for two days, had pain in the left knee. Both joints were hot, swollen and tender. Despite penicillin and streptomycin therapy, chills and fever continued over the next week. Urine cultures became negative. Aspiration of the right knee joint on the 19th day yielded 20 cc. thick, brownish, foul-smelling pus, which on smear and culture showed a nonhemolytic streptococcus and hemolytic E. coli. Sensitivity studies revealed that the E. coli was sensitive to not less than 20 μg. streptomycin/cc. and resistant to 0.15 mg. sulfadiazine/cc. Crepitation on palpation confirmed presence of gas in the soft tissues surrounding the knee. Surgical drainage yielded about 800 cc. brown, foul-smelling pus, which again showed a nonhemolytic streptococcus and hemolytic E. coli. Anaerobic cultures were negative for clostridial organisms and bacteroides. On the 32d day, surgical incision of the left knee released

(8) Am. J. Pub. Health 41:1381, November, 1951.
(9) Am. J. Med. 10:241-248, February, 1951.

400 cc. foul pus which also showed the same two organisms. Despite local instillation of streptomycin the knee wounds continued to drain, the patient became steadily worse and she died on the 35th day. Postmortem culture of heart blood revealed nonhemolytic streptococci and hemolytic E. coli. E. coli was cultured from a liver abscess and from each kidney.

Analysis of clinical and autopsy observations indicated that the patient had a sickling trait without the specific anemic crisis. The major clinical problem was that of combating an overwhelming bacterial infection caused by E. coli and nonhemolytic streptococci. The failure to obtain a positive blood culture during life was probably due to the chemotherapy. The severe chronic pyelonephritis found at autopsy indicated that the kidneys were the primary focus for the bacteremia, with metastatic suppuration in the liver and arthritis. Since sugar fermentation with liberation of gas by E. coli is possible, this organism was regarded as the source of the gas present in the periarticular tissues. The failure of streptomycin may have been due to development of streptomycin resistance during treatment. Aureomycin was not available at the time.

Laboratory Control of Enteric Fevers. According to A. Felix[1] (Central Enteric Reference Laboratory, London), control of enteric fevers depends ultimately on control of the chronic carrier. Recent advances in laboratory methods which facilitate detection of the chronic carrier include: (1) introduction of refined culture mediums for isolation of typhoid and paratyphoid bacilli, (2) typing of typhoid and paratyphoid B bacilli by Vi bacteriophage and (3) the Vi-agglutination test.

The lower the incidence of the disease, the greater is the necessity for using the typing method in every case. When a typhoid outbreak of considerable size occurs, it is usually not difficult to detect the carrier concerned. In sporadic or small groups of cases the greatest difficulties are presented and are most readily overcome with the typing method. The revised typing scheme now in general use and the complete list of references to the 24 typhoid Vi-phage types and subtypes were published by Craigie and Felix in 1947.

It was apparent early that to avoid faulty technic and consequent confusion standardization of the typing procedure in enteric phage was necessary. So far 24 countries have joined the International Scheme for this purpose, and workers from

(1) Brit. M. Bull. 7:153-162, 1951.

many of these countries have studied at the International Reference Laboratory for varying periods. Results have been so satisfactory that efforts will be continued until the Sixth International Congress for Microbiology is held in Rome in 1953, when an additional report will be made.

A necessary prerequisite for success in control of enteric fever in those countries where the incidence is as low as it is in Great Britain is to have a central laboratory and a central bureau serving the whole country. Each country should join the International Scheme and be represented by a national reference laboratory. Within its own sphere of action, each laboratory should make arrangements according to local conditions. With the new laboratory methods, which put epidemiologic investigation of the enteric fevers on an entirely new basis, public health authorities are now in a position to reorganize and intensify the campaign against the chronic carrier. The result of a long term policy based on close co-operation among epidemiologists, clinician and laboratory worker may be complete eradication of enteric infection.

[References to phage typing include: Craigie, J., and Felix, A.: Typing of typhoid bacilli with Vi bacteriophage; suggestions for its standardization (Lancet 1:823-827, June 14, 1947); Desranleau, J. M., and Martin, I.: Bacteriophage typing in the province of Quebec (Canad. J. Pub. Health 41:128-132, March, 1950). The latter authors isolated 49 different strains of Vi-phages of S. typhosa: 7 of type I, 10 of type II, 6 of type III, none of type IV, 2 new strains of type V, one new strain of type VI and 23 not classified.—Ed.]

Food Poisoning and Food Hygiene. Betty C. Hobbs[2] (London) reports that of 3,495 incidents of food poisoning investigated by the Public Health Laboratory Service during 1941-48, about 91 per cent were caused by organisms of the salmonella group, 3 per cent by staphylococcic enterotoxin, 2 per cent by other bacteria and 0.3 per cent by chemicals; 4 per cent were ascribed to unknown causes. The steady increase in recorded food poisoning is due partly to better reporting and partly to the fact that the British public is eating out oftener.

There are no laboratory methods for recognizing the presence of staphylococcic enterotoxin in food. It is essential to isolate and type the responsible organism before it is worth while examining the food handlers. Most staphylococci isolated from toxic food fall into a small number of bacteriophage and serologic types. Unfortunately, these types are fairly

(2) Brit. M. Bull. 7:167-170, 1951.

widespread among the general population. It is therefore not justifiable to incriminate food handlers harboring such types unless the same types have been first isolated from foodstuffs handled by them.

There is no exact information on the minimal number of staphylococci necessary to produce sufficient enterotoxin in foodstuffs to cause symptoms. If the food has been heated after bacterial multiplication has occurred, it may appear to be sterile culturally, but heat-resistant toxins may be present. Direct microscopic examination should reveal the nature and extent of the flora before heat treatment. Coagulase-positive staphylococci, particularly those of the food-poisoning type, are rarely found in normal foodstuffs.

There are numerous outbreaks of food poisoning (the indeterminate group) in which no known pathogenic bacterium or poisons can be found. Many of these may possibly be caused by the inordinate growth of organisms which are harmless when ingested in small numbers. It is too early to say whether the massive bacterial contamination results in formation of toxic products from the food or whether the organisms when present in large numbers give rise to an actual infection of the intestine. Possibly some organisms act in one way and some in the other. A clinical type of food poisoning has been recognized with an incubation period midway between that of staphylococcic enterotoxin food poisoning with its rapid onset and that of salmonella infection with its delayed onset. Anaerobic spore-bearing bacilli have been reported as the causative organism in a few outbreaks, and Clostridium welchii has been suspected. From 50 to 100 per cent of stool samples from patients have been positive for heat-resistant Cl. welchii, and this organism has been isolated from only about 4 per cent of specimens from normal persons. The reservoir of this organism is not yet clear, nor is it known whether the organism is present in meat when it arrives at the kitchen. Persistent carriers are known to exist among kitchen staffs. The practice of cooking meat and gravy in large containers and allowing them to cool slowly overnight before serving the next day favors occurrence of this type of food poisoning.

In 109 of 191 recorded outbreaks meat was the proved or suspected vehicle of infection. In 90, the meat had been made up, manipulated or processed before being served. In 10 outbreaks, prepared fish dishes were cited. Freshly cooked fish

was not mentioned. Puddings or other sweet dishes were associated with 25 outbreaks, milk with 14, eggs with 12 and a miscellaneous group including stews, soups, etc., with 21.

Surveys of kitchens used by the public indicate that too little space is allotted to refrigeration. Washing up methods are usually poor, except when thermostatically controlled sterilizing rinse sinks are used. High bacterial counts are common in large food containers washed without special precautions, but if the containers are sterilized by steam, drained and not wiped with a cloth the count is reduced almost to zero. Partial sterilization of eating and drinking utensils by rinsing in water from 77 to 82 C. is the safest way to protect the public from the unhygienic and potentially dangerous results of haphazard methods. To assure complete co-operation of those working in the kitchen, it is essential that they understand why clean hands, refrigeration and careful washing of utensils are essential.

Bone Marrow Lesions in Human Brucellosis. Ben Fisher[3] (Michael Reese Hosp.) reports two cases.

Case 1.—Man, 41, who always drank unpasteurized milk from untested cattle subject to frequent abortions, had chills, fever and malaise and lost weight. A provisional diagnosis of brucellosis was made. The sedimentation rate was increased, and serum agglutination for Brucella abortus was positive in a titer of 1:320 and later 1:800. Blood cultures were negative. Sternal aspiration showed a

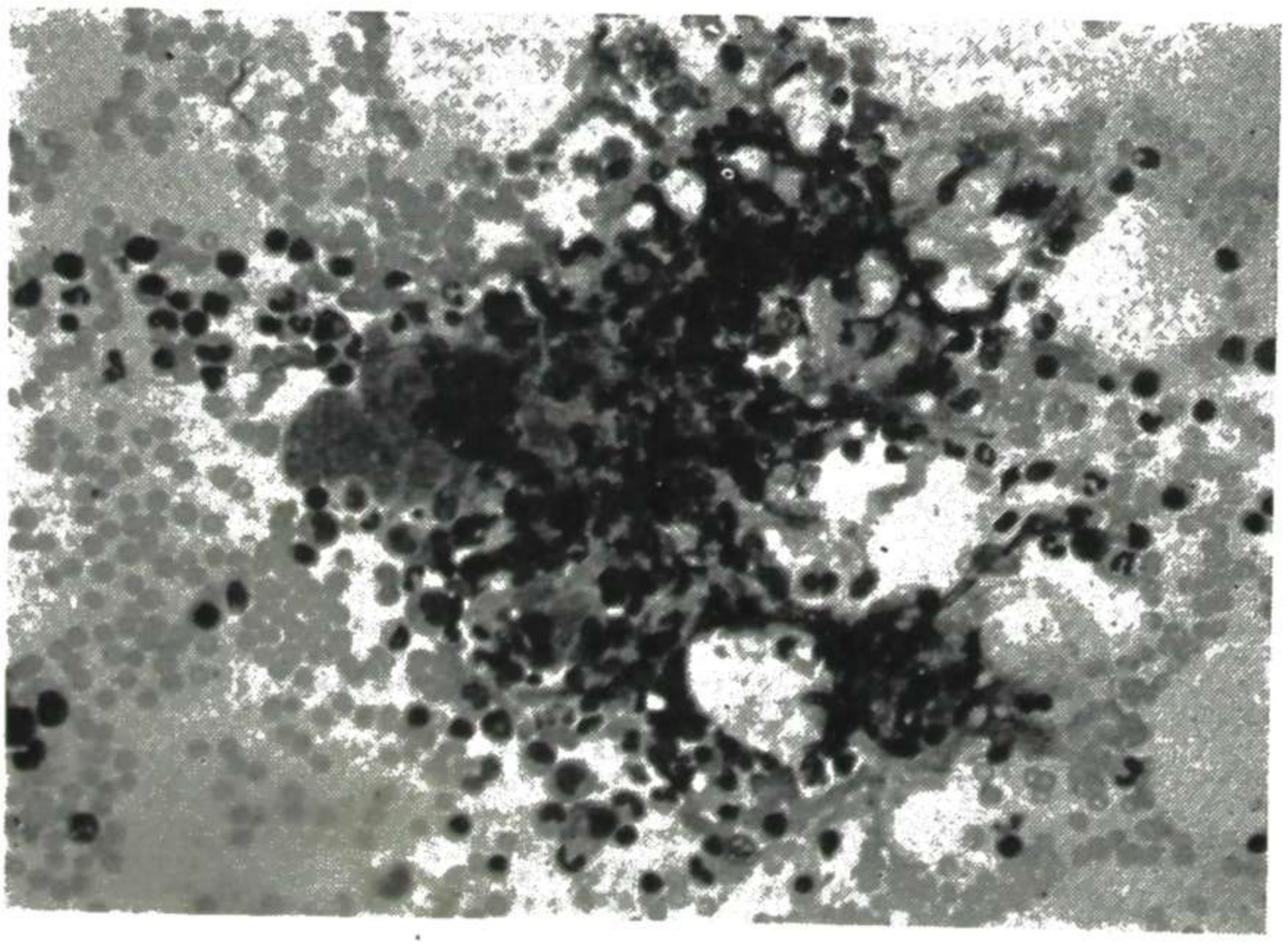


Fig. 127.—Granuloma in direct marrow film. Wright's stain: reduced from × 500. (Courtesy of Fisher, B.: Acta haemat. 6:31-37, July, 1951.)

(3) Acta haemat. 6:31-37, July, 1951.

moderately cellular marrow with an erythrocyte-granulocyte ratio of 1:2. Sections and films of the marrow revealed epithelioid granulomas. Marrow cultures were negative.

Case 2.—Man, 39, had a history of ingestion of raw milk and symptoms of weakness, malaise, chilliness, perspiration, frontal headaches, arthralgia, myalgia and diarrhea. Agglutination was positive in a titer of 1:3,200 and a blood culture was positive. Sedimentation rate was increased. Sternal aspiration revealed an extremely cellular marrow. Erythrocyte-granulocyte ratio was 1:4.4. Sections and films showed small granulomas.

The lesions were similar to nonspecific granulomas and were chiefly composed of epithelioid and reticulum cells. Megakaryocytes and plasma cells were present in and near the granulomas. Many segmented and nonsegmented neutrophils were embedded in the lesions. Uninvolved marrow particles appeared normal; granulocytic and erythroid cells were present in normal numbers and showed no maturation arrest (Fig. 127).

The variability of the manifestations and course of brucellosis, as well as the inconclusiveness of diagnostic tests, makes definite diagnosis difficult. Presence of granulomas in marrow obtained by sternal aspiration offers additional evidence.

Yolk Sac Technic for Routine Isolation of Brucella: Injection of Clotted Blood Specimens into Embryonating Eggs with Recovery of All Three Species is reported by Kathleen Gay and S. R. Damon[4] (Indiana State Board of Health).

Clots from blood received routinely for diagnostic agglutination tests were injected into the yolk sacs of 3, 4 and 5 day old embryos and subcultured periodically. The technic of injection and withdrawal varies with the position of the yolk sac during development (Fig. 128). The older the embryo, the more the yolk shifts from an anterosuperior to a posteroinferior position. Control procedures consisted of crystal violet (C-V) tryptose broth cultures and guinea pig inoculations.

From 96 clots considered satisfactory for egg inoculation, there were 14 recoveries: Brucella melitensis, 1; Br. suis, 4, and Br. abortus, 9. Using the same specimens, guinea pig inoculation yielded one brucella strain and C-V tryptose broth six. Eleven isolations were obtained with specimens from patients with acute symptoms and three from asymptomatic patients. No recoveries were obtained with specimens from patients with chronic complaints. All isolations were obtained with specimens from persons with agglutination titers of at

(4) Pub. Health Rep. 66:1204-1211, Sept. 21, 1951.

least a 1:80 serum dilution. Nearly all recoveries were obtained by the eighth day of incubation of the injected specimens.

Fifteen clots from guinea pig and swine blood were tested and four Br. suis strains were recovered.

The egg embryo yolk sac technic is recommended because

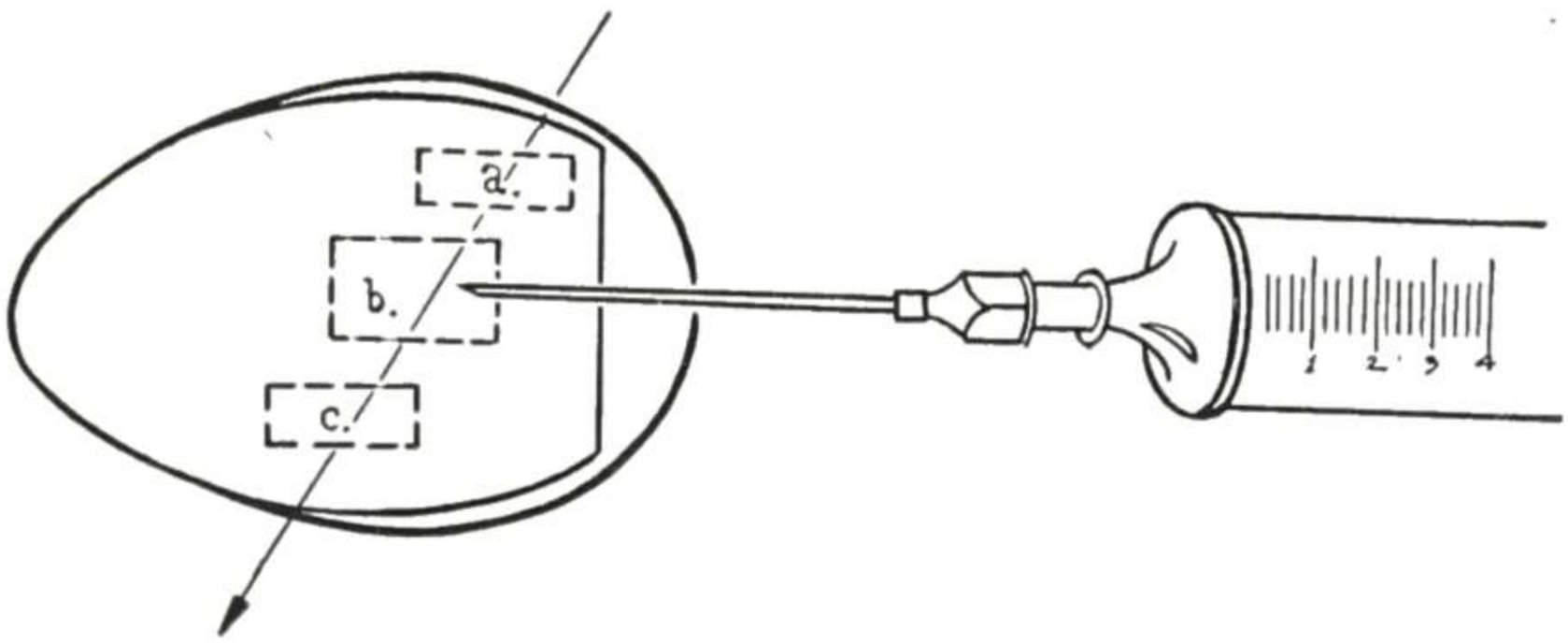


Fig. 128.—Relative positions of yolk sac as embryo develops. Each rectangle represents approximate center of yolk sac at 3 days (*a*), 4-5 and 9-10 days (*b*) and 14 days (*c*). (Courtesy of Gay, K., and Damon, S. R.: Pub. Health Rep. 66:1204-1211, Sept. 21, 1951.)

(1) it provides living tissue cells in a compact medium; (2) permits recovery of all three types of brucella; (3) gives comparatively quick results and (4) entails a minimal amount of danger for laboratory personnel.

Charcoal Agar Culture Medium for Preparing Hemophilus Pertussis Vaccine is described by H. M. Powell, C. G. Culbertson and P. W. Ensminger[5] (Indianapolis). The medium increases greatly the growth of H. pertussis.

METHOD.—The pertussis fluid culture medium described by Cohen-Wheeler was used as the agar base. To this was added 2-3 per cent agar and 0.4 per cent charcoal (Norite sg.). The complete medium is put in large test tubes to make slants for phase I stock cultures and in toxin bottles. They are sterilized in an autoclave. The temperature for incubating cultures should be 35-36 C.

Studies with different strains indicated that a good many subcultures may be made on charcoal agar without degrading the strain as a good vaccine producer.

Acid-Fast Nontubercle Bacilli in Paraffin. Demonstration of acid-fast bacilli in tissue sections is often considered sufficient to establish the diagnosis of tuberculosis, but O. J. Wollenman, Jr., P. O'B. Montgomery and T. B. Foard[6] (Veterans' Admin. Hosp., McKinney, Tex.) point out that such bacilli may be nontubercular. Nontubercle acid-fast bacilli

(5) Pub. Health Rep. 66:346-348, Mar. 16, 1951.
(6) Am. J. Clin. Path. 21:295, March, 1951.

were found in tissue sections which were later proved to be derived from the tissue mat used in preparing microscopic sections. Most of these bacilli were slender, slightly curved rods 1.5-3 μ long and 0.3-0.4 μ wide. Some were short, plump, straight rods,

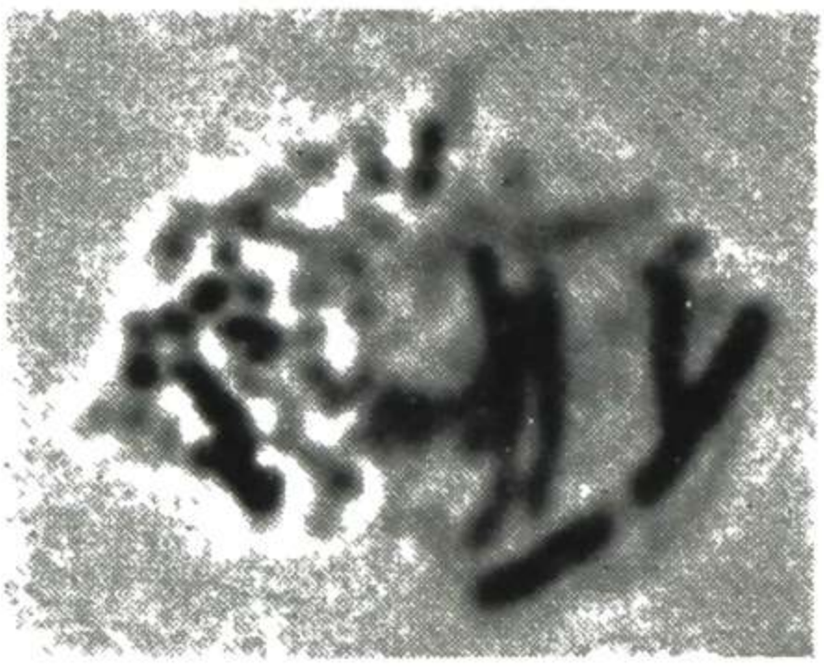


Fig. 129.—Nontubercle acid-fast bacilli found in tissue mat; × 3600. (Courtesy of Wollenman, O. J., Jr., *et al.*: Am. J. Clin. Path. 21:295, March, 1951.)

whereas others showed bipolar swellings and occasional bacilli showed beading (Fig. 129). No branching or spore-forming organisms were seen. The organism could not be grown but its morphologic and staining characteristics suggested that it may be Mycobacterium phlei.

Effective Use of Penicillin to Reduce Contamination in Sputum Concentrates To Be Examined for Tubercle Bacilli is described by John N. Abbott[7] (State Dept. of Health, N. Y.).

METHOD.—All specimens were homogenized in an equal volume of 4 per cent NaOH for 15 minutes in a three dimensional shaking machine. They were centrifuged at a relative centrifugal force of 1,800-2,000 for 20 minutes. The sediment was neutralized with HCl in the presence of phenol red. Several tubes of Löwenstein-Jensen medium were then inoculated. Cultures were incubated at 37 C.

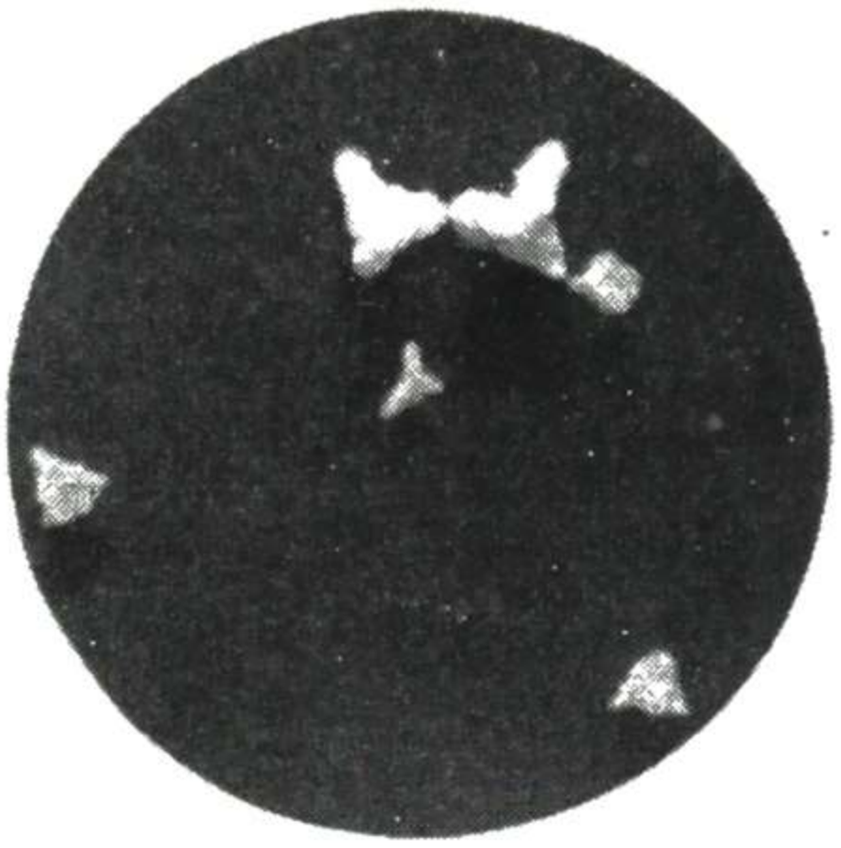
A total of 3,554 sputa were treated in this manner at various intervals throughout the year. In addition, 9,477 sputa were treated by adding commercial crystalline penicillin G in sterile water to the neutralized concentrates just before inoculation of the culture medium. The penicillin solution contained 200 units/0.1 ml. and was added in 0.1 ml. amounts.

Tubercle bacilli survived in the penicillin-treated concentrates and their final recovery by culture on a solid medium was not significantly affected. Rate of contamination of the cultures was reduced 50 per cent by penicillin and the number of positive cultures was increased. Early rate and final volume of growth of bacilli diminished slightly in the penicillin-treated cultures. The action of penicillin lasted 3-10 days.

Growth and Enumeration of Mycobacteria in Transparent Agar Medium. Diran Yegian and Vera Budd[8] (Ray Brook, N. Y., Tuberculosis Hosp.) cultivated mycobacteria on the solid transparent medium described by Dubos and Middle-

(7) Am. J. Pub. Health 41:287-291, March, 1951.
(8) Am. Rev. Tuberc. 64:81-86, July, 1951.

brook. After 30 days' incubation, deep colonies of mammalian tubercle bacilli usually measured 0.5 mm. in diameter. The colony morphology at this time or on further incubation was extremely variable. Some appeared scaly and flat, others raised and rough. A few showed edges more serrated than lobulated and still others resembled odd-shaped crystals. A number of colonies broke through the medium to the surface, and the subsequent growth was heaped up irregularly (Figs. 130-132).



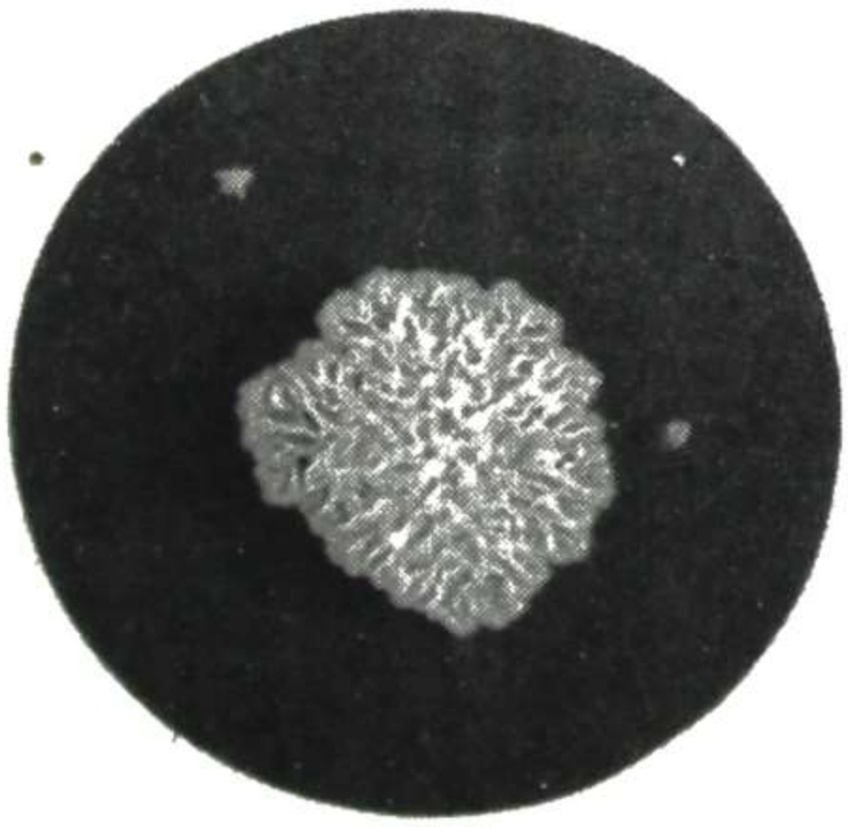


Fig. 130 (above left).—Irregular outline and variable morphology of deep colonies of Mycobacterium tuberculosis, incubated five weeks.

Fig. 131 (above).—Heaped-up appearance of colonies that pierced surface of medium after 10 weeks' incubation.

Fig. 132 (left).—Large surface colony and two deep colonies of Mycobacterium ranae incubated eight days.

(Courtesy of Yegian, D., and Budd, V.: Am. Rev. Tuberc. 64:81-86, July, 1951.)

This variation presumably was the result of the varying depth of the colony in the medium. No deep colonies were lenticular, a form often observed among nonacid-fast bacterial colonies. No changes were observed in the cellular morphology or the staining properties of the bacteria obtained from the deep colonies.

Comparison of pour plate and surface plating methods showed a numerically greater colony growth in the former. This was probably due to better dispersion of bacterial cells in the pour plate and also to the fact that the micro-organisms were not in contact with a diluent for any significant time.

It is generally agreed that tubercle bacilli grow more rapidly in the presence of an ample supply of oxygen, but it should be emphasized that this organism does multiply even when the supply of oxygen is limited. The observations in this study support the statement of Dubos that "any oxygen pressure compatible with the life of adult animal cells will be adequate, not only for the survival of tubercle bacilli but also for their multiplication even though at a slow rate."

Blood Mediums for Culturing Tubercle Bacilli: Evaluation by Means of Serial Dilution Method. Arthur W. Frisch and Maurice S. Tarshis[9] (Univ. of Oregon) used the following mediums in diluting sputum concentrates to the limit at which growth of organisms can be obtained: (1) Corper-Cohn autoclaved in steam-air mixture at 16 lb. pressure for 90 minutes, (2) Löwenstein modified by Jensen and Holm, (3) blood agar base plus 1 per cent glycerin plus 25 per cent citrated human bank blood and (4) agar-agar plus 25 per cent citrated human bank blood.

Sputum specimens, consisting of 24 hour samples from known tuberculous persons, most of whom had been or were receiving streptomycin, para-aminosalicylic acid, or both, were concentrated by the trisodium phosphate technic. Tenfold serial dilutions were prepared in tubes containing 0.85 per cent sodium chloride, a single pipet being used for each dilution.

Each medium received an inoculum of 0.1 ml., beginning with the lowest concentration and progressing to the highest. The tubes were incubated at 37 C. in a slanted position for five days and placed upright for the rest of the test period. The experiment lasted eight weeks.

The sputums were divided into two groups: Group 1 included those sputums from which tubercle bacilli were not recovered in any dilution on one or more of the culture mediums. In 126 trials, growth was obtained 9 times on Löwenstein, 22 times on blood agar, 27 times on agar-agar and not at all on Corper-Cohn medium. In group 2, growth occurred in one or more dilutions on all four mediums. The Löwenstein medium appeared superior in this series with 93 successful isolations in 186 trials.

This method of evaluating culture mediums for their ability to grow tubercle bacilli from serially diluted sputum con-

(9) Am. Rev. Tuberc. 64:551-556, November, 1951.

centrates possesses the following advantages. (1) Organisms are isolated under routine diagnostic conditions. (2) A minimal inoculum is assured at some point in the titration. (3) Specimens which do not contain tubercle bacilli can be safely excluded. (4) The data are comparable, and statistically valid results can be obtained with relatively few cultures.

Mediums containing human blood were equal to the Löwenstein medium for diagnostic purposes. Agar-agar with 25 per cent bank blood scored highest, with 114 positives in a total of 312 trials. It is believed, therefore, that blood-containing mediums are preferable to others because they are economical, readily available, easily prepared, stable on storage and grow tubercle bacilli rapidly from small inocula.

Bacteriology of Leprosy. According to John H. Hanks[1] (Harvard Univ.) the failure to obtain growth in vitro of leprosy bacilli on bacteriologic mediums can be explained adequately by chemical observations which indicate that these organisms cannot obtain energy from any of the carbon sources ordinarily known to serve this purpose. The rapid decline in viability and metabolic activity of leprosy bacilli, even under favorable circumstances, indicates that their metabolic limitations produce extremely unfavorable effects when the organism is outside of host cells. Before there is any reasonable prospect that this organism can be cultivated on bacteriologic mediums, the nature of this difficulty must be defined and its cause removed.

Results of recent investigations suggest that there is a close relation between the viability or infectiousness of leprosy bacilli and their capacity to transfer hydrogen to an artificial acceptor system. Separate biochemical studies on respiration and dehydrogenation by bacterial suspensions have provided evidence which supports this concept. Studies of Mycobacterium phlei, one of the most active of the cultivable mycobacteria, revealed a general correlation between colony counts and endogenous metabolism. It may therefore be anticipated that more rapid and precise methods will be available for investigating murine leprosy than those previously used.

Actinomyces Muris Endocarditis Treated with Chloramphenicol. J. F. Stokes, I. R. Gray and E. J. Stokes[2] (University College Hosp.) report a case. This organism was first

(1) Ann. New York Acad. Sc. 54:12-19, 1951.
(2) Brit. Heart J. 13:247-251, April, 1951.

recovered from the blood and synovial fluid of patients during an epidemic of a febrile illness with arthritis and morbilliform rash at Haverhill, Mass., in 1926. It is also the cause of some cases of rat bite fever.

Woman, 27, had handled a dead rat which her dog caught 1½ years previously. About a month later she had a brief febrile illness. Two months later she had fever, sweating, fatigue and pain in the hands and knees. There was severe pain in the left upper quadrant of the abdomen. The history disclosed that she had had two attacks of rheumatic fever which responded well to salicylates. During the succeeding eight months the temperature rose intermittently for periods of as long as three weeks to levels as high as 103 F. Joint

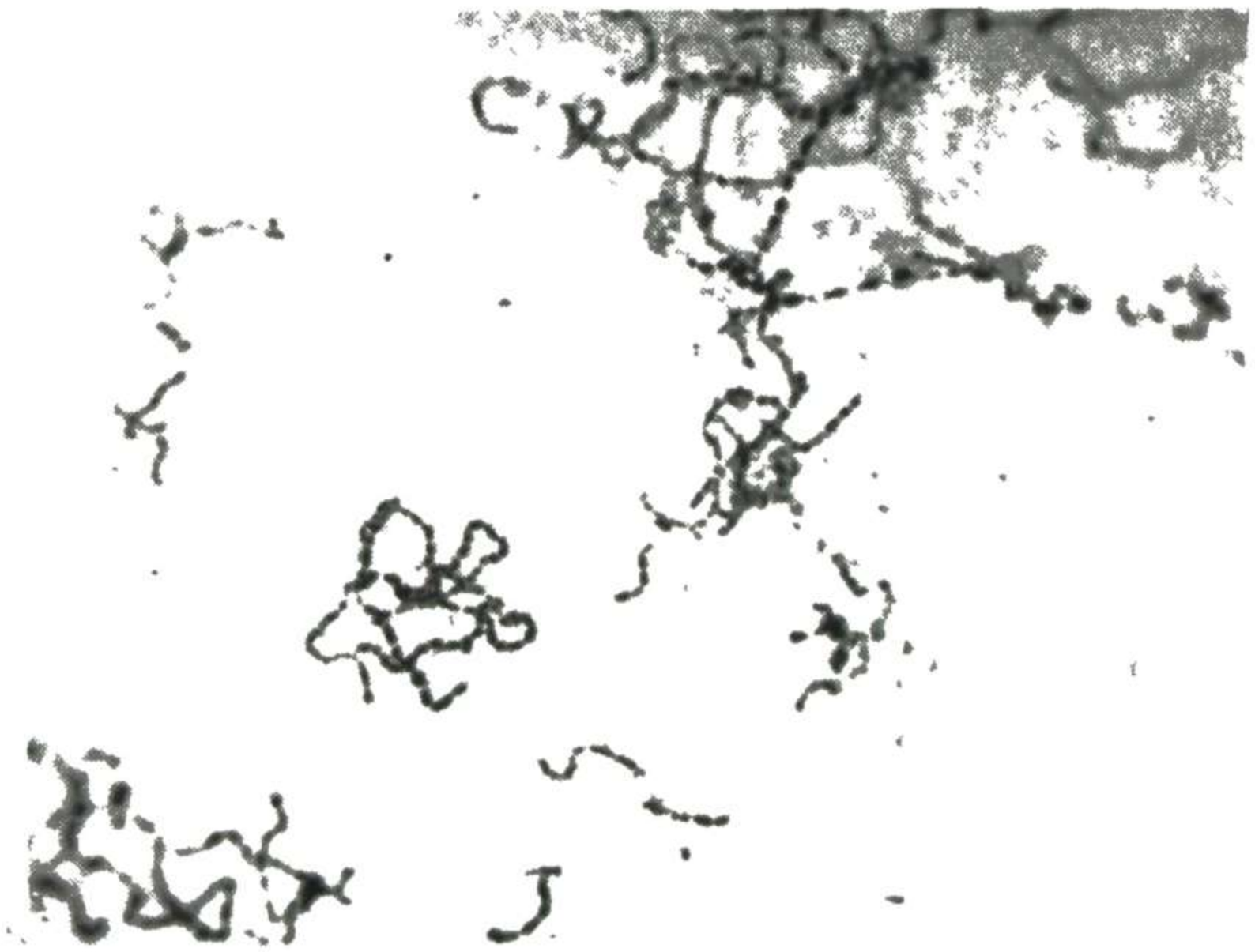


Fig. 133.—Culture from original blood "liquoid" broth. Gram stain; enlarged from × 850. (Courtesy of Stokes, J. F., *et al.*: Brit. Heart J. 13:247-251, April, 1951.)

pains were severe. Many rheumatic nodules appeared on the wrists and elbows, and were present for about two months. Joint pains continued despite salicylate and penicillin therapy. For about six months she remained well but then pain recurred in the fingers and toes and was accompanied by swelling and tenderness in the pulps and bruising under the nails. She became increasingly tired and short of breath. Physical examination showed an enlarged heart, with signs of mitral stenosis and free aortic regurgitation but none of congestive failure. The spleen was palpable. There was a red, tender swelling on the right shin. During the first week of hospitalization, numerous embolic manifestations were seen, including splinter hemorrhages under the nails, Osler's nodes in the pulps of the fingers and toes, and small spots on the palms of the hands. Both anaerobic and aerobic blood cultures revealed very pleomorphic, irregularly staining, but mainly gram-negative organisms, which could be recognized to be Actinomyces muris. A few branching, numerous

serpent and some club forms were seen (Fig. 133), but no moniliform bodies. The organism was a facultative aerobe and preferred an anaerobic atmosphere with carbon dioxide added. It grew best in liquoid (sodium polyanethol sulfonate). Sensitivity tests showed that the organism was resistant to penicillin but was of the same order of sensitivity to chloramphenicol as Klebsiella 41. Over 28 days, 65 Gm. chloramphenicol was administered. By the end of the first week of treatment, emboli had ceased and the patient's general health was much improved. Shortly afterward she was discharged to a convalescent home.

Studies on Etiology of Human Actinomycosis: Do "Other Microbes" of Actinomycosis Possess Virulence? Per Holm[3] (State Serum Inst., Copenhagen) discusses two patients with actinomycosis who were treated with penicillin and from whose lesions the anaerobic ray fungi disappeared. Nevertheless, they remained ill for several months. One had extensive cervicofacial actinomycosis. Pus from two abscesses contained true Actinomyces israeli, Bacillus actinomycetum comitans and a gram-negative rod with characteristic properties (the so-called corroding bacillus). After treatment with penicillin, actinomyces could not be cultured but the other organisms were still present and appeared to have been responsible for an actinomycosis-like disorder during the last phases of the illness. The other patient had actinomycosis of the thorax and lung. True A. israeli was cultured from sputum, but from pus only B. actinomycetum comitans could be grown. After penicillin therapy, actinomyces could not be demonstrated but the patient remained ill for an additional 22 months. Similar observations were made in seven other patients. They support the hypothesis that actinomycotic diseases in man are multiple infections arising through collaboration of anaerobic ray fungi and certain other microbes.

Repeated Recovery of Spirillum by Blood Culture from Two Children with Prolonged and Recurrent Fevers is reported by Gregory Shwartzman, Alfred L. Florman, Murray H. Bass, Samuel Karelitz and Dorothea Richtberg[4] (Mount Sinai Hosp., New York City). The organism resembles Spirillum minus, long associated with one form of rat bite fever, sodoku. In sodoku, spirillum is usually discovered by animal inoculation. No previous record has been found in which it was diagnosed by blood culture. Both patients were seen because of recurrent fever, eruption and symptoms referable to

(3) Acta path. et microbiol. scandinav. 28:391-406, 1951.
(4) Pediatrics 8:227-236, August, 1951.

the central nervous system, respiratory and intestinal tracts. The first child was debilitated and febrile, and spirillum was recovered repeatedly on blood culture. Although there was no history of rat bite, he had received a deep thigh laceration while playing in a rat-infested wood. Arsenicals, streptomycin and penicillin were ineffectual and he died. The second patient had repeated respiratory infections, indolent skin ulcers and hemiplegia. He had been bitten by a rat 3½ years before spirillum was first recovered from his blood. Penicillin, sulfarsphenamine and streptomycin were ineffectual. Aureomycin sterilized the blood stream and healed the ulcers. Coincidentally the general condition improved strikingly.

The mediums routinely used for blood culture and on which spirillum was cultivated consisted of nutrient broth containing para-aminobenzoic acid, glucose and yeast broth in 100 ml. amounts in flasks, a cooked liver anaerobic tube and two glucose and one plain agar pour plates. About 5 ml. blood was placed in each flask, 2 ml. in the liver tube and in each of the plates. Some of the mediums were kept under reduced oxygen tension with increased carbon dioxide. The spirillum recovered from each of these children grew well in broth aerobically and also with added carbon dioxide, but with difficulty anaerobically. On first isolation the broth usually appeared clear although smears revealed the organisms. After repeated subcultures a surface accumulation of material could be observed after about three days and after two more days often fell to the bottom of the flask as a stringy, slimy, mucoid mass. Smears of this material revealed the characteristic form. During five years of subcultures, the spirillum varied in shape, often becoming rodlike and some months later reverting to a more typical spiral shape. The organism grew fairly well on chocolate or blood agar plates, in circular convex colonies which were smooth edged, whitish gray and glistening. The usual biochemical tests were not revealing. No overt disease was produced in mice by intraperitoneal injection with both strains, and the organisms could not be recovered in the animals after 10-14 days. After injection with each strain, rabbits had high agglutinin titers, but there was relatively little cross agglutination by the serums from the two strains.

Survey of Laboratory-Acquired Infections. S. Edward Sulkin and Robert M. Pike[5] (Univ. of Texas) received replies

(5) Am. J. Pub. Health 41:769-781, July, 1951.

to slightly more than half the questionnaires submitted to approximately 5,000 laboratories, including those associated with health departments, hospitals, private clinics, teaching institutions, manufacturers of biologicals and government agencies. Of 1,342 infections (775 bacterial, 265 viral, 200 rickettsial, 39 parasitic and 63 fungus), 39 were fatal, viral infections causing the highest proportion of deaths.

Members of the brucella group outnumbered all other agents in laboratory infections. Brucellosis, tuberculosis, tularemia, typhoid fever and streptococcic infections accounted for 72 per cent of the bacterial infections and 31 per cent of infections due to all agents. Although tuberculosis is listed as the second commonest type of laboratory infection, the 153 cases reported constitute an exceedingly inaccurate estimate. Hepatitis, Q fever, amebiasis and coccidioidomycosis were the leading diseases in their respective groups. Over 1,000 cases appeared in trained scientific personnel, the rest in students, caretakers, janitors and others. Research accounted for about 308 cases, diagnostic work for 455, handling of clinical specimens and infected animals or ectoparasites for 314. Aerogenic transmission and "work with the agent" were frequently recognized sources of infection. There were 98 infections acquired in the autopsy room and 215 due to accidents of various types. Some persons acquired more than one laboratory infection.

Hazard of Acquiring Tuberculosis in Laboratory is discussed by Esmond R. Long[6] (Univ. of Pennsylvania). The skin is the commonest site of known laboratory infection. Infection results from incision and puncture wounds by instruments, injury by contaminated broken glass, penetration by abrasive used in grinding tubercle bacilli, prolonged surface contact or direct contact with infectious material. Alimentary tract infection acquired in the laboratory is relatively rare. Chief sources are pipets used for transferring suspensions of tubercle bacilli and direct transfer of contaminated material to the mouth through carelessness. Infection of the respiratory tract is common though proof of laboratory origin is difficult. Sources include droplets from the discharge of animal secretions, dust from dried contaminated material and droplets of aerosol produced by overenergetic discharge of suspensions of tubercle bacilli from hypodermic needles or pipets. Splashing of infectious materials is also a source.

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(6) Am. J. Pub. Health 41:782-787, July, 1951.

Sulkin and Pike have shown that infections are more common among scientifically trained laboratory workers than among animal workers and less trained assistants. Most infections of apparent laboratory origin have occurred during diagnostic procedures rather than research work. Lesions produced are more often pulmonary than nonpulmonary; exact source is usually undeterminable.

Several rules have been recommended for prevention. Workers should be provided with proper equipment and adequate instruction for its use. Ultraviolet light should be used for sterilizing the atmosphere. A medical and health program for all workers must be instituted. Animals should be handled with utmost caution. Laboratory quarters where tubercle bacilli are used should be properly cleaned. All infected objects should be decontaminated, and workers should make appropriate clothing changes. Perfection in laboratory technic is the most important consideration. A recommended adjunct to other measures is BCG vaccination of tuberculin-negative personnel. If vaccination is not practiced periodic tuberculin testing is essential. Chest x-rays of all personnel must be made at intervals not to exceed six months.

Divided Culture Plate: Its Use in Testing for Sensitivity to Antibiotics is discussed by Joseph Felsen and Alfred J. Weil[7] (Bronx Hosp., New York City). The plate consists of a standard Petri dish bottom which is separated into quarters by two smooth bisecting ridges of glass 5.5 mm. in height. In studies of antibiotic sensitivity these plates are used as well as antibiotic-impregnated paper disks. All of the common antibiotic agents are available in this form in three different concentrations, except for aureomycin which is available in one concentration. Each has a distinctive color. The plates are seeded with the organism to be studied. The three disks of the antibiotic agent are placed in the corners of each quadrant (Fig. 134). By using the apex and left and right corners in this order, strength of the corresponding disks can be identified readily. Two divided plates are used, one quadrant being allocated to each antibiotic agent and one being used as a control. The plates are incubated overnight and the clear zones indicating inhibition of growth and therefore sensitivity of the organism are measured. The agent exhibiting the greatest inhibition of growth is selected for therapy. It takes about 18

(7) A.M.A. Arch. Int. Med. 88:406-408, September, 1951.

hours for completion of the sensitivity test after the organism has been isolated. During the delay in isolating the organism, an agent of wide antibacterial pattern may be used if the patient's condition is critical. The unknown may be set up against the antibiotic agents without waiting for a pure cul-

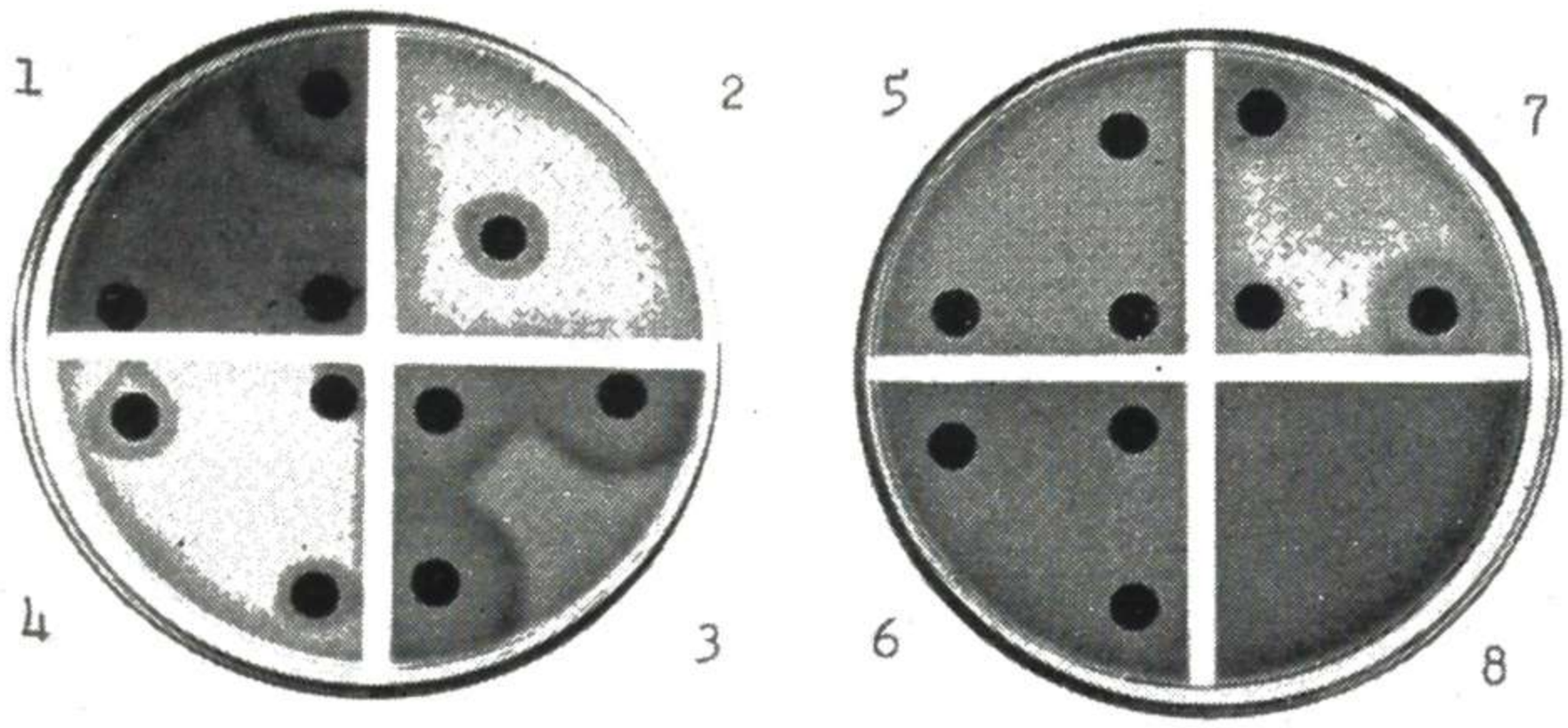

Fig. 134.—Antibiotic sensitivity test with Streptococcus faecalis hemolyticus isolated from patient with empyema of gall bladder. Blood agar plates show zones of inhibition, represented by circular areas around disks, and intervening areas of hemolysis and bacterial growth. *1*, penicillin; *2*, aureomycin; *3*, chloramphenicol; *4*, terramycin; *5*, streptomycin; *6*, dihydrostreptomycin; *7*, bacitracin; *8*, growth control. (Courtesy of Felsen, J., and Weil, A. J.: A.M.A. Arch. Int. Med. 88:406-408, September, 1951.)

ture. Resistant mutants appear as isolated colonies in the zone of inhibition and the clinician is thus advised of impending resistance to therapy.

Trachoma-Psittacosis-Lymphogranuloma Venereum Group of Viruses: Chlamydozoaceae. According to Phillips Thygeson[9] (Univ. of California) a newly defined group of viral agents, occupying an intermediate position between the typical large viruses and rickettsias, carries the family name of chlamydozoaceae. The organisms are small, pleomorphic and often coccoid, usually with a characteristic developmental cycle. They stain with aniline dyes, are ordinarily gram negative, behave as obligate intracytoplasmic parasites and have not been cultivated on cell-free mediums. They are related to typical viruses by virtue of their filtrability and inclusion body formation and are related to the rickettsias by virtue of their size, micro-organism nature, tinctorial properties and susceptibility to chemotherapy. Characteristics of the entire group which set it apart from typical large viruses include ease of staining of elementary and initial bodies with ordinary

(9) Am. J. Ophth. 34 (pt. 2):7-34, May, 1951.

Properties of Chlamydozoaceae

	C. Trachomatis	C. Oculogenitale	M. Lymphogranulomatis	M. Psittaci	C. Conjunctivae
Morphologic variation	Elementary body-initial body	Elementary body-initial body	Elementary body-initial body	Elementary body-initial body	Inconstant elementary body-initial body
Staining					
Gram	Neg.	Neg.	Neg.	Neg.	Neg.
Giemsa	Purplish red to blue	Purplish red to blue	Purplish red to blue	Purplish red to blue	Purplish red to blue
Iodine	Matrix stains	Matrix stains	Does not stain	Does not stain	Does not stain
Filtrable	With great difficulty	+	+	+	+
Tissue tropism	Epithelium of conjunctiva, cornea, lacrimal sac	Epithelium of conjunctiva, urethra, cervix	Prefers cells of mesodermal origin	Prefers cells of mesodermal origin	Conjunctival, corneal epithelium
Cultivability	None with certainty	None with certainty	Tissue culture, chorioallantoic membrane, yolk sac	Tissue culture, chorioallantoic membrane, yolk sac, allantoic sac	Not cultivated
Immunology					
Complement fixation	Common antigen with group	Common antigen with group	Common antigen with group	Common antigen with group	Not known
Frei test	Neg.	Neg.	Pos.	Neg.	Not known
Immunity	None permanent	None permanent	Associated with persistence of virus	Associated with persistence of virus	Not known
Infectivity	Low	Low; eye to eye transmission rare	Low; extragenital infections rare	Extremely infectious	High in herds and flocks
Epidemiology	Eye to eye	Genitourinary tract to eye	Venereal	Air-borne	Not known
Toxin production	Not demon.	Not demon.	+	+	Not known
Leukocytic reaction	Neutrophilic	Neutrophilic	Neutrophilic	Not characteristic	Neutrophilic
Susceptible experimental animals	Monkeys, apes	Monkeys, apes	Laboratory animals, monkeys, apes	Mice, squirrels, gophers, birds, monkeys	Hogs, sheep, cattle, goats, fowl
Sulfonamides	Effective	Effective	Effective	No effect	Not known
Penicillin	Ineffective	Ineffective except in high dosage	Ineffective	Partially effective	Not known
Aureomycin	Effective	Effective	Effective	Effective	Not known
Chloromycetin	Not known	Not known	Effective	Effective	Not known
Terramycin	Effective	Effective	Effective	Effective	Not known

dyes; basophilic character of the bodies, especially the initial ones; character of the inclusion matrix which permits ready identification of the virus particles in contradistinction to the acidophilic staining of the matrix of the typical large virus inclusions which tends to mask the individual virus particles; sequence of morphologic variation, and susceptibility to chemotherapy with the sulfonamide and antibiotic agents. The important properties of the chlamydozoaceae are compared in the table.

Miyagawanella lymphogranulomatis can be grown in tissue culture but isolation from man is best accomplished by intracerebral inoculation of mice or injection into the yolk sac of the developing chick embryo with biopsy material. Inoculum contaminated by bacteria can be treated with penicillin-streptomycin mixtures before being introduced into the yolk sac. The virus is not highly infectious, and epidemiology of the disease parallels that of syphilis. Ocular forms include an oculoglandular conjunctivitis, sometimes with elephantiasis of the lids, and a sclerokeratitis with special characteristics.

Inclusion conjunctivitis is a benign form of ophthalmia neonatorum, unassociated with pathogenic bacteria. Inclusions morphologically identical to those found in trachoma can be observed in conjunctival scrapings from conjunctivitis of the newborn. The elementary body is a minute coccoid body about 250 mμ. in diameter when stained by Giemsa's method. It is gram negative and stains poorly with ordinary aniline dyes. With Giemsa or other blood stains it has the same reddish blue color as the elementary body of trachoma.

Nature of Inclusion Bodies in Trachoma. Henry Grossfeld[1] (New York City) states that between appearance of inclusion bodies in the epithelial cells and formation of typical follicles several days to several weeks must pass. It is just at this stage when trachoma cannot be diagnosed clinically that inclusion bodies are usually found in abundance in the epithelial cells in conjunctival scrapings and that microscopic diagnosis is very easy. The trachoma virus exists in only one form, the elementary body, and divides without a complicated cycle. "Initial bodies" are not present in the inclusion bodies. Figure 135 shows that the inclusion body consists of particles of equal size, i.e., of elementary bodies only. Histochemical studies showed that the elementary bodies give positive Feulgen re-

(1) Am. J. Ophth. 33:1831-1836, December, 1950.

actions; therefore, they contain desoxyribonucleic acid. This implies that these elementary bodies belong to the class of highly organized viruses which may be considered the most

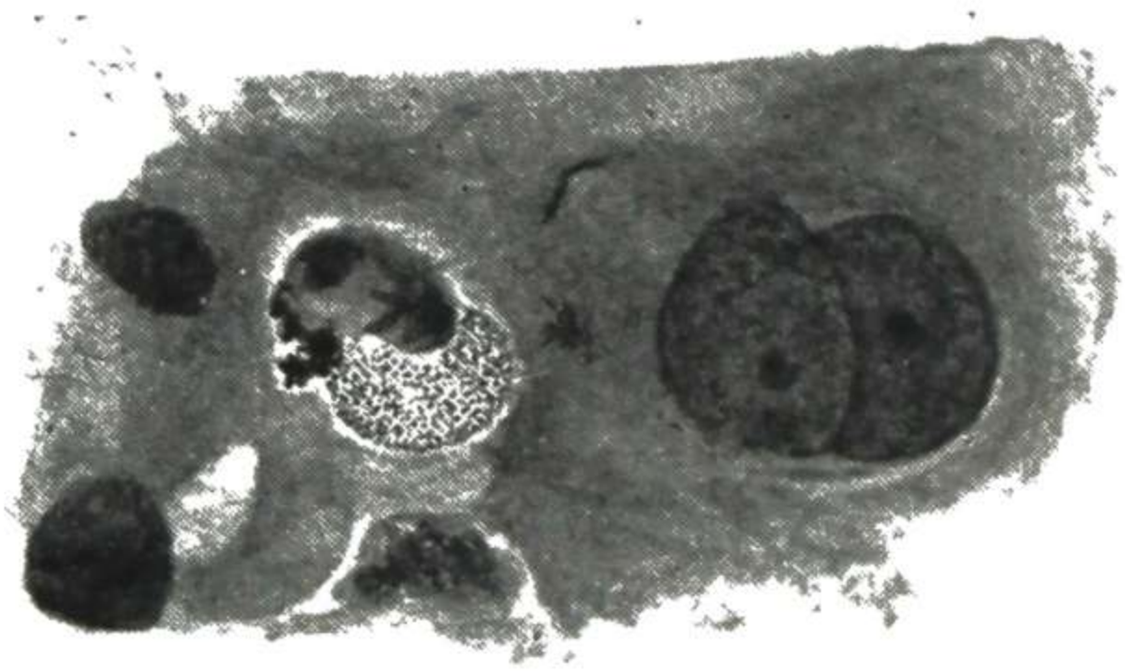

Fig. 135.—Elementary bodies of inclusion body impregnated according to Foot. (Courtesy of Grossfeld, H.: Am. J. Ophth. 33:1831-1836, December, 1950.)

primitive cellular units. Electron microscopic study of elementary bodies, in purified material, from a case of fresh pre-follicular trachoma indicated that their size is about 200 mμ.

Isolation and Characterization of Rocky Mountain Spotted Fever Rickettsias from Rabbit Tick Haemaphysalis Leporis-Palustris Packard. In 1945 Jellison reported that spotted fever rickettsias had never been recovered from any mammal in the highly endemic area of the western part of the United States. R. R. Parker, E. G. Pickens, D. B. Lackman, E. J. Bell and F. B. Thraikill[2] made saline suspensions of rabbit ticks, H. leporis-palustris, obtained from cottontail rabbits caught in the Bitter Root Valley of western Montana. They were injected into guinea pigs and eggs. From the guinea pigs, three strains of rickettsias were recovered; from the eggs, five.

On establishment of the strains in guinea pigs, pronounced differences were noted when the clinical picture was compared with that of a stock laboratory strain of Rocky Mountain spotted fever rickettsia. With prolonged passage in guinea pigs there was a tendency for them to decrease in virulence, which made it difficult to maintain a guinea pig passage strain. Initial injection of the H. leporis-palustris or laboratory strain produced complete immunity against boutonneuse fever, South African tick bite fever and maculatum disease, as well as against each other. Inoculation with these rickettsias protected against challenge with either the H. leporis-palustris or laboratory strain.

Although the rabbit tick, H. leporis-palustris, does not

(2) Pub. Health. Rep. 66:455-463, Apr. 13, 1951.

usually bite man, the finding of rickettsias in this tick, which can be classified as Rocky Mountain spotted fever rickettsias, indicates that it may be of importance in maintaining spotted fever rickettsias in nature among rabbits. The observation that all stages of Dermacentor andersoni, the tick most commonly involved in transmission of the infection to man in the western part of the United States, are also found on rabbits is of additional significance in assessing the role of the rabbit tick in maintaining this infection among a probable mammalian reservoir.

MYCOLOGY

Unsealed Hanging-Drop Technic for Investigation of Microsporum in Hair. C. J. La Touche and L. A. H. Dublin[3] (Univ. of Leeds) present a simple hanging-drop method which, within a few days, enables one to distinguish between Microsporum audouini and M. canis on infected hair from patients with tinea capitis.

PROCEDURE.—Into a Petri dish, 9 cm. in diameter, a disk of filter paper is placed from which four or five circular areas have been cut to accommodate glass cylinders, 1.5 cm. in internal diameter and 0.5 cm. high. Circular cover slips are placed on the cylinders (Fig. 136). Before use the apparatus is sterilized in a dry oven at 140-150 C. for two hours.

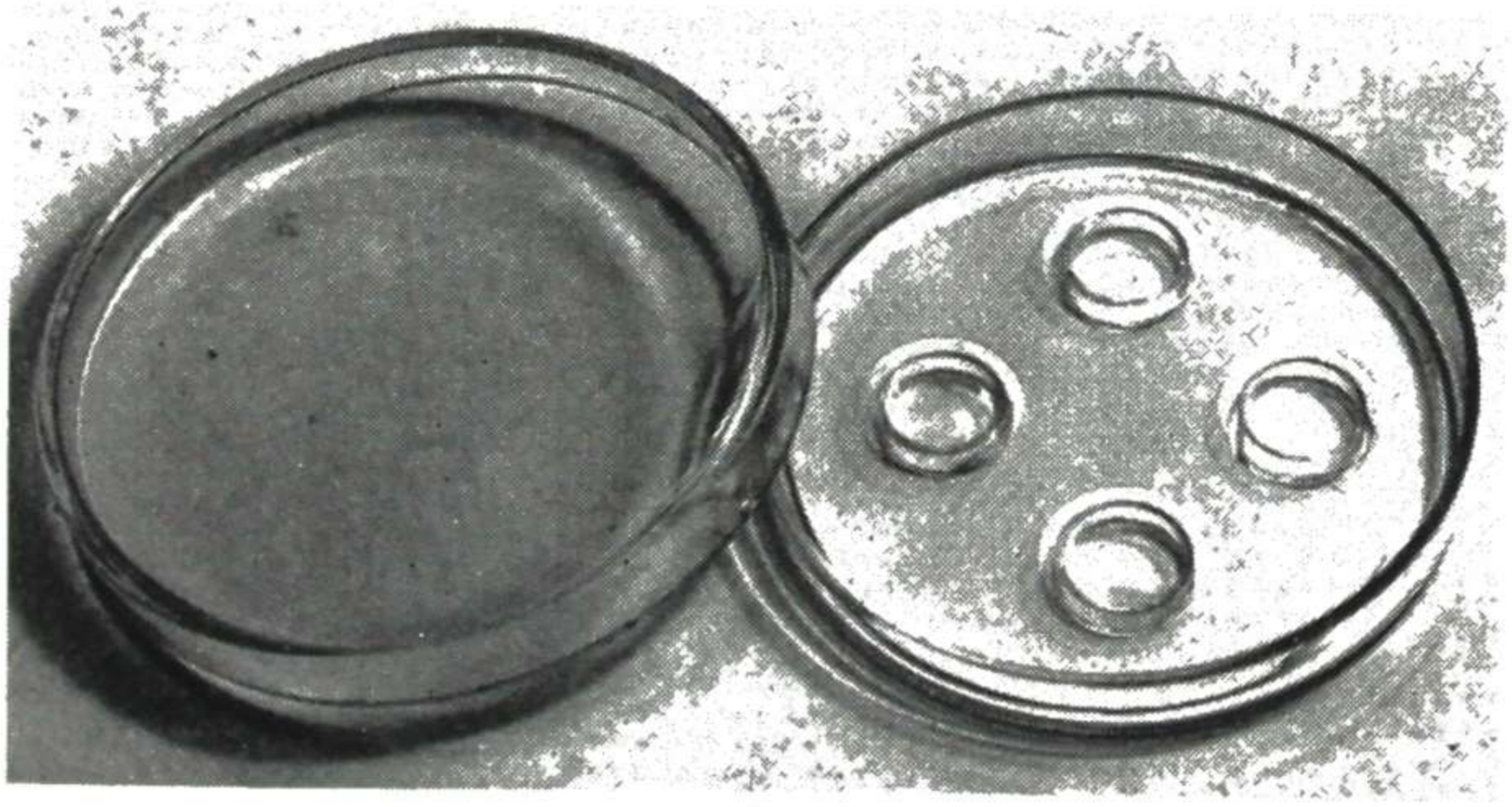

Fig. 136.—Apparatus used in preparation of hanging-drop cultures. (Courtesy of La Touche, C. J., and Dublin, L. A. H.: Brit. J. Dermat. 63:8-15, January, 1951.)

The culture medium is a modification of that used by Kligman and Rebell: honey, 5 Gm.; peptone, 1 Gm.; agar, 0.15 Gm.; water, 100 ml. Reaction: pH 5-5.3. The infected hair is suspended in a

(3) Brit. J. Dermat. 63:8-15, January, 1951.

drop of culture medium on the cover slip of the cylinder. Infected material, placed on black photographic paper and held close to a Wood's lamp, fluoresces distinctly. Cultures should be left at room temperature (15-20 C.) for about 24 hours. Usually, germination

Fig. 137.—Macroconidia in unstained, living 4½ day culture of M. canis; × 180. (Courtesy of La Touche, C. J., and Dublin, L. A. H.: Brit. J. Dermat. 63:8-15, January, 1951.)

Fig. 138.—Vesicular chlamydospore in 11 day culture of M. audouini; reduced from × 640. (Courtesy of La Touche, C. J., and Dublin, L. A. H.: Brit. J. Dermat. 63:8-15, January, 1951.)

of the spores will then have taken place, but with M. audouini or with old material this may be delayed several days.

Hyphae may grow from all parts of the hair or appear as isolated tufts. Between the fourth and fifth days, and sometimes earlier, the large multiseptate, spindle-shaped macroconidia, characteristic of the genus Microsporum, may be observed in the aerial hyphae growing from hair (Fig. 137).

The occurrence of many macroconidia at an early stage and development of a yellow pigment distinguishes M. canis from M. audouini. Cultures of M. audouini kept for over six days have in some cases produced a remarkable number of pectinate branches and in others large numbers of vesicular chlamydospores (Fig. 138). Microcultures may be preserved permanently by the following method.

PROCEDURE.—The cover slip is placed culture side up on a slide. A drop of 70 per cent alcohol is dropped on the culture to fix it and exclude air. The slide and cover slip are placed on a heated surface until the alcohol has evaporated. A drop of lactophenol, containing cotton blue 0.02 per cent, is placed on the culture, which is left on the heated surface for some hours until staining has been effected. The cover slip is mounted, culture side up, on a clean slide in a drop of polyvinyl lactophenol mixture, containing 0.02 per cent cotton blue; more of this is placed over the culture and cover slip and the whole is then sealed with a square cover slip. The preparation is left to harden on a heated surface.

Mycologic and Histologic Technics in Study of Superficial Fungous Infections are discussed by George M. Lewis, Wilbert Sachs and Mary E. Hopper[4] (Cornell Univ.). The actual demonstration of a pathogenic organism in culture is irrefutable evidence of the etiology and such information is always useful before beginning therapy. To demonstrate fungi in a direct mount, an untreated lesion of recent origin should be selected. Material from the periphery is most likely to contain the fungous elements. The area is sponged with either 70 per cent alcohol or 0.1 per cent zephiran.® The first scrapings, obtained with a dull blade, are discarded; the second specimen is placed on a clean glass slide for direct examination after treatment with KOH, and the third specimen is implanted directly onto the culture medium.

Filtered ultraviolet rays are valuable chiefly for diagnosis of tinea capitis and in determining when cure has taken place.

A useful isolation medium is composed of 4 per cent dextrose (technical), 1 per cent peptone (Difco), 2 per cent agar (powdered) and tap water sufficient to make 100 per cent. Cornmeal agar and other special mediums are also useful.

There is no staining technic advisable for routine use in isolating fungi from superficial mycotic infections. For preserving a specimen permanently or for preserving culture mounts, methylene blue counterstained with a weak alcoholic solution of eosin has been found adequate.

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(4) A.M.A. Arch. Dermat. & Syph. 63:622-632, May, 1951.

Preliminary studies indicate that study of histologic sections is of little help in the diagnosis of superficial fungous disease. It is impossible to distinguish differences in the histologic picture according to species of infecting organisms. Even in the direct mount it is seldom possible to identify a fungus as pathogenic.

Use of Mineral Oil in Maintenance of Cultures of Fungi Pathogenic for Humans is reported by Libero Ajello, Virginia Q. Grant and Mark A. Gutzke[5] (U. S. Pub. Health Service).

METHOD.—Fungi are subcultured on fresh slants of Sabouraud's dextrose agar. After colony size has become about 1 cm. in diameter at room temperature, heavy mineral oil (autoclaved 30 minutes at 15 lb. pressure) should be added in quantities sufficient to cover completely both the fungus growth and surface of the agar slope. The tubes may then be stored on sloping racks in a cabinet at room temperature.

Species such as Candida stellatoidea, Epidermophyton floccusum and Microsporum audouini which do not survive cold storage were still viable after storage under oil for 19-21 months. Pleomorphic changes were not prevented in several strains of E. floccosum, which lost their original powdery, olive green form and became white, cottony and nonsporulating. Colony changes also were noted in cultures of Trichophyton tonsurans. In 264 strains of fungi representing 15 genera and 34 species viability was 9-22 months. Thus, mineral oil is recommended for preservation of human pathogenic fungi in the viable state.

Improved Technic for Diagnosing Ringworm Infections and Moniliasis is described by A. M. Klingman, D. M. Pillsbury and H. Mescon[6] (Univ. of Pennsylvania).

METHOD.—In collecting the material, 1 or 2 drops of Mayer's glycerin-albumin reagent is placed directly over the lesion, which is then vigorously scraped with a blunt knife. Horny material is removed in the form of macerated thin shavings. Thick pieces should be avoided. The macerated material on the knife is transferred to a slide and rubbed over the surface so as to effect an even distribution. The albumin aids adherence of the material to the slide during the staining procedure. Such slides can be shipped by mail and stained without loss of brilliance weeks later. Scrapings from nails may be similarly collected. Mucous surfaces should be swabbed and the sample rubbed over an albuminized slide. The following

(5) A.M.A. Arch. Dermat. & Syph. 63:747-749, June, 1951.
(6) J. A. M. A. 146:1563-1565, Aug. 25, 1951.

staining procedures are best carried out in a series of Coplin staining jars that may be set up permanently for routine use.

The slide is immersed for one minute in 95 per cent alcohol and then for five minutes in a 5 per cent aqueous solution of periodic acid. It is transferred to a solution containing 0.1 Gm. basic fuchsin, 5 cc. of 95 per cent ethyl alcohol and 95 cc. water for two minutes. After brief rinsing in tap water it is transferred to a solution containing 1.0 Gm. zinc hydrosulfite, 0.5 Gm. tartaric acid and 100 cc. tap water for 10 minutes. (This solution is stable for at least a

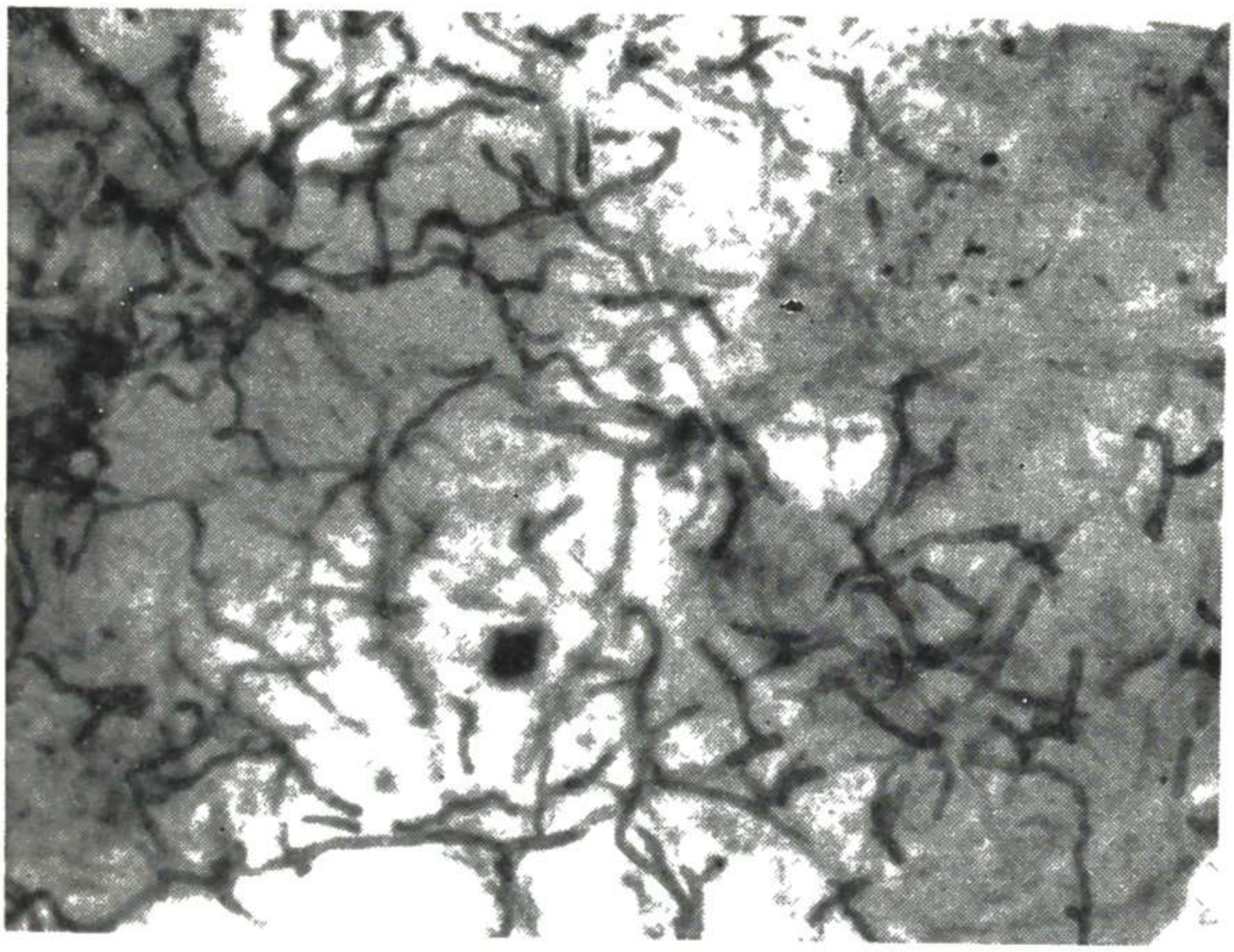

Fig. 139.—Stained scrapings from case of ringworm of skin. Highly branched threads permit immediate diagnosis. (Courtesy of Klingman, A. M., *et al.*)

month if it is kept in a closed jar and can be used over and over again.) The slide is rinsed briefly in water, dehydrated and mounted (Fig. 139). For semipermanent preparations, dehydrating and clearing may be omitted and the specimen mounted in 1 or 2 drops of polyvinyl alcohol.

As a result of this procedure, fungi are stained red and horny tissue is either colorless if fairly thin or light red if thick. Even in thick smears there is sufficient contrast between the fungi and keratin to afford ready detection of the fungus. The method is superior to the potassium hydroxide technic.

Selective Medium for Isolation of Coccidioides Immitis. Lucille K. Georg, Libero Ajello and Morris A. Gordon[7] (U. S. Pub. Health Service) selected sabouraud dextrose agar fortified with penicillin (20 units/ml.) and streptomycin (40 units/ml.) as the basal medium. This combination inhibits most

(7) Science 114:387-389, Oct. 12, 1951.

bacteria but does not prevent growth of fungi, except the actinomycetes. Because other investigators have demonstrated the selective antifungal activity of actidione,® it was added to the basal medium.

Attempts were made to isolate C. immitis in culture from a mixture of its spores with those of 15 different saprophytes. On the first of a series of plates containing 0.1 mg. actidione®/ml. basal medium, colonies of C. immitis were recognizable

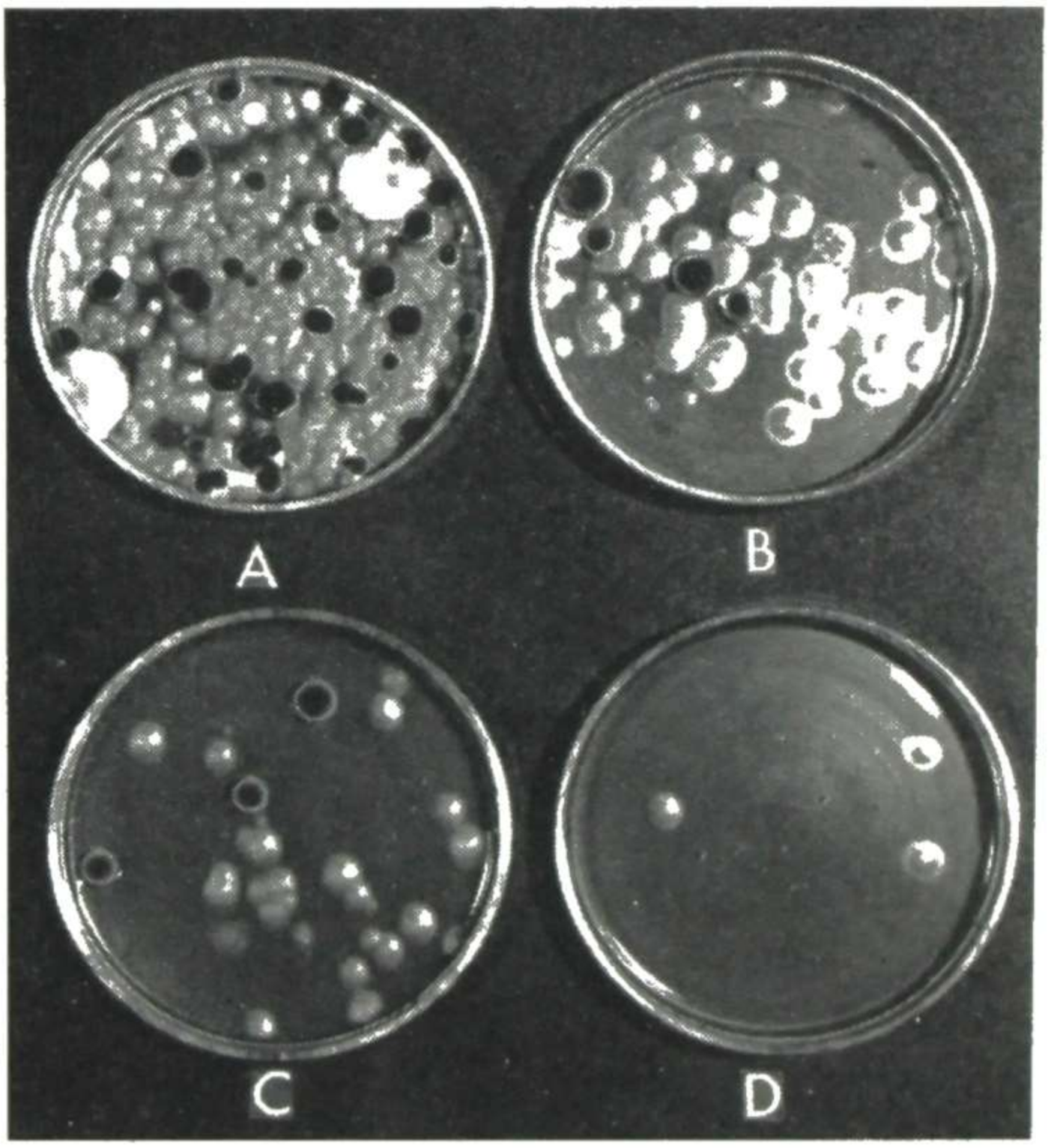

Fig. 140.—Isolation of C. immitis from mixture of saprophytic spores. Some saprophytic colonies can be seen on plates *A*, *B* and *C*, but gray, slightly moist, dome-shaped colonies of C. immitis can be seen on all plates and appear in pure culture on plate *D*. (Courtesy of Georg, L. K., *et al.*: Science 114:387-389, Oct. 12, 1951.)

but were overgrown with saprophytes, whereas on the remaining plates of the series saprophytic growth was negligible and many isolated colonies of the pathogen appeared. On a series containing 0.5 mg. actidione®/ml., growth of the saprophytes was further restricted and, even after 12 days, the plates contained C. immitis in almost pure culture (Fig. 140).

The results indicate that 0.1 mg. actidione®/ml. basal agar may be of value in isolation of C. immitis from the air, but higher concentrations might be required for isolation from more heavily contaminated materials.

Diagnosis of Pulmonary Coccidioidal Infections. According to Charles E. Smith[8] (Univ. of California), about 40 per cent of naturally acquired coccidioidal infections in man are accompanied by symptoms. The commonest are pleurisy, fever, malaise, cough, anorexia, backache, and night sweats. The disease is endemic in southwestern Texas, southern part of New Mexico, southern and central parts of Arizona, San Joaquin Valley and region around San Diego County in California, and the area around Las Vegas, Nev. A careful residence history often provides highly significant evidence for diagnosis.

Proof that a pulmonary lesion is coccidioidal depends on laboratory evidence. The first procedure should be the coccidioidin skin test. Now available commercially, coccidioidin is generally used in 1:100 dilution of a Berkefeld filtrate of multiple cultures of coccidioides grown on asparagine synthetic medium. It does not activate old, quiescent infections, nor is the currently provided material antigenic. It does not evoke humoral antibodies, and therefore does not interfere with coccidioidal serologic tests. The only significant systemic complication which may occur is exacerbation or precipitation of erythema nodosa or erythema multiforme in association with a primary infection. Since the active principle is mainly carbohydrate, coccidioidin is quite stable and when kept refrigerated and uncontaminated remains potent at least two years in 1:100 dilution. Syringes which have contained other biologic material must not be used unless the equipment is soaked overnight or boiled in dichromate cleaning solution. Intradermal coccidioidin test results should be read at 24 and 48 hours. Induration greater than 5 mm. in diameter should be considered a positive reaction. The significance of the results of the coccidioidin test are comparable to those of the tuberculin test. If coccidioidal sensitivity has been demonstrated, or if the test result is negative but the patient is suspected to be undergoing coccidioidal dissemination or has a very thin-walled nonreactive cavity and an accusative residence history, serologic tests should be carried out next. If a patient merely has a respiratory illness suspected to be coccidioidal, serologic tests should be deferred until conversion of the coccidioidal test reaction to positive takes place, because

(8) California Med. 75:385-391, December, 1951.

diagnostic humoral antibodies develop more slowly than dermal sensitivity. In precipitin tests made with a constant quantity of serum and increasing dilutions of appropriate antigen, results are indicated by buttons which develop over five days. This test is important when the acute phase of infection is suspected. Precipitin antibodies begin reverting in the third week of the disease and seldom persist longer than four months. A complement fixation test may be done with serial dilutions of serum as in any quantitative Kolmer test. Positive reactions may continue for a few months to an indefinite period. With the acute pneumonic type illness, both tests are appropriate; with more long-standing infections, only complement fixation tests need be done.

Demonstration of C. immitis is irrefutable proof of coccidioidal infection. The most damning objection to sputum cultures is the hazard of laboratory infection. Culture on malt, Sabouraud's agar or differential mediums with inoculation of material from suspicious cultures intraperitoneally into white mice or intratesticularly into guinea pigs is necessary to complete identification.

SEROLOGY

Studies on Effect of Anti-Bone-Marrow Serum. Bertil Björklund and Lennart Hellström[9] (Karolinska Inst.) immunized horses with rabbit bone marrow preparation and subsequently injected the immune horse serum into rabbits. Hemoglobin values and red cell counts rapidly decreased, whereas controls given normal horse serum retained normal values. The animals given immune serum showed an evident increase in the number of white blood cells. Smears of the peripheral blood revealed a large output of not quite mature cells of abnormal appearance and signs of accelerated destruction. All three groups of blood cells were affected. There were polychromasia, anisocytosis, basophilic stippling, Cabot rings and Howell-Jolly bodies. Nucleated red cells were also present.

These observations and changes seen at autopsy indicated that the anti-bone-marrow serum had a selective effect on the bone marrow and a general toxic effect which was mainly vascular. They also indicated that the immune serum con-

(9) Acta med. scandinav. 139:123-132, 1951.

tained antibodies which reacted with essential substances, specific for the bone marrow, and that these antibodies were responsible for the selective, distinctly structural and functional damage to this organ.

Mazzini Cardiolipin Microflocculation Test for Syphilis. L. Y. Mazzini[1] (Indiana Univ.) states that in adapting the cardiolipin to the technic the basic characteristics, including simplicity in preparation of the emulsion and performance of the test, predetermination of optimal ratios of the test components and use of a buffered diluent, remain unchanged. A variation of the cardiolipin antigen emulsion is used which, although the same total quantities of reagent are used as in the original technic, allows the emulsion to reach optimal sensitivity immediately after preparation.

MATERIALS.—The approximate optimal combination of reagents for the Mazzini technic is 0.025 per cent cardiolipin, 0.2 per cent lecithin (1:8 ratio) and 0.75-0.9 per cent cholesterol. The ultimate ratio depends entirely on the particular lots of lecithin and cholesterol used. Titration of each lot of a reagent is essential to produce antigens of uniform sensitivity and specificity. After the ratio which produces optimal dispersibility and which will not cause flocculation with negative serums is selected, its sensitivity is evaluated. For this purpose, 50 weakly positive, 50 strongly positive and as many false-positive reacting serums as are available are selected. The ratio producing the strongest reactions with the weakly positive serums and the weakest positive reactions with the false-positive serums is chosen as the titer for that particular lot. Once the desired level of sensitivity has been established, future lots are standardized against the "standard" antigen.

A buffered saline solution with pH of 6.3-6.4 and salt concentration of 10 Gm./L. is prepared by mixing 8.1 Gm. NaCl (C.P.), 0.2 Gm. primary K_2HPO_4, 1.7 Gm. secondary $Na_2HPO_4 \cdot 12\ H_2O$, 1,000 ml. distilled water, 1.0 ml. formaldehyde (Merck's reagent) and 3.2 ml. 1 N HCl. The solution is filtered, and pH checked and adjusted.

The patient's serum is separated from the clot by centrifuging and heated for 30 minutes in a water bath at 56 C. Any precipitate should be removed by centrifugation.

To prepare the antigen for the daily test, 0.4 ml. buffered saline solution is pipetted to the bottom of a 30 ml. round bottle. With a 1.0 ml. pipet, 1.4 ml. cholesterolized antigen is measured (measurement is made from tip of pipet). The bottle is held in the left hand and, while it is rotated rapidly and constantly, the antigen is added directly and at once; any antigen left in the pipet is blown out. The emulsion is drawn into and out of the pipet six times, all emulsion left in the pipet being returned on the last mixture. After 2.6 ml.

(1) J. Immunol. 66:261-275, February, 1951.

buffered saline solution is added, a paraffin-coated cork is placed in the bottle, which is then shaken from bottom to cork and back 15 times in 15 seconds. The emulsion is then ready for immediate use and continues usable for the entire day. It should be mixed gently each time it is used.

The qualitative and quantitative tests with serum follow.

METHODS.—As a check on the antigen and saline solution, known positive serum, negative serum, weakly positive serum and the saline solution should be tested before the qualitative test is performed. With a 1 ml. pipet, 0.03 ml. serum is placed in a chamber of a glass slide which is free from grease and lint; 0.01 ml. (1 drop) antigen emulsion is discharged from a syringe held at a 45 degree angle to the serum sample. The slide is rotated on a mechanical shaker for four minutes at 160-180 rpm or by hand at 120. Immediately after this, 1 drop of 0.9 per cent salt solution is added from a medicine dropper which delivers about 0.05 ml./drop. The slides are then re-rotated for four minutes at 100-120 rpm, either by hand or mechanical rotater. Results are examined under the low power of a microscope. They are recorded as follows: no clumping, negative (reactions which give the antigen particles a coarse appearance but not distinguishable aggregation are also read as negative); very small but definite clumps, 1+; small clumps, 2+; medium-sized clumps, 3+; large clumps, 4+. If zonal reactions are suspected the serum should be diluted serially from 1:2 to 1:64 or higher and retested quantitatively as follows.

Serum dilutions of 1:2 to 1:64 and higher are prepared by pipetting 0.5 ml. buffered saline solution into each of six or more test tubes. To the first tube, 0.5 ml. heated serum is added and mixed thoroughly; 0.5 ml. diluted serum is transferred from the first to the second tube and mixed thoroughly. The transferring is continued from one tube to the next until all dilutions have been made; 0.05 ml. of each dilution is then pipetted into a chamber of a glass slide and 1 drop of antigen emulsion added to each. The slide is rotated by a mechanical shaker for four minutes at 160-180 rpm or by hand at 120. Results are read as for the qualitative test and reported in terms of dilution units according to the highest dilution producing a positive reaction (3+ or 4+).

Optimal results in study of cerebrospinal fluid can be obtained with this method; it is as sensitive and reliable as the complement fixation test. Bloody fluid is unsatisfactory for examination.

METHOD.—The fluid is centrifuged at 2,000 rpm for five minutes and the clear supernatant liquid decanted into a clean tube. The cerebrospinal fluid is not heated. Known positive and negative fluids should be included as controls. The quantities 0.05 ml., 0.2 ml. and 0.5 ml. cerebrospinal fluid are pipetted into separate chambers of a glass slide. One drop (0.01 ml.) of antigen emulsion is added to each chamber. The slide is rotated in a mechanical shaker for 10 minutes at 100-120 rpm. Then 2 drops of 0.9 per cent saline solution is added

from a medicine dropper into the chamber containing 0.05 ml. cerebrospinal fluid. The slide is rotated for 20 minutes at 80-100 rpm. Reactions are read and recorded in the same manner as described for the serum test. Result of the strongest reaction is reported, regardless of which of the three quantities it may be.

Strongly positive cerebrospinal fluid should be quantitated by preparing serial dilutions of the fluid (1:2 to 1:16 or higher); 0.5 ml. of each dilution is pipetted into the chamber of a glass slide and 1 drop of antigen emulsion added to each. The slide is rotated on a mechanical shaker for 10 minutes at 100-120 rpm and rerotated for an additional 20 minutes at 80-100. Reactions are read for each dilution. The highest dilution giving a positive result is reported.

Antiglobulin (Coombs) Test in Brucellosis. The method described by M. M. Wilson and E. V. O. Merrifield[2] (Univ. of Melbourne) is particularly applicable to cases in which the conventional agglutination test is negative or the prozone phenomenon conspicuous.

METHOD.—The standard agglutination test is put up in Dreyer tubes by making serial doubling dilutions of the patient's serum, 1:10 to 1:2,560 with saline solution, in 0.3 ml. quantities. To each tube, 0.3 ml. standard formolized suspension of Brucella abortus is added, giving a final dilution range of 1:20 to 1:5,120. The rack is placed in the 37 C. incubator overnight. Tubes are examined for agglutination and readings made from 4 plus, representing complete agglutination and clear supernatant fluid, to 2 plus, the standard agglutination, with fine macroscopic agglutination and incomplete clearing of the supernatant fluid to negative with no deposit and no clearing of the supernatant fluid. All tubes showing less than 2 plus agglutination are centrifuged; the supernatant fluid is removed; 0.6 to 0.8 ml. saline solution is added, and the solution and sediment are thoroughly mixed. The tubes are then centrifuged and the washings repeated twice so that the suspended organisms are washed three times to remove every trace of free human protein. The organisms are then resuspended in 0.5 ml. saline, and 1 drop of Coombs serum of 1:100 titer or higher is added to each tube. The tubes are replaced in the 37 C. incubator overnight and examined for agglutination next day.

Among 130 serums there was a significant titer in 17 from patients with probable or possible brucellosis in which the conventional test was negative from 1:20 to 1:5,120. The method eliminated the prozone which was present in 13 cases in which the conventional test gave positive results. Possibly this procedure makes safe the use of a screening test at 1/20 dilution. The modified Coombs test may be of value in the future study of brucellosis.

Factors Influencing Agglutination Titration in Human Brucellosis. Richard J. Feinberg and George G. Wright[3]

(2) Lancet 2:913-914, Nov. 17, 1951.
(3) J. Immunol. 67:115-122, August, 1951.

(Camp Detrick, Frederick, Md.), used as antigens 22 different smooth clones of brucella grown in a like manner and killed by three different technics. The serums included Brucella suis, abortus and melitensis and cross-reacting serums obtained from subjects immunized with cholera vaccine and from patients who had recovered from tularemia.

All 22 strains when killed by the same method gave essentially identical titers with each serum and method of incubation. These results supported the conclusion that any smooth strain of brucella is satisfactory for preparation of antigens. The method of killing the antigen suspension and the temperature of incubation of the titrations influenced the titers, especially in titrations of cross-reacting serums. Titers of cross-reacting serums with phenol-killed antigen suspension were much lower when titrations were incubated at 56 C. than when they were incubated at 45 C. or below; titers of brucellosis serums were essentially independent of incubation temperatures from 24 to 60 C. The lower titers of cross-reacting serums at 56 C. were attributed to a reversible inhibition of the nonspecific agglutination. Titers with formalin-killed antigen suspension were less influenced by incubation temperature; these antigens, however, favored the appearance of prozones, particularly with serums from persons recovered from brucellosis rather than from those with active brucellosis.

The results provided additional evidence of the significance of the cholera and tularemia cross-reactions when titrations are incubated at 37 C., since 66 per cent of the serums, representing 13 of the 19 cases of brucellosis, had titers within the range covered by the cross-reacting serums. The possibility that the specificity of other diagnostic agglutination titrations is similarly influenced by incubation temperature merits investigation.

Slide Test for Modification of Hemagglutination Test for Antibodies against Tubercle Bacilli is described by William Thalhimer and Charlotte Rowe[4] (Grasslands Hosp., Valhalla, N. Y.)

METHOD.—Human group O, Rh-negative cells were prepared by collecting blood in bottles containing acid citrate-dextrose solution. After centrifuging, the supernatant fluid was discarded and cells were washed three times with phosphate buffered saline (pH 7). On the last washing the cells were packed for 10 minutes at 2,000 rpm.

(4) Am. Rev. Tuberc. 63:667-671, June, 1951.

For sensitization of cells, tuberculin 4 times International Standard (Lederle) was diluted 1:12. To 48 parts of diluted tuberculin, 1 part of washed, packed cells was added. Cells were incubated for two hours in a water bath at 37 C. and agitated every 15 minutes. After two hours sensitized cells were centrifuged and supernatant fluid discarded. Cells were further washed three times with buffered saline, centrifuged five minutes at 2,000 rpm. After the last washing the cells were made up to volume with buffered saline to obtain 15 per cent suspension of cells.

A 15 per cent suspension of untreated cells, made from the washed, packed cells and buffered saline, was used as a control with each serum tested.

For the test 2 small drops of undiluted serum inactivated at 56 C. for 30 minutes were placed in the center of a ruled half of a microscope slide. Several slides may be set up at a time.

To the serum at the labeled end of the slides were added 2 drops of sensitized cell suspension; to the serum at the other end, 2 drops of untreated cell suspension. These were mixed rapidly with wooden applicators, spreading to form an oval, lengthwise of the slide, about 2.5×2 cm. in size. Slides were placed immediately on a special warmed illuminating box tilted by hand from side to side to cause the serum-cell mixture to run from one end of the oval to the other. Results were read after three minutes. Agglutination began quickly and was usually maximal in three minutes. Lack of agglutination was interpreted as a negative, any degree of definite agglutination as a positive reaction; if agglutination was indefinite, result was recorded as doubtful.

Evaluation of Hemagglutination Test for Tuberculosis is presented by Jack W. Fleming, Ernest H. Runyon and Martin M. Cummings,[5] who applied the Scott and Smith modification of this test to serums from normal subjects, patients with tuberculosis, sarcoid and miscellaneous diseases and BCG-vaccinated persons. Repeated tests on the same serum showed that the method used gives consistent results (table). Hemagglutination titers of 53 normal adults were mostly low or negative. The tuberculin-positive group had somewhat higher titers than the tuberculin-negative group.

Titers were higher in patients with active than in those with inactive tuberculosis but extremes were found in both groups. Average titers in patients with active minimal and moderately advanced tuberculosis were significantly higher than those in normal subjects but many patients with minimal disease had low titers. Highest average titer was that for the group with far advanced tuberculosis.

Patients with sarcoidosis had titers similar to those in the normal group. Response following BCG vaccination of tuber-

(5) Am. J. Med. 10:704-710, June, 1951.

culin-negative persons was inconsistent. No false positives were detected in patients with syphilis, pulmonary or miscellaneous diseases. The hemagglutination test by itself seems of limited value. Elevation of titer suggests tuberculosis in

RESULTS OF 2 TESTS ON 46 POSITIVE SERUMS USING DIFFERENT LOTS OF SENSITIZED CELLS

	No.	%
Identical titer, 1st and 2d test	28	61
1 tube difference	17	37
2 tube difference	1	2
More than 2 tube difference	0	0

cases in which the differential diagnosis includes this disease. Absence of demonstrable circulating agglutinins does not rule out active tuberculosis.

Lysis by Complement of Sheep Erythrocytes Sensitized with Extract of Mycobacterium Tuberculosis and Anti-BCG Rabbit Serum. Stephen Fisher[6] (Melbourne) confirms the report of Middlebrook and Dubos that sheep erythrocytes when coated with an extract of phenol-insoluble residue of tubercle bacilli became agglutinable by specific antiserums. It is also shown that hemolysis occurs when guinea pig complement is added to the suspension of sensitized cells in immune serum.

Amounts of antibody and complement necessary to cause lysis of a given quantity of erythrocytes are reciprocally interdependent in the conventional sheep cell-amboceptor-complement system as well as in the hemolytic system reported. In both systems more antibody is required to agglutinate a specified quantity of the appropriate cells than is necessary for lysis in the presence of excess of complement. Sheep erythrocytes sensitized with extract of Myco. tuberculosis behave in some respects in the same way in the presence of anti-BCG rabbit serum and complement as do normal sheep cells with amboceptor and complement.

[See the 1950 YEAR BOOK, pages 399-401, for this and other technics described by Middlebrook.—Ed.]

New Toxoplasma Antigen for Complement Fixation Test. The antigen described by Emil Steen and Erik Kåss[7] (Oslo) is water soluble, not anticomplementary and fixes complement specifically in the presence of human immune serum.

METHOD.—White mice are inoculated intra-abdominally with Toxoplasma gondi and killed the fourth day. The peritoneal exudate is pooled and centrifuged at 3,000 rpm for 30 minutes and the super-

(6) Australian J. Exper. Biol. & M. Sc. 28:613-618, November, 1950.
(7) Acta path. et microbiol. scandinav. 28:36-39, 1951.

natant fluid removed. The centrifugate is suspended in physiologic salt solution, volume being equal to that of the original exudate. The suspension is divided into six parts which are frozen in a mixture of solid carbon dioxide and alcohol and then thawed in tap water; this procedure is repeated 5 or 10 times (it yields a better antigen in a shorter time than slow freezing and thawing). The samples are then centrifuged at 2,000 rpm for 10 minutes. The supernatant fluid constitutes the antigen.

The antigens may be used undiluted or diluted in a volume of 0.2 ml. As complement fresh guinea pig serum diluted in saline solution is used, 0.2 ml. containing 2 units of complement is added to each tube. The mixtures are incubated in a water bath at 37 C. for 60 minutes. Then 0.2 ml. hemolysin (4 units) and 0.2 ml. of a saline suspension of 3 per cent washed sheep red cells are added. The tubes are incubated at 37 C. for 10 minutes and the reactions read.

Specific Complement Fixation Test for Infection with Poliomyelitis Virus was developed by Jordi Casals, Peter K. Olitsky and Ralph O. Anslow[8] (Rockefeller Inst. for Med. Research). The antigen was prepared from central nervous system tissue of newborn mice infected with the MEF1 strain of the Lansing type poliomyelitis virus. Specific reactions were obtained with serums of mice, cotton rats and monkeys immunized with the Lansing type virus and from monkeys and chimpanzees convalescent from infection with this virus. Of serums obtained from 35 persons convalescent from poliomyelitis, 21 were positive when tested with this antigen. Of 22 apparently normal persons with Lansing-neutralizing antibody, 6 had positive reactions, whereas only 1 of 19 without Lansing-neutralizing antibodies had a positive reaction.

With this test, serums were positive which were obtained from patients without Lansing-neutralizing antibody but from whom a Brunhilde poliomyelitis virus strain was isolated. This suggests that a cross reaction may exist between the Lansing and Brunhilde types.

Further study is necessary to discover whether this test can be applied practically to the diagnosis of poliomyelitis.

Mechanism of Hemolysis in Paroxysmal Cold Hemoglobinuria: Role of Complement and Its Components in Donath-Landsteiner Reaction. Donath and Landsteiner demonstrated that the abnormal factor responsible for hemolysis in paroxysmal cold hemoglobinuria is in the patient's serum. Inactivation of the serum by heat prevented hemolysis but addition of fresh serum restored the activity. The hemolytic reaction in vitro was shown to occur in two phases: (1) a cold phase

(8) J. Exper. Med. 94:123-137, August, 1951.

during which antibody (hemolysin) was adsorbed on the erythrocytes, and (2) a warm phase during which the sensitized cells were lysed in the presence of complement (fresh serum).

William S. Jordan, Jr., Louis Pillemer and John H. Dingle[9] (Western Reserve Univ.) studied the serum of two patients with paroxysmal cold hemoglobinuria and found that the Donath-Landsteiner reaction requires a large amount of complement. By using an adequate amount of complement the hemolysins from both patients were stable at 62 C. Further studies showed that complement must be present during both the cold and warm phases. A reciprocal relation was discovered between complement requirements in the two phases and amount of complement and degree of antibody fixation. It was further ascertained that the hemolytic reaction requires only two components of complement. Hemolysis occurs in the absence of fresh guinea pig fractions C'1 and C'3. Hemolysis does not occur when C'4 is missing in the cold phase or when C'2 is missing in the warm. Since only two components of complement are required for hemolysis in paroxysmal cold hemoglobinuria, titration of complement in a serum by the sheep cell-amboceptor system, which requires all four components, may not measure the capacity of that serum to produce hemolysis with paroxysmal cold hemoglobinuria antibody.

Mechanism of Hemolysis in Paroxysmal Cold Hemoglobinuria: Observations on Behavior and Nature of Antibody. William S. Jordan, Jr., Louis Pillemer and John H. Dingle[1] (Western Reserve Univ.) studied the erythrocytes of one patient and the serum of two.

The erythrocytes were agglutinable in antiglobulin serum (direct Coombs' test). Although the erythrocytes had been sensitized in vivo, they were not hemolyzed when warmed with complement. They were hemolyzed both in vivo when the patient was chilled and in vitro when they were chilled and warmed in paroxysmal cold hemoglobinuria serum. The cells were no longer agglutinable in antiglobulin serum six hours after hemolysis in vivo. No concomitant change in either serum antibody or complement level was detected. Further characterization of the "cell antibody" responsible for agglutination in the direct Coombs test was impossible since it was

(9) J. Clin. Invest. 30:11-21, January, 1951.
(1) Ibid., pp. 22-30.

not demonstrable in eluates from the patient's erythrocytes.

Antiglobulin serum (indirect Coombs' test) and the Donath-Landsteiner reaction were used to study the abnormal antibodies present in the serum of the two patients. Antibody adsorbed in vitro from serum with a high antibody titer could be eluted from erythrocytes by heating the cells at 56 C. The serum factor which reacts with antiglobulin serum was identified as the paroxysmal cold hemoglobinuria hemolysin. Fractionation of the serums in alcohol-water systems revealed that the paroxysmal cold hemoglobinuria antibody is a water-soluble (pseudoglobulin) gamma globulin. It resembles most other human antibodies in this respect.

CEREBROSPINAL FLUID

Effect of Head Posture on Manometrics of Cerebrospinal Fluid in Cervical Lesions: New Diagnostic Test. Lawrence Kaplan and Foster Kennedy[2] (Bellevue Hosp.) state that variation of head posture during manometric studies aids in detection of early cervical cord compression not revealed by the original Queckenstedt test heretofore applied. The new technic was used in 294 cases and 341 spinal punctures.

METHOD.—With the patient in the lateral supine position on either side, lumbar puncture is performed with an 18 gauge needle. Position of the patient is rechecked after successful puncture. No more than 90 degrees of flexion of the thighs on the abdomen is necessary. The head is centered in relation to the trunk by placing a small pillow under the parietal surface next to the table. One observer must be present for the sole purpose of managing the changes in head posture and compression of jugular veins. Before manometric studies are begun, the head is placed in "neutral position," in line with the trunk or very slightly flexed. The manometer is then attached and initial pressure recorded. The rise in pressure with firm abdominal compression must first be estimated while the patient avoids straining or holding his breath. If a free connection is established, manometric response to manual jugular compression, applied bilaterally, is recorded. The head is then fully flexed while the observer's hands maintain contact without pressure on the neck structures. In this position the jugular compression test is repeated and the results noted. Finally the head is fully extended on the neck and the test repeated for the third time. The thoracic cage should be immobile during these manipulations. In each of the head positions, abdominal compression is applied after jugular compression to assure free connection.

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(2) Brain 73:337-345, September, 1950.

The purpose of the test is to determine whether subarachnoid block, as manifested by pressure studies, exists in only one, two or all head positions. Manipulation of the head is contraindicated in any recent acute cervical spine injury and in any suspected high cervical-foramen magnum lesion.

Of 31 patients with clinical diagnosis of cord-compressing lesions in the cervical region, 3 showed complete subarachnoid block unchanged by variation in posture, 16 had normal test results in all positions and 12 showed abnormalities in only one position. The phenomenon of intermittent block, as demonstrated in the last group of patients, is pathologic, not physiologic, since normal results were encountered in all 253 patients with neurologic disorders without suspicion of cord compression.

Four of the 12 patients were treated nonoperatively, but exploratory laparotomy in the rest revealed extrinsic cord lesions in the cervical region. Seven of the 16 with cervical lesions who had normal test results underwent exploratory laminectomy; no demonstrable lesion was found in 6 and slight, filmy, cervical arachnoiditis in 1.

Of the 12 patients in whom the test showed abnormality, 6 had normal protein levels, 3 slight protein elevations and 3 moderate elevations. This discrepancy between subarachnoid block and protein elevation is now explained on the basis of "intermittency" of the block depending on position of the head and neck.

Tryptophan Test in Cerebrospinal Fluid has been advocated as helpful in the difficult differentiation of tuberculous from abacterial meningitis, meningism and preparalytic poliomyelitis. In the study of this reaction, Gerhard Stalder[3] (Univ. of Basel), used one quantitative and two qualitative technics. Since the former determines only free tryptophan, enzymatic breakdown of cerebrospinal fluid proteins was necessary for accurate tryptophan estimation. Analyses were made on casein, old tuberculin, brain, hemoglobin, fibrin, fibrinogen, thrombin, serum albumin and globulin. The most intense qualitative reactions were given by human thrombin and brain.

Positive reactions were obtained in 104 of 273 patients at Children's Hospital, Basel: 45 had tuberculous meningitis; 34, purulent, bloody or xanthochromic fluid, and 25, various other ailments, including meningism, poliomyelitis, encephali-

(3) Ann. paediat. 176:270-291, April, 1951.

tis and syphilis of the nervous system. There was no measurable free tryptophan in any of the fluids: tryptophan content increase was due in each instance to increase in tryptophan-containing proteins. In tuberculous meningitis, tryptophan values were 0.2-11.7 mg./100 cc. cerebrospinal fluid and the tryptophan content of cerebrospinal fluid protein, 0.9-1.95 per cent. The tryptophan fluid content in various other meningitides was 0.2-2.4 mg. per cent, whereas cerebrospinal fluid protein contained 0.75-2.7 per cent tryptophan.

In general, whenever total protein increases to 0.5-1 per cent, results of the tryptophan test are positive. This may occur in a variety of neurologic conditions. It remains to be seen to what degree the presence of tryptophan-rich proteins in cerebrospinal fluid indicates brain destruction.

Cerebrospinal Fluid: Comparative Study of Specimens Taken from Cisterna Magna and Lumbar Subarachnoid Space. M. R. Davis, G. R. Cannefax and E. B. Johnwick[4] (U. S. Pub. Health Service) report results on material obtained from simultaneous cisternal and lumbar punctures on 102 unselected adults. In 21 instances cell counts were slightly higher in cisternal than in lumbar specimens, and the reverse was true in 26. In instances in which pleocytosis was pronounced, the count was appreciably greater in the lumbar fluid. Specimens from nine patients showed a positive reaction to the Kolmer complement fixation test. In eight instances the readings were comparable; in the ninth, the cisternal fluid was negative, whereas the first tube of the test on the lumbar fluid showed a 4 plus reaction. Most cisternal fluids examined contained less than 26 mg. per cent protein, and relatively few lumbar specimens exceeded a value of 35 mg. per cent. In the absence of appreciable elevation of protein, the cisternal to lumbar total protein ratio approximates 2:3 in most instances. Positive colloidal mastic reactions were noted on specimens taken from 24 adults. In 13, only the lumbar fluid was positive. No cisternal fluids were positive in the absence of positive lumbar specimens. The more consistent reactivity of lumbar specimens probably reflects the higher total protein values in lumbar than in cisternal fluids in given persons.

Comparison of Results of Spinal Fluid Examination by Two Separate Laboratories on Duplicate Specimens of Spinal Fluid from 400 Patients with Syphilis is presented by William

(4) J. Ven. Dis. Inform. 32:284-288, October, 1951.

T. Ford, John H. Stokes, Herman Beerman and C. J. Gentzkow[5] (Philadelphia). Conflicting results were obtained on 18 per cent of the 400 fluids with one or more of the four tests used. There was conflict between results with the Kolmer and Kline tests in 13.7 per cent and between results with colloidal gold and colloidal mastic tests in 9 per cent. Cell counts were conflicting in 1.5 per cent and total protein determination in 1 per cent.

Patients known to have neurosyphilis contributed 264 specimens; the others were from patients with latent syphilis. Of those with neurosyphilis, 218 had positive cerebrospinal fluid findings. Among these, results were conflicting in 33 per cent. Kline and Kolmer reactions were conflicting in 28.7 per cent and results with colloidal gold and mastic tests in 17.5 per cent. Cell counts were conflicting in 14.7 per cent and total protein determinations in 7.7 per cent.

The Kolmer test was about twice as sensitive as the diagnostic Kline test (using cardiolipin antigen) on fluids from penicillin-treated patients with neurosyphilis. There was little difference between their sensitivity in untreated neurosyphilis. The mastic test appeared to be more sensitive than the colloidal gold test on the treated patients, but there was no difference between the tests on untreated ones.

Although both the Kolmer and the Kline test gave a high degree of specificity on the fluids examined, it is recommended that both a complement fixation and a flocculation test be performed on cerebrospinal fluids of patients suspected of having central nervous system involvement.

[It is remarkable that there was complete agreement of the results reported by two laboratories with all four tests in 82 per cent of fluids from 400 patients, rather than that there were 18 per cent conflicting reports with one or more of the four tests.—Ed.]

Critique of Methods for Determination of Protein in Cerebrospinal Fluid is presented by H. L. Wikoff and P. Kazdan[6] (Ohio State Univ.). The protein concentration of 50 samples of cerebrospinal fluid was analyzed by common qualitative methods and results checked against Kjeldahl analyses. Since results obtained with the standard qualitative methods, Pandy test, K. O. Newman test and Ross-Jones test were often inaccurate, it was concluded that these methods are unreliable. Results obtained by the colorimetric procedures of Johnston

(5) Am. J. Syph. 35:553-558, November, 1951.
(6) Am. J. Clin. Path. 21:1173-1177, December, 1951.

and Gibson, Matz and Novick, and Denis and Ayer correlated satisfactorily with those obtained by the Kjeldahl method. Statistical analysis revealed that no one quantitative method was greatly superior to the other. The final choice of the method should depend on the skill of the technician who performs the test. The Johnston-Gibson method is the simplest, whereas the Matz-Novick method also permits calculation of albumin-globulin ratios. The Denis-Ayer method is too complicated for the average technician. In both the Johnston-Gibson and the Matz-Novick method, the improved phenol reagent developed by Folin and Ciocalteu, containing lithium sulfate, produced more satisfactory results than did Folin's original phenol reagent.

PARASITOLOGY

Rapid Flocculation Test for Diagnosis of Trichinosis. The test described by John Bozicevich, John E. Tobie, Elizabeth H. Thomas, Helen M. Hoyem and Stanley B. Ward[7] is equally as sensitive as the complement fixation test and apparently gives no false positive reactions. It can be performed rapidly; personnel need not be highly trained. The antigen remains stable for months.

METHOD.—Trichina extract is prepared by inoculating medium-sized adult rats intragastrically with infective larvae of Trichinella spiralis. After two months the animals are killed, muscle samples examined for larvae and only heavily infected carcasses put through a meat grinder. To a battery jar containing 3 L. artificial gastric juice is added 70 Gm. ground meat. The digestive fluid is prepared by adding 21 ml. HCl (specific gravity 1.10-1.19) and then 15 Gm. pepsin to 3 L. tap water (37-40 C.). The mixture containing meat is slowly agitated for six hours at 37 C. It is poured through one layer of surgical gauze (35-40 mesh apertures/in.) into a 3 L. glass funnel, to the stem of which is attached a 15 ml. conical centrifuge tube by means of a rubber tube, fitted with a pinchcock. The mixture is allowed to stand 1½ hours so that larvae will settle to the bottom of the centrifuge tube. The pinchcock is closed and the tube containing larvae removed. The supernatant is pipetted off as closely as possible to the settled larvae and the tube refilled with physiologic saline and allowed to settle 10-15 minutes. This procedure is repeated at least five to six times until the larvae are free from rat protein. Two ml. larvae is suspended in 8 ml. physiologic saline and transferred to a 10 ml. Ten Broeck tissue grinder. The larvae are thoroughly ground, transferred to a flask containing 90 ml.

(7) Pub. Health Rep. 66:806-814, June 22, 1951.

physiologic saline and allowed to extract at 4 C. for 24 hours. The suspension is centrifuged in 20 ml. culture tubes at about 3,000 rpm for 15 minutes (International centrifuge, size 2, no. 240 head). The sediment is discarded and the supernatant saved and centrifuged at 15,000 rpm for 15 minutes; if a high speed centrifuge is not available a sintered glass filter of fine porosity (UF) may be used. The supernatant fluid (or the filtrate) is the trichina extract.

Stock bentonite is prepared by suspending 0.5 Gm. BC micron or no. 200 standard Volclay (Wyoming bentonite) in 100 ml. distilled water. This is homogenized in a Waring blendor for one minute, the bentonite suspension transferred to a 500 ml. glass-stoppered graduate and distilled water added to make 500 ml. After thorough shaking it is allowed to settle for one hour. The supernatant is placed in 100 ml. centrifuge tubes and centrifuged by tachometer at 1,300 rpm for 15 minutes (International centrifuge size 2, no. 240 head). The supernatant is saved and centrifuged in 100 ml. test tubes at 1,600 rpm for 15 minutes and the supernatant discarded. The sediment is resuspended in 100 ml. distilled water and homogenized in a Waring blendor for one minute; this stock bentonite will remain stable for four months without losing adsorptive properties.

To prepare the stock antigen, 10 ml. trichina extract is added to 20 ml. stock bentonite and allowed to absorb at 4 C. for 15-18 hours. Five ml. of a 0.1 per cent thionine blue O (C. I. no. 926) solution is prepared in distilled water and added to the 30 ml. sensitized bentonite. After thorough shaking one hour is allowed for adsorption of the dye.

To prepare the test antigen, the stock antigen is thoroughly shaken and 8 ml. transferred to a 16 × 150 mm. culture tube; 10 ml. physiologic saline is added, the tube shaken thoroughly and spun five minutes at 2,500 rpm in the International centrifuge. The supernatant is discarded and the sediment resuspended in 15 ml. physiologic saline. After five minutes at 2,500 rpm the supernatant is discarded and sediment resuspended in 4 ml. physiologic saline. To this is added 0.5 ml. of 1 per cent distilled water solution of tween® 80 and the mixture shaken thoroughly.

Saline and negative serum control must be performed to determine proper adjustment of the spontaneous flocculating properties of the antigen. For saline control, 1 drop of antigen is added to 0.1 ml. of physiologic saline and the slide placed on a Boerner type rotating apparatus and rotated 100-120 times/minute for 15 minutes. If reaction is greater than 2+, additional 0.1 ml. amounts of tween 80® must be added not to exceed 0.5-1.0 ml. in total until a 2+ or less reading is obtained. If the antigen does not adjust it should be washed by centrifugation several times in distilled water, then 4 ml. physiologic saline and 0.5 ml. tween 80® added as before and again tested. For negative serum control, 1 drop of antigen is added to 0.1 ml. of twofold serial dilutions of negative serum. The dilutions, made in physiologic saline, should be the same as used for positive serum in the test proper. The slide is rotated for 15 minutes and read. There must be no flocculation in the dilution of the negative control serum which corresponds to the highest dilution

of the positive serum giving a 3+ or greater reaction. The antigen is tested against twofold serial dilutions of a positive serum according to the technic for the test proper. The antigen is now ready for use as the test antigen.

For the test proper, 1:5, 1:10 and 1:20 serum dilutions are prepared with physiologic saline. The twofold serial dilutions may be continued to the end point; 0.1 ml. of each dilution is pipetted onto a slide by putting the pipet in contact with the slide, delivering the 0.1 ml. amount and withdrawing. The same pipet is used for all dilutions, proceeding from highest to lowest. The test antigen, kept at 4 C., should reach room temperature before use. The antigen is shaken vigorously to obtain a homogeneous suspension, and with an antigen pipet 1 drop is added to each portion of diluted serum. The slide is rotated at 100-120 rpm for 15 minutes and flocculation reactions then read. For each series a negative serum control with 1 drop antigen and 0.1 ml. of the same serum dilutions should be set up. Saline control with 1 drop antigen and 0.1 ml. physiologic saline in the dilution of the serums should also be prepared. The specimen should also be examined under low power magnification, taking care to examine the entire area.

In a 4+ reaction all sensitized particles will flocculate into separate masses. The fields between the flocs are clear. About three fourths of the sensitized particles are flocculated in a 3+ reaction, one-half in a 2+ reaction and one-fourth in a 1+ reaction. None are flocculated in a negative reaction. A serum which gives a 4 or 3+ reaction is termed positive whereas that giving a 2 or 1+ reaction is negative.

Carbol Toluidine Blue for Staining of Blood Protozoa, described by Viola Mae Young[8] (Cook County Hosp.), is simple to prepare and use and gives a satisfactory stain for both trypanosomes and plasmodia.

PROCEDURE.—To 1 Gm. toluidine blue and 2 Gm. phenol, dissolved in 5 cc. alcohol, 100 cc. distilled water is added. After standing for 48 hours, the solution is filtered. Smears are fixed with methyl alcohol for three minutes, drained, covered for five minutes with stain, washed with distilled water, again drained and allowed to dry.

Structural characteristics are well distinguished, the nuclear material appearing pink in contrast to the pale blue cytoplasm. The undulating membrane stains particularly well.

In Vitro and in Vivo Studies of New Antibiotic, Fumagillin, with Endamoeba Histolytica were carried out by Arseny K. Hrenoff and Mitsuru Nakamura[9] (Univ. of California). Fumagillin has been isolated from Aspergillus fumigatus. Its "amebacidal" level was detected in dilutions of 1:10,000,000 to 1:15,000,000. Trypanosomes were not affected

(8) Lab. Digest 15:15, September, 1951.
(9) Proc. Soc. Exper. Biol. & Med. 77:162-164, May, 1951.

by these concentrations. When 0.01 N NaOH was used as a solvent for the antibiotic agent, it was not toxic for E. histolytica. Fumagillin exerted the most active in vitro effect of all the numerous antibiotic and other agents tested.

Fourteen monkeys infected with E. histolytica were treated with fumagillin, receiving 50-125 mg. /kg. body weight orally for five days. Follow-up examinations disclosed that the stools of four contained E. histolytica. No toxic manifestations were observed. Bromsulfalein retention tests indicated that 125 mg./kg. might cause liver damage. Blood urea nitrogen level increased in only one monkey. ECG's of all animals were normal after therapy.

Acquired Toxoplasmosis: Report of Seven Cases with Strongly Positive Serologic Reactions. J. Chr. Siim[1] (Copenhagen) performed the Sabin-Feldman serum dye test and complement fixation tests for toxoplasmosis on six children and one adult with generalized lymphadenopathy or suspected infectious mononucleosis and obtained positive results in all (five cases occurred in two familial outbreaks). The principal clinical change with hitherto unnoticed, enlarged painless lymph nodes in the neck, groins and axillas. Some patients complained of excessive lassitude for no obvious cause, elevation of temperature or nonspecific catarrhal symptoms. Lymphocyte counts were increased in three patients, and smears showed atypical lymphocytes with increased cytoplasmic basophilia. No patient had exanthems. Wassermann and Paul-Bunnell reactions were negative. Although in congenital toxoplasmosis eye and central nervous system lesions are dominant, no abnormality of these organs could be found.

Microscopic examination and animal inoculation of a lymph node removed at biopsy gave negative results. Although these methods require positive results to confirm diagnosis, the results of the serologic tests made the diagnosis probable, since the titers were of a level hitherto observed only in confirmed congenital toxoplasmosis.

The natural route of infection with this organism is unknown, but the possibility of infection via the alimentary or upper respiratory tracts as well as by bites of blood-sucking insects should be considered. The viscera of rabbits and urine of infected dogs contain the organisms. It is possible that these sources play a role in natural transmission.

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(1) J. A. M. A. 147:1641-1645, Dec. 22, 1951.

Chromatographic Analysis of Ragweed Pollen Extract. Ely Perlman[2] (Mount Sinai Hosp., New York City) used this method to separate the numerous constituents present in crude extracts of giant ragweed pollen. The components were de-

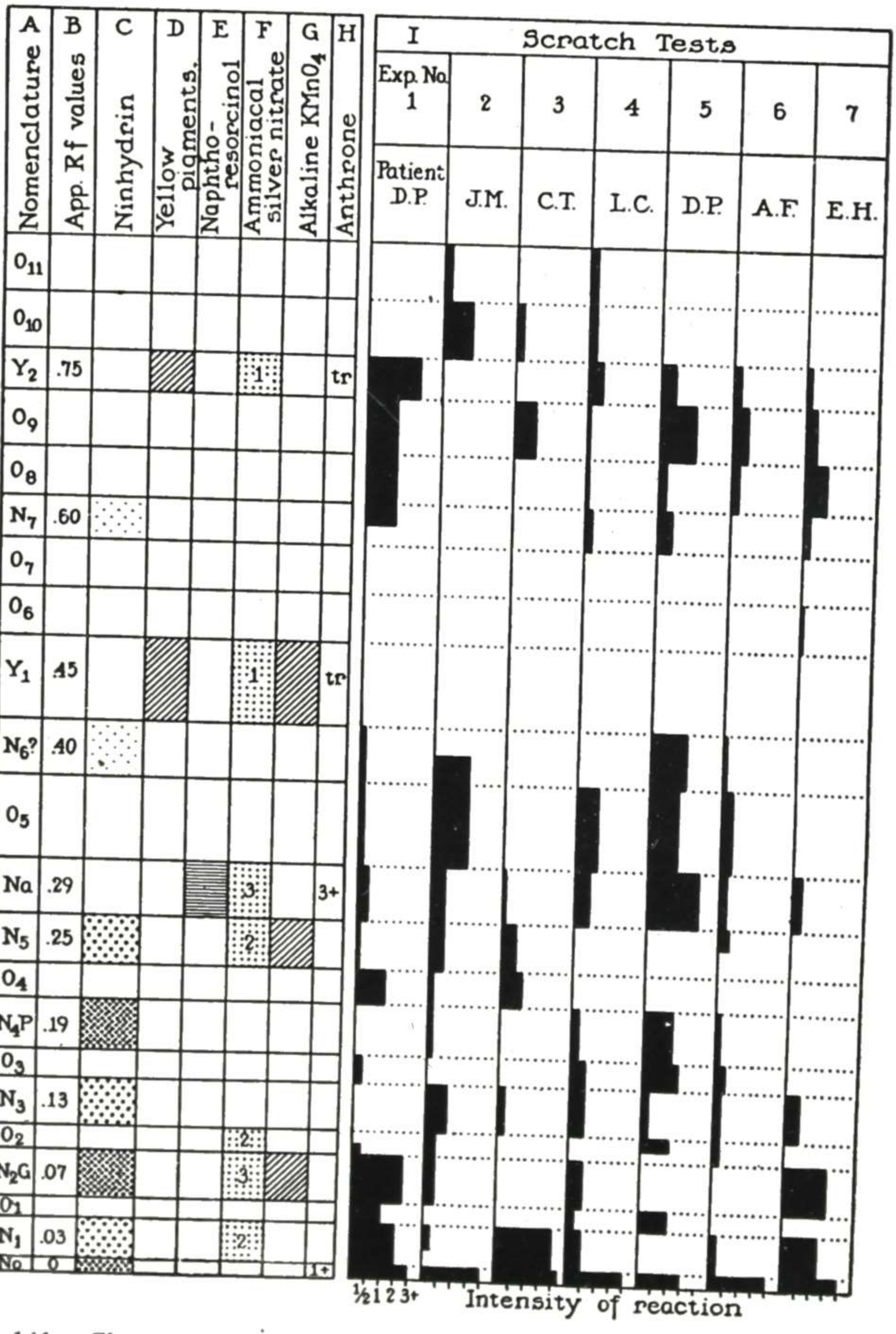

Fig. 141.—Chromatographic analysis of ragweed pollen extract, showing bands revealed by various chemical tests and results of skin tests with fractions eluted from each corresponding region of chromatogram. (Courtesy of Perlman, E.: Bull. New York Acad. Med. 27:586-589, September, 1951.)

(2) Bull. New York Acad. Med. 27:586-589, September, 1951.

tected by a variety of tests and their activity determined by elution from the paper and scratch testing suitable sensitive patients. A chromatogram is shown in Figure 141, in which it is seen that at least two naturally occurring yellow pigments separated (Y_1 and Y_2, column D). Components revealed by spraying with ninhydrin reagent are shown in column C. Reducing substances detected after treatment with ammoniacal silver nitrate are shown in column F. Presence of polysaccharides was detected by Dreywood's anthrone reagent (column H).

Activity of the components was determined by performing scratch tests with the eluates on sensitive and control subjects. The control subjects showed no reaction; the reactions of the sensitive subjects are recorded alongside the region where the eluates were obtained. Eluates obtained from regions Na, O_5 and N_6 were active; the eluate from No gave the strongest reaction. Additional active material may occur in region N_4P. Nitrogen content of the various eluates in no way correlated with their activity.

Vaginal Smears in Evaluation of Ovarian Development and Activity. After examining many vaginal smears, Ralph G. Bonime[3] (New York City) concluded that stratification and cornification proceed and recede in direct proportion to the amount of ovarian hormones involved, whether intrinsically elaborated or therapeutically administered.

Smears with low levels of stratification which reveal no evidence of activity over long periods constitute the criteria of the infantile ovarian age, no matter at what chronological age they are found. The prepuberal ovarian age is characterized by vaginal smears with moderate levels of stratification and cornification but no evidence of cyclic or rhythmic alterations which usually lead to bleeding phenomena. In the puberal ovarian age, during follicle ripening followed by prolonged functional activity, the rate of stratification is higher than that of desquamation and no precornified cells are found. Follicle ripening followed by early collapse and deterioration is characterized by gradually increasing stratification and cornification. Smears reveal a poised state of highly cornified cells which soon begin to show signs of deterioration. During desquamation, deep cells from the outer basal zone may be found. Between these two extremes is follicle inertia, during

(3) Am. J. Obst. & Gynec. 60:1306-1314, December, 1950.

which alterations in the smear vary according to individual changes.

In the mature ovarian age the follicle-ripening phase is characterized by a closely packed zone of cornification; pyknosis and polygonal shapes are relatively uniform, egress of leukocytes is inhibited and a high acidity inhibits bacterial growth. In the follicle transition phase, estrogen withdrawal causes deterioration and desquamation of the cornified zone. Leukocytes appear abruptly, followed in a day or two by noncornified cells. The picture of a clean smear of cornified cells on which a sudden leukocytosis occurs indicates ovulation. In the stage of luteinization, cornified cells decrease in number as noncornified cells appear. During premenstrual deterioration, cellular elements are no longer discernible. In the menopausal ovarian age, smears reveal "atrophy," desquamation to the lower levels of stratification without signs of regrowth. There is a resemblance to the infantile type of smear, which suggests that ovarian activity is absent.

Role of Salientia in Human and Mammalian Pregnancy Tests. J. L. Bhaduri[4] (Univ. of Calcutta) states that while the female Xenopus laevis, a South African clawed toad, may be useful for diagnosing human pregnancy, several investigators have found that spontaneous ovulations often occur among animals kept in single glass jars under ordinary laboratory conditions. Thus far the only other female toad which has shown a hormonal affinity is the Discoglossus, also from South Africa.

Although it was well known that both sexes of Salientia would react to mammalian gonadotrophic hormones, it was Galli Mainini who fully realized the significance of this fact and utilized the males of the South American giant toad, Bufo arenarum, for urine test in pregnancy diagnosis. From 1 to 20 ml. urine is injected into the dorsal lateral subcutaneous lymph sacs of an adult male frog or toad, and after ½-2 hours the urine is sampled and examined under the microscope. The presence of sperm in any quantity, dead or alive, motile or nonmotile, indicates a positive reaction. It is wise to test the animal's urine before the unknown is injected. The animal can be used successfully several times after a rest of four to seven days. Results from the experimental injection of chori-

(4) Proc. 38th Indian Sc. Cong., pt. 2, pp. 171-202, 1951.

onic gonadotrophin into these animals indicate that the reaction set up in male Salientia under the influence of pregnancy urinary gonadotrophins is due to the synergistic action of luteinizing hormone and follicle-stimulating hormone.

There is no doubt that, while the first morning specimen of urine is preferable, no less satisfactory results may be obtained with urine specimens collected during any part of the day. Concentration of the specimen probably is not necessary if the maximal quantity a frog or toad can accommodate without detrimental effect and absorb within ½-1 hour is given initially. For example, in a group of animals with a negative response a second 5 ml. dose of urine was uniformly used with successful results. Toads tested with toxic urine may yield a definite result.

Although results with serum may be as satisfactory as with urine, the latter is considered the most suitable material for all practical purposes. With the Galli Mainini procedure, pregnancy may be diagnosed as early as the fourth to fifth day after the first missed period. Even earlier positive responses have been reported. A negative result should always be checked with a second injection of urine, the observation continuing for two to three hours. In abnormal cases results should be analyzed carefully by obtaining several samplings of the toad's urine at intervals of one-half hour. Frequently the tests should be repeated from day to day or from week to week. In such cases conclusions should not be based on a single finding.

Experimental work with various species of animals has indicated that specimens of feces may provide suitable test material.

New Biochemical Test for Pregnancy: Study of 2,560 Tests on 1,640 Patients. Garwood C. Richardson[5] (Chicago) found a simple office procedure involving identification of free estrone to have an accuracy of 99.1 per cent in diagnosis of pregnancy. Among 500 nonpregnant women there was no false positive result. Positive reactions were obtained in three women before they had missed a period. Return to a negative reaction after delivery depends on complete removal of placental tissue, which requires but a few days. Only four patients were still positive six weeks post partum. Of 13 patients with threatened abortion, 4 were positive and abortion was in-

(5) Am. J. Obst. & Gynec. 61:1317-1323, June, 1951.

complete, whereas 9 were negative and curettage produced no living chorionic tissue. A negative reaction therefore indicates complete abortion, complete placental separation or placental death, and intervention would be indicated only for bleeding. Four of five tubal pregnancies gave positive reactions. Hydatidiform moles and chorionepitheliomas also produce positive reactions.

This test is based on the fact that although estrone is normally present in female urine, it is not present in sufficiently high concentration to give a positive response to the reagent which is carefully calibrated to react only with the significantly higher levels found in pregnancy. Blood serum may be used also. The diagnostic agent is 2, 4-dinitrophenylhydrazine in solution with ethanol. A standard screw cap test tube with 2 and 5 cc. calibration marks and a specially constructed tube to fit in it are the only apparatus required. The insert tube has a perforation at a proper level above the 5 cc. calibration mark to permit withdrawal of an upper stratum of the fluid which separates as a result of the extraction process.

METHOD.—Two ml. filtered urine is placed in the outer chamber. Two drops of 0.5 N NaOH is admixed. Because of its phenolic hydroxyl group, estrone is sufficiently acid to react and form sodium estronate, which is not soluble in chloroform U.S.P. Progesterone does not separate. Chloroform is added to the 5 cc. mark. The tube is capped and shaken vigorously for 30 seconds and then allowed to stand until the two layers separate (about 1 minute). The inner tube is then inserted, with consequent rising around its outside of the sodium estronate-containing liquid, which enters the inner tube through the special opening. The tubes are separated, the larger one is rinsed out and the contents of the inner tube are emptied into it. Four drops of 0.5 N H_2SO_4 is admixed for conversion of the estrone salt to free esterone and to assure an acid medium to allow its chemical coupling with 5 drops of saturated 2,4-dinitrophenylhydrazine in 70 per cent ethanol, prepared within 24 hours of the test. This is allowed to stand for 10-15 minutes. Two cc. of 0.5 N NaOH is admixed for alkalinization to allow development of the stable brown color of the sodium salt of 2,4-dinitrophenylhydrazine of estrone.

In an alternative procedure, which is the same until after the sodium estronate-containing liquid enters the inner tube, 5 drops of freshly prepared 1 per cent meta-dinitrobenzene in ethanol and 1 ml. of 15 per cent potassium hydroxide are added. The contents are mixed and allowed to stand about 10 minutes. Estrone forms a chemical complex with potassium hydroxide, giving a reddish violet color.

The alternative procedure is subject to some errors due to certain drug reactions and is used only for comparisons. The

principal procedure, in addition to its high degree of accuracy and early recognition of pregnancy, is inexpensive, simple, requires a minimum of equipment and can be completed in 30 minutes.

Seasonal Variation in Pregnancy Test Using Rana Pipiens. J. B. Holyoke and E. E. Hoag[6] (Dartmouth Med. School) conducted a study to determine the seasonal effect on the minimal reactive dose of chorionic gonadotrophin, using male frogs as subjects. During March, April and May and the first half of June, about 75 per cent of the animals reacted to 10 I.U. of hormone; a significant number of reactions occurred with 7.5 and 5 I.U. In the second half of June, only a fourth of the frogs reacted to 10 units and none to less. During the second half of July, 80 I.U. was needed to induce reactions in 75 per cent of the frogs, though one reaction occurred with 20 units. During the last half of August, there were no reactions with 80 units. In September, 75 per cent of the frogs reacted to 40 I.U., and in October, 100 per cent reacted to 20 units and 50 per cent to 10 units. By November the frogs were reacting similarly to their performance of the previous winter.

Comparison of these data with results of 211 pregnancy tests suggests that poor results can usually be traced to seasonal variability in the sensitivity of the frog to chorionic gonadotrophin. During summer the amount of hormone injected should probably be increased through the use of urine concentrates equivalent to 40-100 ml. whole urine. Production of any sperm in fresh frogs during summer should be interpreted as a positive reaction.

MICROSCOPY

Electron Micrograph of Plasma Lipoprotein Molecule (S_f 10-20). John J. Prendergast and D. Maxwell Teague[8] (Detroit) present an electron micrograph (Fig. 142) showing individual molecules of the blood lipoprotein which Gofman and co-workers have shown to be associated frequently with atherosclerosis. The concentrates in which this lipoprotein complex was separated contained both the normal cholesterol molecule of 3-7 Svedberg flotation units and the abnormal

(6) Am. J. Clin. Path. 21:1121-1126, December, 1951.
(8) Circulation 4:23-24, July, 1951.

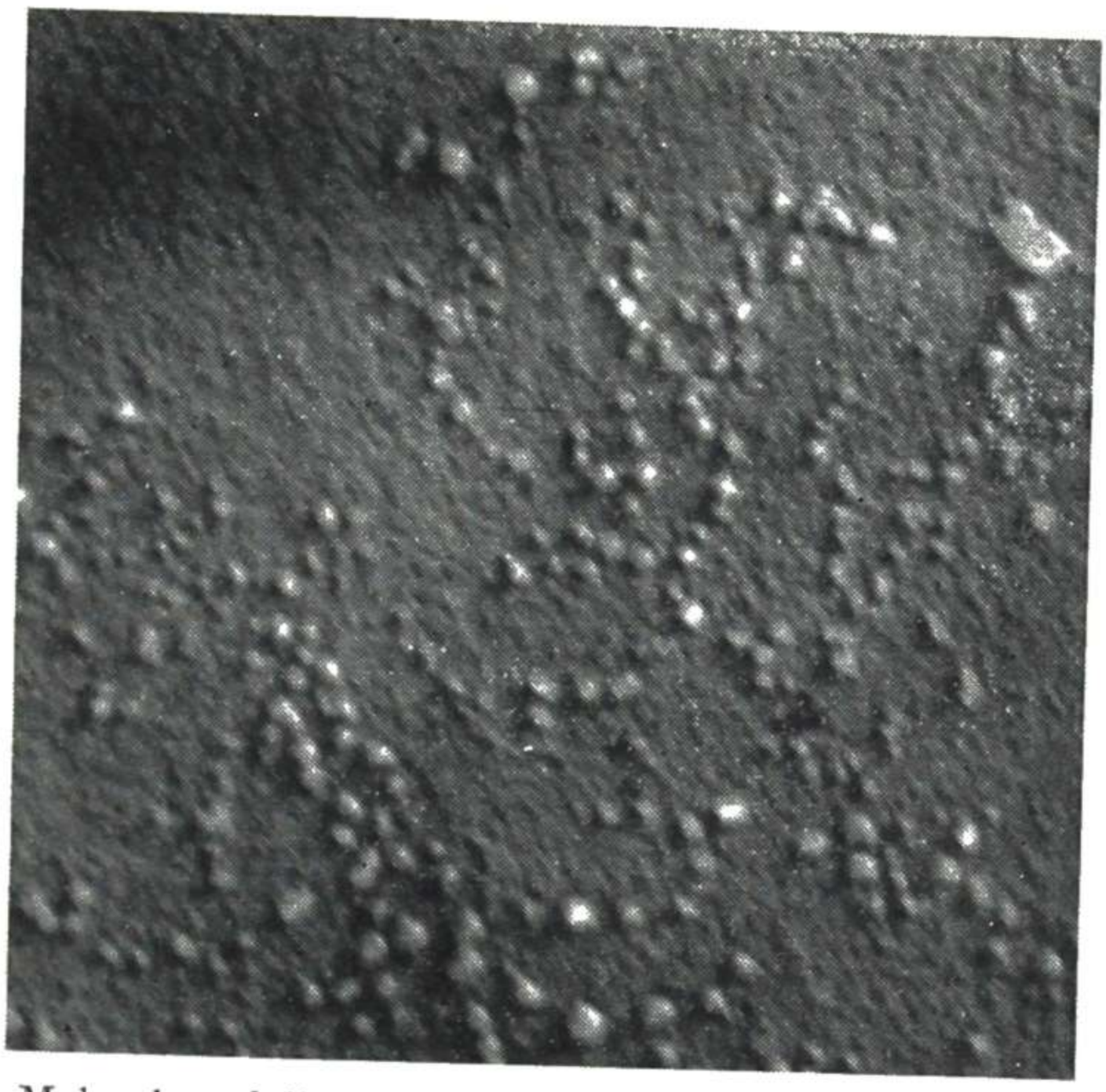

Fig. 142.—Molecules of lipoprotein presumed to be associated with atherosclerosis; × 25,000. (Courtesy of Prendergast, J. J., and Teague, D. M.: Circulation 4:23-24, July, 1951.)

lipoprotein complex of 10-20 Svedberg flotation units. Careful measurements of single particles were made on several micrographs with the resultant mean particle size diameter of this abnormal lipoprotein of 25 mμ. This supports Gofman's previous estimates of the size of this particle.

Electron Microscope Study of Red Cell Membranes after Experimental Infection with Virus of Foot and Mouth Disease. B. Epstein, N. M. Fonseca and E. De Robertis[9] (Montevideo, Uruguay) inoculated guinea pigs with the Vallée O type strain. Red cells obtained 42-48 hours after inoculation and washed in saline solution were infective in normal guinea pigs. Infection was no longer obtained at 92 hours when general symptoms of the disease had regressed.

By direct examination and also shadow casting with palladium, normal red cell membranes showed only a very fine structure on the surface (Fig. 143). Rounded masses of high electron density appeared in the ghosts 24-72 hours after inoculation with virus (Fig. 144). In some cases the masses were distributed preferentially in single lines forming ring figures and in others at random. At 42 hours, dense particles of 20-70 mμ could be detected in the masses. On the basis of

(9) J. Exper. Med. 94:171-176, Sept. 1, 1951.

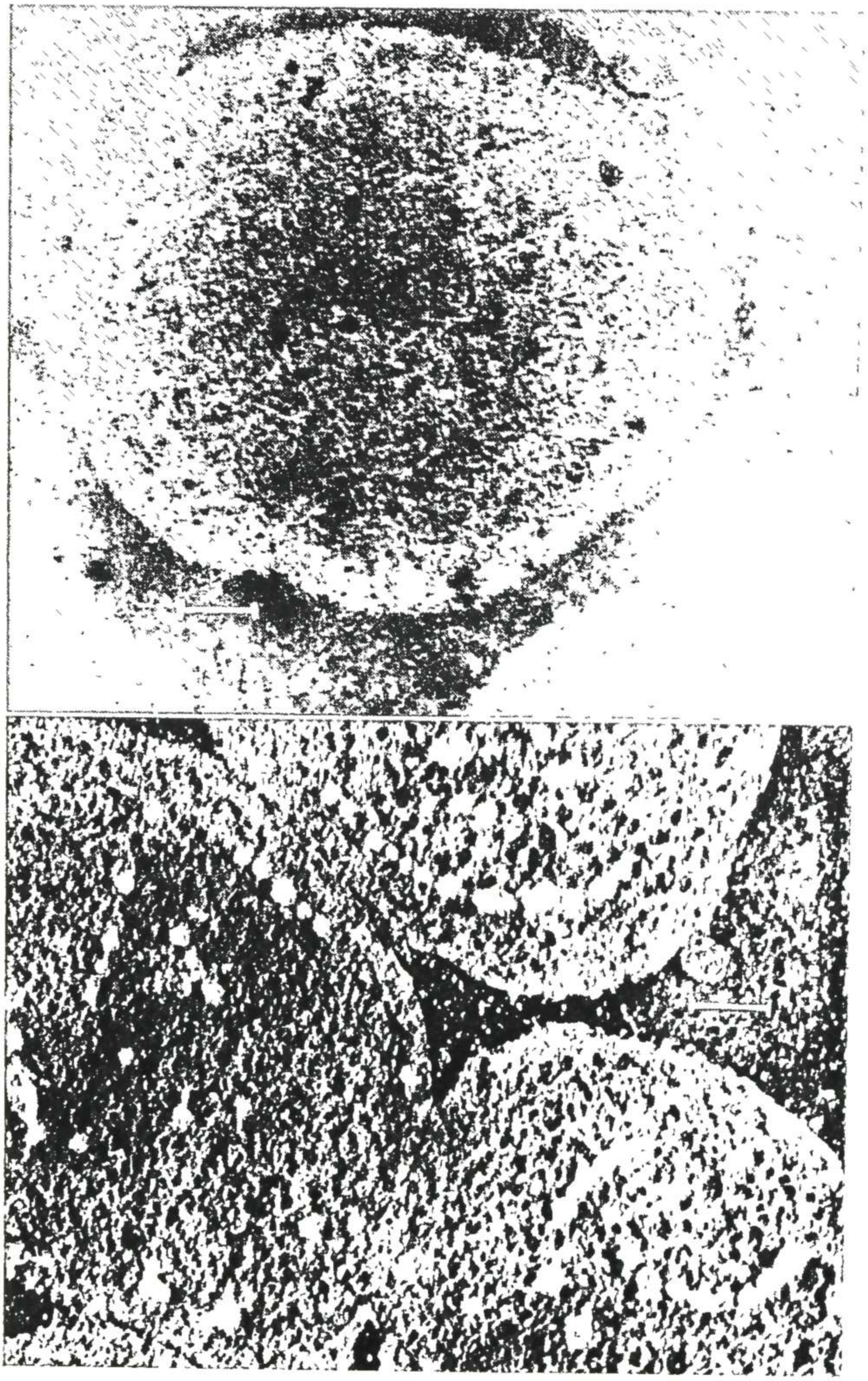

Fig. 143 (top).—Normal red cell membranes; reduced from × 10,800.
Fig. 144 (bottom).—Red cell membranes 72 hours after inoculation; reduced from × 10,500.
(Courtesy of Epstein, B., *et al.*: J. Exper. Med. 94:171-176, Sept. 1, 1951.)

105 electron micrographs and study of thousands of cells, it was concluded that practically all red cell membranes contain dense masses 24-72 hours after inoculation, but at 92 hours,

coinciding with disappearance of infectivity of the erythrocytes, the masses can no longer be seen and the membranes look entirely normal. Although the observations suggest a relation between the dense masses in the red cell membranes and presence of virus entities in washed erythrocytes, no definite theory is advanced.

"Phase Contrast" Methods and Birefringence. R. Barer[7] has found it advantageous to employ polarized light when using phase contrast methods. In the case of certain liquid crystals, areas containing sharp interference fringes alternated with quite large clear areas completely devoid of fringes when monochromatic unpolarized light was used. When a polarizer was introduced the fringes extended into the clear areas, and on rotating the polarizer 90 degrees a dark fringe was replaced by a bright one. This provides an interesting illustration of the fact that a birefringent crystal actually resolves light into two components and also that in such an object the contrast may be zero unless polarized light is used. Therefore, in using any variety of phase contrast method, a polarizer should always be available as considerable improvements in contrast may be revealed as well as the presence of birefringence.

With some types of interference microscopy, objects producing phase changes of several wavelengths can be observed. Birefringence can be measured by counting the changes in the number of fringes across the object. This is not normally possible in phase contrast microscopy, since phase changes differing by a whole number of wavelengths cannot be distinguished easily.

With some binocular microscopes the plane of polarization differs by 90 degrees in the two eyepieces. Thus, under suitable conditions, very different images may be seen in each eyepiece, even when no other polarizer is deliberately introduced. Some caution is therefore necessary in using these instruments.

APPARATUS

Electrically Operated Bunsen Type Burner. The heating unit of the burner described by Sol Roy Rosenthal[2] (Univ. of

(7) Nature, London 167:642-643, Apr. 21, 1951.
(2) J. Lab. & Clin. Med. 37:327-330, February, 1951.

Illinois) is composed of a lava cylinder 3¼ in. long with outside diameter ⅞ in. and a central cavity ½ in. in diameter which runs the entire length of the core. The heater wire (28 gauge) is wound around the threaded column submerging the wire from the surface. The heater is operated by a toggle switch in the base. A movable deflector can be rotated 360 degrees and functions to concentrate the heat radiation away from the operator. Slots in the base are provided for needle

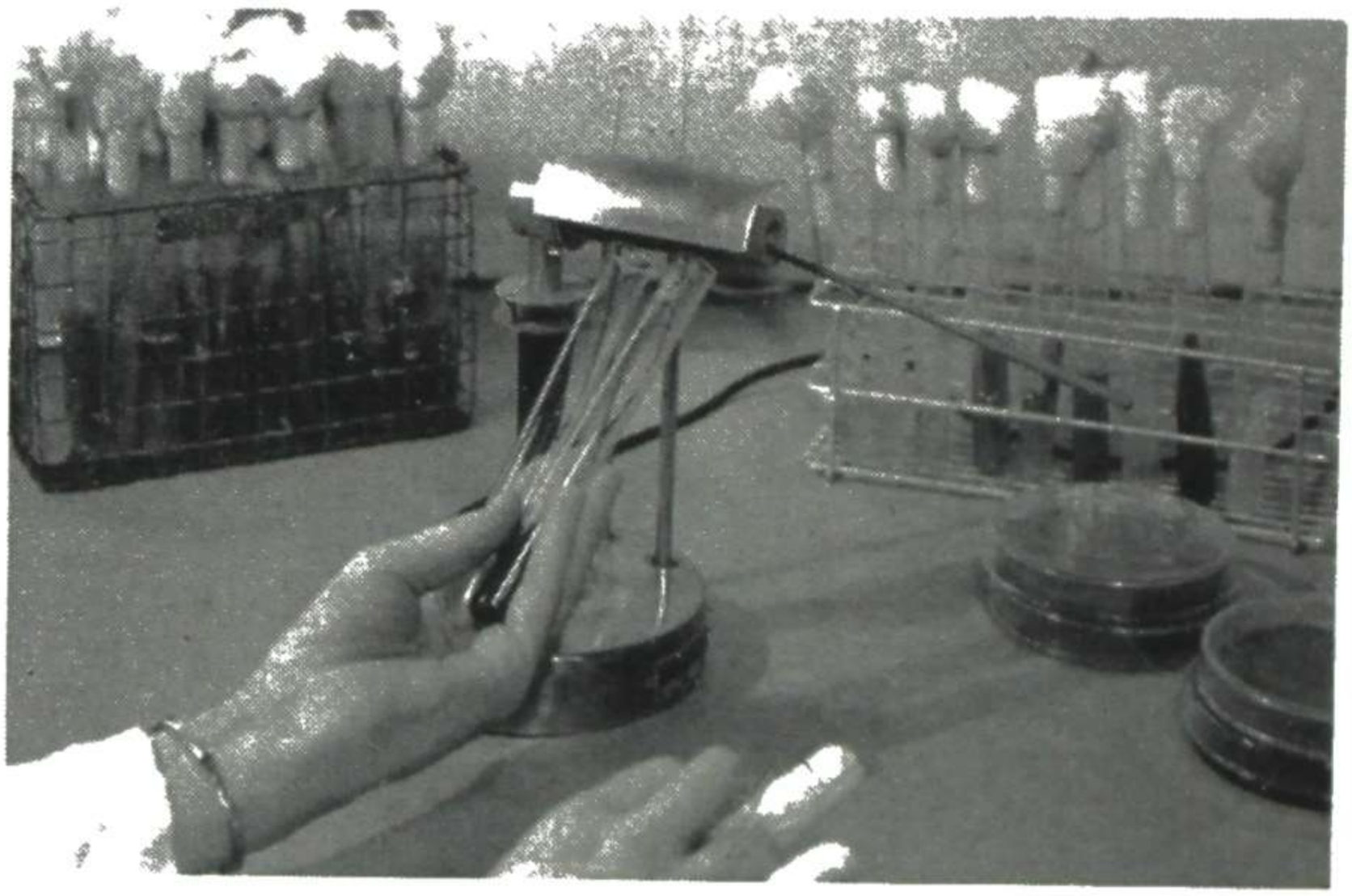

Fig. 145.—Use of electrically operated Bunsen type burner in bacteriology. (Courtesy of Rosenthal, S. R.: J. Lab. & Clin. Med. 37:327-330, February, 1951.)

holders. This burner can perform practically all of the functions of the gas-operated type but has the advantages of flexibility, ease of operation, time saving, easy availability of electricity in comparison to the expense of gas installations and reduction of hazards to the operator.

Method.—For bacteriologic procedures the deflector is rotated toward the operator and in the upper quadrant (Fig. 145). The bacteriologic loop or needle is placed in the cavity of the core and allowed to rest there without holding. By incinerating within a cavity, spattering is confined to it, thus reducing the hazards of working with virulent organisms. If test tubes are used, the mouths are sterilized either by holding them directly against the outside of the core or by rolling the end of the tube over the core. For a long needle or loop the handle is held so that the wire touches the glowing inside wall of the core until it glows; it is then passed back and forth slowly for sterilization.

For heating fluids in a test tube, the deflector is moved away from the operator to the lower quadrant. Any portion of the column of fluid may be heated by simply rolling the tube over it. Heating of fluids in beakers is accomplished by permitting the bottom of the

container to rest on a stand which keeps it not more than 1/8 in. above the core.

Automatic Pipet is described by H. W. Dalton[3] and illustrated in Figure 146. The excursion of the moving piece is limited inward by the stop and outward by the adjustable screw. The glass part of the pipet is held to the fixed part by means of a metal band which can easily be slipped down to remove the glass part for cleaning. To use the instrument, the fingers are placed around the glass and the thumb is placed against the end of the moving piece. By closing the fingers the rubber bulb is compressed to a degree predetermined by adjustment of the screw. On opening the fingers a coiled spring pushes the moving piece back into its former position and allows the bulb to expand until the moving piece is stopped by the screw. The apparatus saves a great deal of time in carrying out doubling dilutions of serum and other fluids.

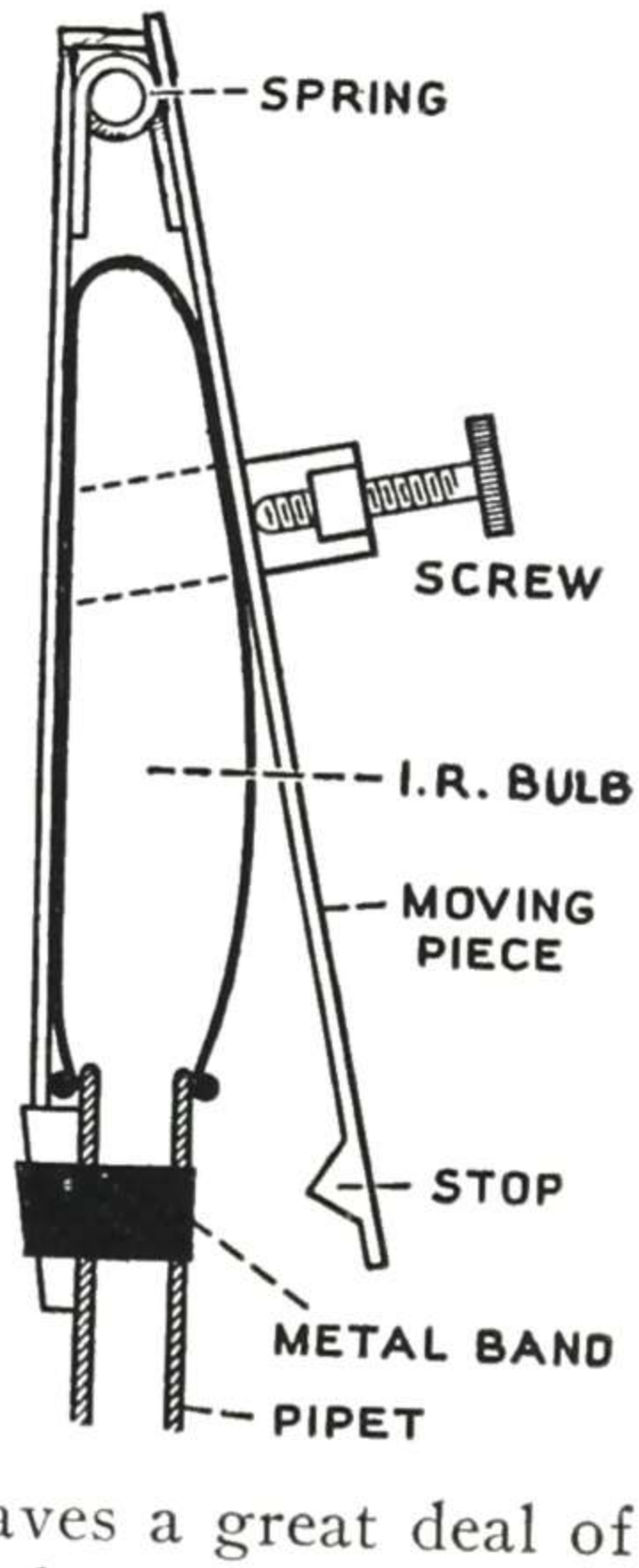

Fig. 146.—Automatic pipet. (Courtesy of Dalton, H. W.: Lancet 1:273, Feb. 3, 1951.)

Easily Constructed Apparatus for Preparing Ultrafiltrates is described by Leland C. Clark, Jr.[4] (Antioch College). Its dimensions can be varied readily to meet the needs of a particular problem.

Apparatus.—The apparatus which has been most generally useful is 15 cm. square with gaskets 2 cm. wide, leaving an 11 cm. square effective filtering area. The basic design is shown in Figure 147. The end plates are best constructed of 1/4 in. chrome-plated brass plates, although aluminum or Plexiglas can be used. A sheet of polyethylene film may be placed between the end plates and the gasket to prevent contamination of the filtrate by the metal. Gaskets are cut from 1/8 in. neoprene sheet and equipped with pieces of 15 gauge needles. Sharp edges which protrude are filed smooth. Short pieces of fine polyethylene tubing may be used in place of the needles if contact with metal must be avoided. After assembly the rubber gaskets are cleaned well and preferably, though it is not essential, coated with silicone. In the center of the filtration gaskets

(3) Lancet 1:273, Feb. 3, 1951.
(4) J. Lab. & Clin. Med. 37:481-484, March, 1951.

are placed three or four sheets of rayon base filter paper which serves superbly to separate the cellophane sheets when they are under pressure during operation and to provide numerous longitudinal pores for escape of the ultrafiltrates. When wet, this paper expands in the direction of the fibers; this should be taken into account when the paper is being cut. Such paper separators may be used repeatedly since they can be washed and dried. To facilitate flow in filters with a large surface, a piece of plastic or stainless steel screen may be placed between the sheets of paper. The cellophane of choice is

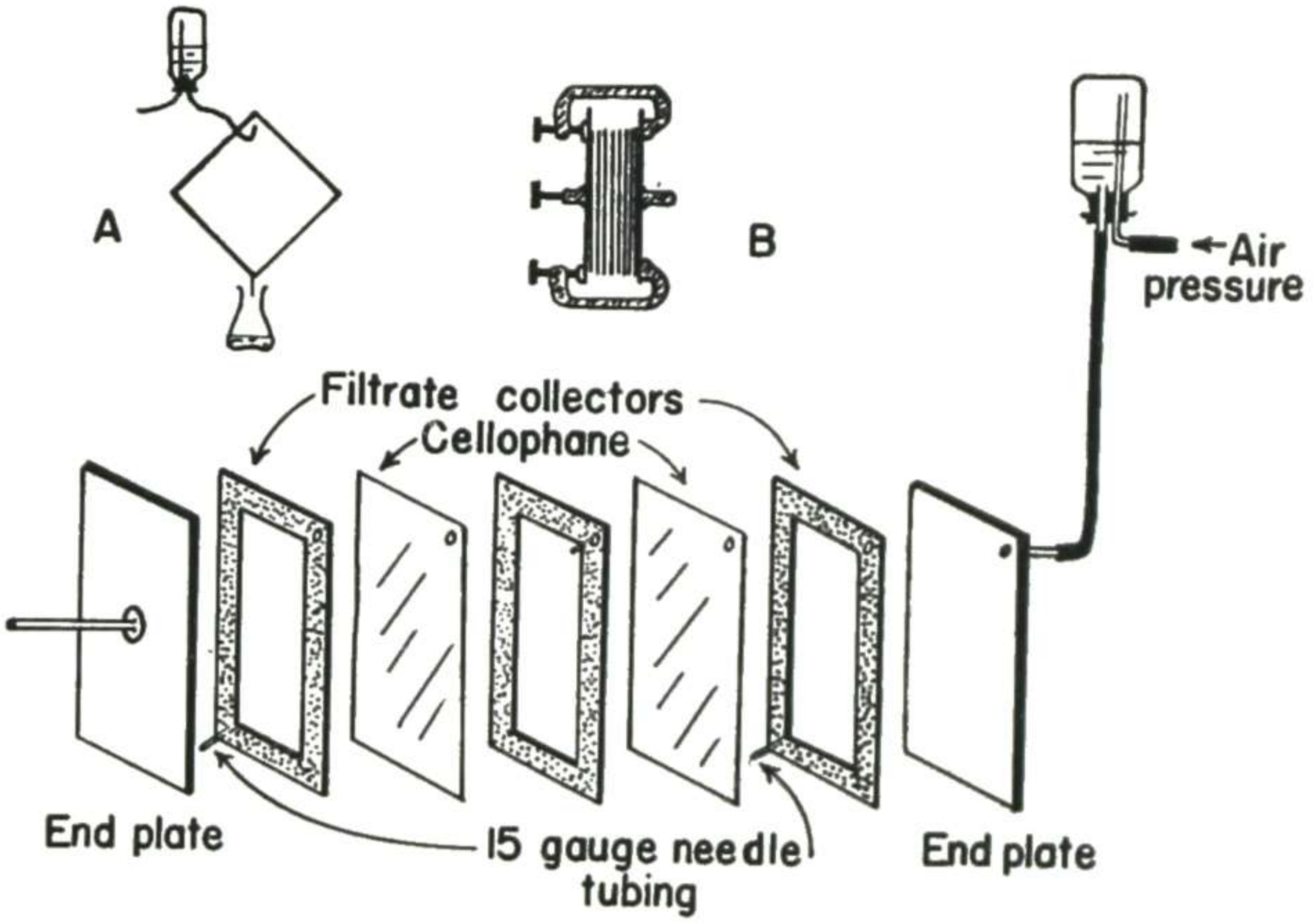

Fig. 147.—Pressure ultrafilter. *A*, general view of apparatus; *B*, clamping method and detailed "exploded" view of construction of filter. (Courtesy of Clark, L. C., Jr.: J. Lab. & Clin. Med. 37:481-484, March, 1951.)

"300 PUT-71" (DuPont Co.). After the apparatus is assembled, a sharp probe is introduced through the holes at the top of the gaskets so as to pierce the cellophane at this point for subsequent admission of the solution to be filtered. The tubing from the container holding the solution to be filtered is then connected and pressure slowly increased up to 20 lb./sq. in. The pressure should be alternately applied and released several times or until all the trapped air has bubbled back into the pressure bottle. For certain labile biologic compounds, nitrogen gas is recommended for pressure. The completely assembled apparatus may be sterilized in an autoclave for 20 minutes at 120 C. without significant change in porosity of the cellophane.

Gravimetric Method for Calibration of Hemoglobin Micropipets is described by George F. Stevenson, George W. Smetters and John A. D. Cooper[5] (Northwestern Univ.). The method lends itself well to rapid consecutive calibration of a large number of pipets. Analysis of 100 consecutive calibra-

(5) Am. J. Clin. Path. 21:489-491, May, 1951.

tions showed that the standard deviation did not exceed ±0.06 cu. mm.

METHOD.—The tip of a tuberculin syringe, the barrel of which has been heavily lubricated with stopcock grease, is fitted tightly into one end of a rubber stopper and the stopper clamped to a heavy retort stand so that it is fixed firmly about 18 in. above desk level. The base of the pipet to be calibrated is inserted as far as possible into the other end of the stopper and the whole assembly adjusted so that the pipet occupies a vertical position. The plunger of the syringe is withdrawn slightly, and a beaker containing mercury of the same temperature as the pipet is held beneath the apparatus so that the tip of the pipet is well immersed. Further aspiration of the syringe fills the pipet with mercury. When the mercury has reached the 20 cu. mm. mark, the beaker is removed rapidly. If there is a change in the level of the mercury in the pipet, the entire procedure must be repeated. A weighing bottle, the weight of which has been determined, is held beneath the pipet and the measured amount of mercury delivered into it by manipulation of the syringe. The weight of the bottle plus the mercury is determined and the weight of the mercury alone obtained by subtraction. The volume occupied by this weight of mercury at the prevailing temperature is calculated by dividing the weight by a temperature correction factor. Calibration should be performed in duplicate.

Improved Blood Coagulation Time Apparatus has been developed by P. Hedenius[6] (Stockholm). It is uncomplicated to use, yields reproducible values of good reliability, is sensitive enough to give coagulation time readings of adequate accuracy and does not waste the operator's time. The normal mean coagulation time for men is 5.47 ± 0.26 minutes and for women 4.91 ± 0.27 minutes. The apparatus is composed of the following items: a flat, box section metal holder with three keyhole type apertures on one side and a piece of foam rubber inside which serves as a soft cushion stopper for test tubes; precision made test tubes with an inner diameter of 5.52 ± 0.01 mm., capable of being locked in the keyhole apertures against the rubber pad; precision ground glass beads with a diameter of 5.230 ± 0.001 mm., and a pair of special pincers for handling the beads.

All parts of the apparatus must be cleaned scrupulously after each determination and then dried. No chemicals may be used that will interfere with the coagulation process. After the tube is filled with blood, the bead inserted and the tube locked in the metal holder, the whole should not be inverted more than once every half- or whole minute. The developing

(6) Scandinav. J. Clin. & Lab. Invest. 3:80-81, 1951.

threads of fibrin must be given the opportunity to become strong enough to stop the passage of the bead. It is important that the glass beads be dropped by means of the pincers into the tubes after the blood has been added.

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