CHAPTER EIGHTEEN

The Handling, Care, and Management of Farm Animals

Domestic animals belong to several species, each of which embraces a number of different breeds. These species, and to a lesser degree the breeds that make them up, differ in body conformation, in physiology, and in temperament. It follows that techniques of handling and management must be suited to the particular kind of animal involved. Efficient methods with one species may be quite unsuitable for another. Again there are important differences between the sexes. The male is usually stronger and more difficult to handle than the female, and is often rendered more amenable by castration. In the female striking differences in behaviour are often associated with oestrus, pregnancy, and nursing. Finally, it is important to remember that each animal is an individual, by virtue of both its inherent make-up and its experience, and should be treated as such.

To a large extent the successful control of large and powerful animals depends upon training them to react in particular ways to man-made routines and instructions. Usually a well-trained animal is easily controlled. The extent to which training can be effective is well illustrated by circus animals, for even those that are normally wild and highly dangerous can be made to perform complicated acts. As a result of training, patterns of behaviour may become so well conditioned that lay observers often attribute them to the influence of an intelligent mind, and believe that some animals possess a near human capacity to reason and to act in the interests of their handlers. This is not so, and it is well to remember that all animals are fundamentally selfish and self-centred, and most probably have no conception of right and wrong. The provision of adequate food and shelter by man leads them to modify their behaviour and plays a large part in training, and a good knowledge of these requirements is essential. Some knowledge of animal behaviour and a good deal of common sense are also necessary. The handler should always give warning of his approach. He should always be kind, firm, patient, and assured. He should never make sudden changes of routine, should never show fear, and should never take risks. He should have confidence in his own ability to handle and control the animal. Animals remember instances of cruelty for a very long time, and many difficulties in management result from this. Some animals are quick to sense fear in their handlers and are quick to take advantage of it. Some that are normally quiet and manageable may suddenly become unmanageable and dangerous.

A further important aspect of handling large animals concerns the use of various methods of physical restraint which must be resorted to when training alone does not suffice. These devices are designed to secure animals in such a way that manipulation of various kinds can be carried out without injury to either the animal or the operator. Foolhardiness and the taking of risks when dealing with the larger and stronger animals nearly always becomes corrected with time and experience.

THE HORSE

The horse is used chiefly for traction and for riding. There are big differences in temperament between the various breeds, and thoroughbreds are much more likely to be nervous and difficult than are heavy draught horses. The mature stallion can be difficult and dangerous to handle, and males not required for breeding are usually castrated at about eighteen months to two years of age. Earlier castration results in a loss of physical strength. The gelding is not only more docile and amenable than the stallion but also behaves in a less active and 'intelligent' way. For this reason circus horses are never castrated.

Training

Training should start at an early age, and should be characterized by kindness. Pleasure should be associated with early lessons and obedience inculcated by firmness. Much of the horse's disposition is established during his early training, and only good training produces the prompt and willing responses to commands which endow the horse with so much of its value. Cruelty often results in a sullen, spiritless submission, and lack of firmness often gives rise to temper and obstinacy.

Handling

When approaching a horse the handler should always speak before touching the animal. He should then pat the animal's neck and attempt to reassure it. Strange horses should be approached with special care, but should never be allowed to suspect that the handler is afraid.

Before examining a horse a halter or head collar should be fitted and held by an assistant. For simple operations like blood taking, the assistant stands on the opposite side to the operator and holds the horse's head, one hand on the nasal bone and with the other hand, a good grip on the ear. No additional restraint should be necessary. If the fore-quarters of a horse are to be examined the horse should be turned so that his hind-quarters are in a corner, or at least against a wall. In this position the horse is unable to back away and the examiner is left with maximum freedom of movement.

If the hind-quarters are to be examined it is wise to have an assistant pick up the front leg on the side which the examiner will stand. The front foot should be raised so that the leg is well flexed and does not provide the horse with support. In this position the horse will find it difficult to kick with the hind-leg that is on the same side as the raised front leg.

When painful operations, such as the dressing of wounds, are to be carried out it may be necessary to apply a counter-irritant that will divert the horse's attention. This is best done by applying a twitch loop to the sensitive area of the upper lip. The twitch loop consists of a loop of $\frac{3}{8}$-in. cord (total length about 16 in.) attached to the end of a stout wooden pole about 3 ft long. The twitch is applied by slipping the loop over the upper lip of the horse, care being taken to leave the nostrils free, then twisting up the lip by turning the pole until slight pain is inflicted. The operator should stand close to the horse's shoulder, in which position he cannot be kicked by the front feet, and should hold the pole pointing backwards. The horse should be held by an assistant by means of a halter. (The horse should never be tied up when a twitch is applied.) The twitch should only be applied immediately before restraint is required, and should be used only when absolutely necessary. There are several other types of twitch, most of them meant to bring pressure on the sensitive area of the lip, but the twitch loop is more generally used, and is simple to apply.

For a number of manipulations a horse must be cast and its feet secured by ropes and hobbles. This is carried out on a suitable surface, such as a grass meadow, a bed of peat moss, or straw, and in such a way that the horse falls on the required side and so that the available light can be used to the best advantage. It is important that adequate man-power should be available and that there should be as little noise and fuss as possible. It is common practice to administer tranquillizing drugs beforehand. These minimize struggling, and hobbles are applied easily and enable the horse to be cast more safely and by fewer men. There are several methods of casting, and the method chosen should be the one suitable to the job in hand. This, of course, depends on the type of horse and the particular operation. A method which has been used for many years and with good effect is the 'London Method'. This method is suitable for horses weighing up to about 1,100–1,200 lb. Two ropes (about $\frac{5}{8}$ in. × 25 ft) are required, each with a spliced noose at one end. Three hobbles made of strong woven cotton webbing with leather piping at the loop end and a strong brass 'D' at the other end are used. To cast the horse on to its left side, a fixed loop is placed over the neck. The rope is then passed round the chest, where a half-hitch is made. The hobbles are put on both fore and the near hind pasterns. A loop is made in the other rope and put round the right fore cannon bone of the leg which will lie uppermost when the horse is down. The other end is then threaded through the brass loop of the near hind-leg, then through the brass loop of the near fore-leg, than through the brass loop of the off fore-leg and held on the right side. The head should be held by a man and, when all is ready, the horse is brought down by a steady pull on both ropes. When down, the rope used about the chest is then looped round the right hind pastern and passed through the neck loop so that the right hind leg can be pulled well forward and secured by a half-hitch. The three hobbled legs are made secure by taking a couple of half-hitches round the right fore pastern with the rope used in the hobbles.

Vices

The chief vices of the horse are temper and obstinacy. Stable vices are many, such as kicking the heel post, crib-biting, wind sucking, licking walls, tearing clothing, weaving, and sleeping standing. Once these vices are established, they are difficult or near impossible to remedy. The stable vices result mainly from lack of work, boredom, and lack of human attention, and they will spread rapidly from one horse to others in the same stable.

Food

Horses have small stomachs and should be fed frequently; three or four feeds a day are usual. The staple indoor diet consists of oats, bran, chaff, and hay. The total amount of food and the proportion of its constituents vary with the type of horse and the nature of its work. For heavy slow work the proportion of grain is less and the proportion of roughage is greater. Horses weighing between 1,100 and 1,200 lb require about 26 lb of food daily. A van horse working at a slow rate would get about 10 lb of oats, 1 lb of bran, and 15 lb of hay. A hunter doing fast work would get about 15 lb of oats, 1 lb of bran, and 10 lb of hay.

Water

If water is not continuously available it should be offered before feeding. If this is not possible, then at least 1 hour should elapse after feeding before water is offered. Some horses drink 8–9 gallons a day and more in hot weather.

Bedding

Good bedding is necessary for horses, for providing them with warmth and comfort. It encourages them to lie down and rest their legs. Wheat straw is the best bedding material, because it is longer than other straws, and because its toughness and bitter taste discourage most horses from eating it.

Grooming

When not out at grass horses must be groomed regularly. This removes dirt, loose hair and scurf, stimulates the circulation of the skin, and discourages parasites.

Reproduction

The breeding season normally extends from early February to the end of June. During this time oestrus recurs every 21 days, unless pregnancy intervenes, and on each occasion lasts 5–6 days. The gestation period is 340 days. The foal is usually suckled for about 6 months. It reaches maturity at about 4½–5 years.

CATTLE

In this country cattle are kept for two main purposes: beef production and milk production. There are again differences in behaviour between breeds. Mature bulls are often difficult and dangerous, and the utmost care should always be taken in handling them. Males not required for breeding are usually castrated at about four to six months of age. Both sexes are often de-horned within a few days of birth. The details of management vary greatly with different systems of farming.

Training

Cows do not have the capacity to respond to training that horses have. They do, however, easily become conditioned to regular routines, and patterns of behaviour established in this way play an important part in management. At milking time, for example, dairy cows at pasture usually gather at the field gate, and when this is opened they proceed, unguided, to the cow byre and into their own stalls. Training does not normally extend beyond the establishment of simple patterns of behaviour except in countries where cattle are still used for traction. Temperament, especially in bulls, may be greatly influenced by early treatment.

FIG. 1

FIG. 2

Handling

Cows are usually driven rather than led. Bulls are usually led by a pole attached to a ring through the nose. The ring is usually inserted at about nine to twelve months of age. Dangerous bulls are led by two handlers. One takes the pole and the other takes the rope that is fastened round the horns and then passed through the ring.

Cows should be approached quietly. When the head is to be examined a hand is placed on the animal's back and rubbed or scratched along the back until the head is reached. A horn or ear is then grasped with one hand and the nostrils with the other. The nostrils are held by placing the thumb in one nostril and the first two fingers in the other (Figs. 1 and 2). If the examination is prolonged, or if the animal becomes restive, a 'bull-dog' holder is attached to the nostrils. This relieves strain on the fingers and thumb, and the swivel handle allows the animal to twist its head without twisting the operator's wrist.

Cattle do not usually allow their feet to be picked up by hand. To lift a front foot it is best to attach a rope above the fetlock and pass the free end of the rope over a sack (for protection on the animal's back) to an assistant on the other side. As the operator leans against the shoulder of the leg to be raised the assistant pulls on the rope. To lift a hind-leg it is best to pass a pole in front of the hock to be lifted and behind the other one. The operator and his assistant each take an end of the pole and lift it backwards and upwards at the same time, leaning towards each other.

When taking blood from a cow a loop of cord is passed around the lower part of the neck and drawn tight enough to obstruct and raise the jugular vein. An assistant holds the cow by the right horn and the nostrils and pulls the cow's head to the right and backwards. This leaves plenty of room for the operator to take blood from the left jugular vein.

Casting cattle as a means of restraint

One of the best methods of casting cattle is by the side-line method of Reuff. A rope about 40 ft long is tied to the horns, or to a head collar, and is passed backwards along one side of the animal. A half-hitch is made around the neck, a second half-hitch around the chest, and a third around the abdomen. The remainder of the rope is trailed behind the animal, and all slack rope is taken up. The cow is brought down by two men pulling with a strong and steady pull on the rope. Hobbles are then quickly placed around the cannon bones and all four legs are pulled together and secured by two half-hitches.

Feeding

Cattle are fed in a variety of different ways, depending on the need. A cow needs about 3 acres of land to support her; 1¼ acres for grazing and 1¾ acres for hay, oats, and roots. Dairy cattle are usually fed having regard to the amount of milk they produce and the foodstuff available. The common practice is to feed 4 lb of concentrates to each gallon of milk produced, with about 16–20 lb of hay per day. Intensive feeding results in cows giving three times more milk than is needed to feed a calf, and lactation ailments are common. Drinking-water must always be available.

Reproduction

The cow comes into oestrus throughout the year, but more regularly in summer. Heat last from 6 to 30 hours and recurs every three weeks. The gestation period is 280 days. The cow licks the calf as soon as it is born. A strong calf will be up on its feet in 20–30 minutes. Some dairy farmers wean the calf at once, some about 3–4 days after the calf has had the first milk (colostrum). The calf depends entirely on milk for the first 3 weeks of its life. Subsequently the calf should have access to small amounts of good hay and clean water. If it is to be weaned the calf must be taught to drink from a pail. It is usual to start it drinking by dipping a finger in the milk and letting the calf suck the finger. The hand is then lowered into the milk, the calf still sucking the finger and at the same time taking a little milk. Gradually the finger is withdrawn as the calf learns to drink without help. Calfhood is usually reckoned to be up to about 6 months of age. Maturity is reached at about 18 months, at which time heifers are usually served so as to calve at about 2½ years of age.

SHEEP

Sheep are chiefly used to supply meat and wool. There are many breeds, and the choice of breed depends mainly upon the type of country on which the sheep are to be kept. There are again many differences in temperament between breeds. Mountain sheep, for example, tend to be much more active and excitable than lowland sheep. Males not required for breeding are usually castrated at about two to three weeks of age, the operation usually being carried out at the same time as 'docking'. 'Docking' consists of removing about two-thirds of the tail. This is done to prevent the accumulation of dirt and faeces and to reduce the risk of blow-fly strike. 'Docking' may be done by cutting the tail with a sharp knife. Bleeding is usually negligible, but if it persists the wound may be dressed with Friars Balsam. Sheep are dipped usually twice a year, in August and November, to eradicate parasites. Some farmers dip their sheep once before shearing and a second time when the fleece has grown long enough to retain in it a certain amount of dip. By law all sheep in some areas must be dipped twice a year in the presence of a police officer.

Handling

Sheep tend to flock together, and for this reason can be driven easily in flocks by shepherds and trained dogs. They quickly learn to recognize their handlers, and will follow people well known to them. Sheep should never be caught by holding on to the wool, but by gently sliding one hand under the base of the neck and lifting the chin high. Horned sheep may be caught by the horns. To restrain a sheep the handler stands astride the animal and lifts its head high by the chin or horns (Fig. 3). To turn a sheep 'up' the handler reaches well

FIG. 3

FIG. 4

under the sheep's belly with his right hand and lifts the chin with his left hand. The sheep is then lifted clear of the ground, fore-quarters first, and is lowered into a sitting position with its back against the handler's knees. The handler then holds the fore-legs (Fig. 4).

Bedding

Wheat straw is the best bedding for sheep, but sawdust and peat moss can also be used. Concrete floors are suitable for pens, and can be hosed regularly. If it is necessary that the sheep eat nothing but the ration given, then slatted floors should be used with sawdust beneath. The slats should be about $\frac{3}{4}$ in. apart.

Feeding

A normal ration consists of rough fodder, such as hay, and concentrates. Adult sheep will eat up to about 4 lb per day. Drinking-water must always be available.

Reproduction

The normal age for the first mating of female sheep is about eighteen months, so that the first lamb is dropped at nearly two years of age. The gestation period is 147 days. The oestrus period varies from breed to breed and with local climatic and food conditions. Some sheep come into oestrus as early as July, while others may not do so until October.

Mountain sheep rarely have more than one lamb per year, but lowland flocks may be expected to give 50 per cent twins. The lambs are usually weaned at about three to four months of age and are then transferred to good grazing.

PIGS

Pigs are kept chiefly to supply bacon and pork. There are again many breeds and systems of management. Males not required for breeding are usually castrated at about six weeks of age, or two weeks before weaning.

Mature boars may be extremely dangerous on account of their powerful tusks, and should always be handled with great care. Nursing sows will attack anyone interfering with the litter, and should always be secured before the piglets are handled.

Handling

Pigs can usually be driven, but when this is not effective they can be backed into any desired position by putting a large bucket over the head. Small pigs may be caught by the hind-legs above the hock, or by their ears. They should not be held by the tail, for in young pigs the skin of the tail tears easily. Large pigs are best restrained by driving them into narrow crates, or by pinning them in a corner with a hurdle or table top. Restraint can also be obtained by applying a twitch loop around the upper jaw.

Bedding

The best type of bedding for pigs is short straw or sawdust. This kind of bedding will not become entangled about the legs of the piglets. With suitable bedding pigs will keep themselves as clean as do other animals.

Feeding

At weaning time the young pigs should weigh about 25–30 lb. They will eat about $1\frac{1}{2}$–2 lb of meal per day, and they should gain weight at the rate of 1 lb per day. At about 16 weeks of age the rate of growth increases to 10–12 lb per week up to bacon weight, which is about 200–210 lb and the intake of meal per day will rise to about 6–7 lb. The meal can be fed dry or as a mash, with water *ad libitum*.

Reproduction

Sows will breed throughout the year, but oestrus is more prolonged in the spring and autumn. Oestrus recurs every 21 days, and lasts for 3–4 days. The gestation period is 115 days, and the litter is usually weaned at about 8 weeks. The average litter size is about 8, but numbers of up to 20 have been known. Maturity is reached at about 12 months of age. Piglets should be given iron (usually by injection) during the first few days of life to prevent anaemia.

GOATS

Since the turn of the century, the goat has been used increasingly as an experimental animal in Physiology, Pharmacology, Endocrinology, Biochemistry, Dairy Research, and latterly experimental orthopaedic surgery. For orthopaedic purposes the goat is preferred to the sheep and dog (Barnett, 1958).

Handling

The goat learns quickly, providing it is handled gently and firmly. Careless handling and the use of force will encourage stubbornness in the animal. Horns are an advantage when handling goats, as they afford a useful handgrip. It is satisfactory to run horned and non-horned goats together for exercise, provided there is enough room for the non-horned goats to take evasive action. It is advisable to train newcomers to the herd to walk with a collar and lead, remembering that the goat is a very inquisitive animal (as well as a nervous one) and should be allowed to satisfy its natural curiosity.

Housing

Goats are best kept in individual pens that are warm, dry, draught-proof, and strong. Beard and Duncombe (1951) recommend a size of 8 ft × 5 ft, with 4 ft 6 in. × 4 ft 6 in. as the minimum. These pens should be adjacent to an extensive exercise yard, which is essential in winter. In summer strip grazing in grass paddocks is desirable.

Bedding

Goats need plenty of bedding; straw is recommended, but peat moss and sawdust may also be used.

Feeding

Food may be divided into maintenance and productive rations. For maintenance goats require 0·6 lb starch equivalent and 0·15 lb protein daily, while for production 0·3 lb starch equivalent and 0·07 lb protein are needed per pint of milk. A good maintenance ration per day is supplied as follows:

5 lb good meadow hay
3 lb marrow stem kale
1 lb of any of the following meal mixtures—

	MIXTURE 1	MIXTURE 2	MIXTURE 3
Linseed cake	4	1	1
Flaked maize	3	—	1
Bran	2	—	2
Crushed oats	1	1	1

Parts by weight (Beard and Duncombe, 1951)

For milk production 6 oz of the meal should be fed per pint of milk produced.

Reproduction

Sexual maturity in the female is reached at 5–6 months of age, but the first mating should be delayed until the animal is 15–18 months old. The goat has an oestrus cycle of 21 days and the heat period lasts 2–3 days. The gestation period is 5 months, and the breeding season is from September to February. Recurrence of oestrus after the young are born is next season.

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CHAPTER NINETEEN

Care and Management of Amphibians, Reptiles, and Fish

AMPHIBIANS *

There are some 1,500 species known to exist in the world today, with a few new species being discovered each year, and it is hardly an exaggeration to say that they all require different treatment if the aim is to establish a breeding colony. Really adequate knowledge exists only in respect of the few species which have already been bred in captivity in large numbers. Such general information should only be regarded as a basis for further detailed research with selected species, and mention will be made of a number of problems which deserve further investigation.

Modern amphibians are divided into three orders:

(i) Apoda or Gymnophiona

These are the caecilians—legless, wormlike, burrowing amphibians, found only in certain tropical areas.

(ii) Caudata or Urodela

This order comprises the tailed amphibians, including the salamanders and newts as well as a number of permanently aquatic forms.

(iii) Salienta or Anura

The tailless amphibians, i.e., the frogs and toads.

CAECILIANS

Very little indeed is known about these primitive, secretive creatures, of which some fifty species have been discovered in the tropics of Africa, South-East Asia, and the New World. They all have the shape of a worm, are limbless and blind, and spend almost their entire lives underground. In most cases they lay large-yoked eggs in an underground burrow and the eggs are guarded by the female until they hatch. Knowledge of their requirements in captivity is almost non-existent, but presumably they would need to be kept in moist, soft soil or leaf-mould at a reasonably high temperature. Their food, as far as is known, consists of worms and grubs. Besides being difficult to obtain and probably unsuitable in general as laboratory animals, their requirements as such would

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need to be determined in far more detail than is already known, and further speculation at this stage might well be misleading.

TAILED AMPHIBIANS

For practical purposes regarding their requirements in captivity, these may be divided into three broad groups: the salamanders, the newts, and the permanently aquatic forms.

Salamanders

The salamanders proper spend most or all of their adult lives on land, and some New World species even live to a greater or lesser extent in trees. Many, but not all, resort to water for a limited breeding season each year. They require a certain amount of humidity at all times, and most of them are secretive, at least in the daytime. These requirements are best met by some such container as a normal glass aquarium with a few inches of damp soil or preferably leaf-mould on the bottom. One or two flat stones or pieces of wood under which the creatures can hide is advisable, and a plant or two in a pot could be added.

It is important to keep the soil at the right humidity, for if it is allowed to dry out the salamanders may well perish. At the same time, a very damp or wet soil can be dangerous, particularly if it becomes sour. Little or no research has been carried out to ascertain the pH values most suitable for salamanders, but it is well known that in a wild state some can live in tolerably acid soils, while others are limited to limestone regions. With many species it may well be a factor of some importance to provide optimum pH conditions, and a study of the natural habitat should give some indication of these. What is certainly important is to avoid conditions which promote the growth of fungus, as certain forms are dangerous to salamanders. They are, in fact, susceptible to a particular kind of fungoid infection which can quickly kill and which appears to be highly contagious, overcrowding will lead to a higher incidence of this infection. Periodic changing of the soil is to be recommended to avoid this and other infections, as amphibians are prone to various skin troubles.

Some salamanders lay their eggs on land, the Alpine Salamander, *Salamandra atra*, even gives birth to living young in the adult form. Such species do not require water containers, as they are often poor swimmers and can easily drown. Most species, however, enter water to lay their eggs, which hatch as tadpoles. The well-known European Salamander, *Salamandra salamandra*, deposits its tadpoles alive in water at a fairly advanced stage of development. For the breeding of these species a water container is necessary; only a few inches of water is necessary, and the container should be arranged so that the salamanders can climb out of the water if they so wish. Small plastic bowls are suitable if a few stones or crocks are placed in the water to overcome the difficulty of the smooth sides. The water should be kept clean, and pond water is generally better than tap water, though this varies according to the district. The eggs or tadpoles should be removed immediately, and with most species it is advisable to keep the tadpoles in separate containers (small plastic bags are suitable), as they frequently attack each other. A little water-weed, such as

Elodea, in each bag helps to provide cover, oxygenation of the water, and purchase for moving around. The tadpoles which hatch from eggs have no limbs at first. but soon begin to swim sufficiently well to capture small crustaceans such as *Cyclops* and *Daphnia* after an initial short period of feeding on infusoria either already present if pond water is used or introduced with the water-weed. In due course the front and then the hind limbs appear, by which time the larvae are capable of taking *Tubifex* and similar-sized small worms, which may, if necessary, be chopped up to a smaller size. At this stage almost any kind of water-life small enough to be swallowed may be given, cultured brine-shrimps are a good stand-by provided not too many are given at a time, as any not eaten will quickly die and foul the water. Mosquito and gnat larvae can also be used. By the time the tadpoles are approaching full larval size most are capable of taking very small garden worms and water-shrimps. As the time of metamorphosis approaches the larvae should be taken out of the plastic bags and put into shallow water in a tank or bowl so arranged that they can leave the water without difficulty. This container should be furnished with a well-fitting lid, as young salamanders are excellent climbers. As soon as they leave the water they can be put into a similar tank to that prescribed for the adults, but without any water-container. The most suitable food at this time is white-worm or very small earthworms, though some species prefer small insects such as *Aphis* or *Drosophila.*

The food of adult salamanders varies: some are specialized insect-feeders and catch their prey by means of a sticky tongue. Most of the larger salamanders seize their prey in their jaws and feed readily on earthworms. Some, such as *S. salamandra*, are very fond of slugs, particularly the soft, white form frequently found in gardens. Very few will tackle hard-bodied insects or even meal-worms.

Newts

The name 'newt' is commonly, though loosely, applied to a number of salamanders, most fairly closely related, which are generally more at home in water than most of the typical salamanders. They are confined to the temperate zones of the Northern Hemisphere, and as a rule the adults repair to water each year for a breeding season lasting several months and spend the rest of the year on land. In some, such as the Asian hynobid salamanders, the eggs are laid in small sacs with a gelatinous cover, and subsequently fertilized by the male in a similar manner to most fish. In others, such as the various European newts of the genus *Triturus*, the males develop various skin appendages, particularly dorsal crests, during the breeding season and indulge in characteristic courtship dances in the presence of the females; following which, a spermatophore is deposited by the male and picked up by the female, so that the eggs are fertilized internally before being laid.

The requirements of these newts in captivity are similar to those of the salamanders proper, except that many species can be kept permanently aquatic, and this makes feeding them much easier. The water in which they are kept need not be more than a few inches deep, and provision should be made, possibly by stones, for the newts to leave the water whenever they wish. It should be remembered, if the newts are kept in ordinary glass-sided fish-tanks, that they are extremely good climbers; and when wet the smaller species, at least, can walk up a vertical glass surface with comparative ease. The tanks should therefore be fitted with well-fitting covers, which need rarely be removed if a small hole is left in the middle for the introduction of food.

It is advisable to have a quantity of water-weed in the tank, not only to provide welcome cover for the newts and encourage them to stay in the water but also to provide them with suitable attachment for the eggs when they are laid. This particularly applies to the *Triturus* species, the females of which lay their eggs singly and, when possible, carefully wrap each egg in a leaf as it is laid. *Elodea* is a most suitable weed for this purpose. The eggs should be removed to another container before hatching, and this is best achieved by taking out any weed to which eggs are attached and replacing it with fresh. Once the eggs have hatched, the larvae can be reared in the manner already outlined for the salamanders proper.

While some newts, such as the Ribbed Newt, *Pleurodeles waltl*, and the Japanese Fire-bellied Newt, *Triturus* (*Cynops*) *pyrrogaster*, have been bred in captivity with considerable success, with most species breeding is frequently achieved only during their first season in captivity (they are normally captured during the breeding season when concentrated in ponds). When the next breeding season comes around they fail to react in any way, even the males failing to produce their crests and other appendages. Recent experience suggests that this is in some way connected with diet, and that feeding the newts throughout the year on a wide range of natural foods, particularly small water-life such as aquatic crustacea and insect larvae, overcomes this difficulty. It is also possible that failure to provide any period of hibernation or semi-hibernation in captivity reduces the breeding urge, particularly in those species with a more northerly range, which normally spend several months in hibernation each year. There is considerable scope for research in this matter, taking into account such factors as temperature and light.

Both on land and in the water, newts will devour almost anything small enough to be swallowed, provided it moves and has no hard covering. In the water some species hunt their prey to a considerable extent by smell as well as by sight, and will readily swallow small pieces of raw meat, though this is not recommended as a staple food. On land most of the smaller species will take quite small insects, such as *Aphis* and *Drosophila*, catching them on their sticky tongue, but the larger species react only to large prey, such as earthworms, slugs, and caterpillars.

While some salamanders are best kept at temperatures of around 70–80°F (21·1–26·7°C) newts generally should be kept fairly cool. They nearly all feed well in water at temperatures down to about 40°F (4·4°C) and the water should not rise above 60–65°F (15·6–18·3°C), as higher temperatures may be fatal over a period. On land, 50–60°F (10–15·6°C) is suitable; below about 45°F (7·2°C) the newts start to get sluggish and stop feeding.

The permanently aquatic salamanders are those which, even in a natural state, never leave the water except perhaps in an emergency such as the drying up of the pond or stream in which they live. Most are neotonic forms in which the external gills are retained throughout life. Where the external gills are reduced or absent, normally some other means is available for obtaining oxygen from the water, such as the highly vascularized skin of the cryptobranchid salamanders. With all these permanently aquatic species there is no need to provide means of leaving the water, and the simplest form of container is a normal fish-tank. The only addition need be a bed of fine sand for one or two species which like to burrow in the bottom, and for some of the more secretive species a 'hide-away', such as a large flat stone firmly supported on a smaller one at each end, a piece of concrete or earthenware drain-pipe of suitable size, or even half a large flower-pot split vertically and placed with the hollow side downwards. It is advisable to check on the natural habitat of the species concerned, as some live in sluggish or even stagnant water, and others in streams or mountain torrents rich in oxygen. The former are quite happy in just a tank of water with a little water-weed to assist oxygenation, but the latter must have the water artificially aerated.

The most hardy of the aquatic species is undoubtedly the Axolotl, *Siredon mexicana*. It has been bred in large numbers in captivity, and much has been published about it. It can withstand a wide range of temperature and feeds readily on earthworms, water insects of all kinds, and even strips of meat. Fertilization is internal by means of a spermatophore, and the tadpoles which hatch from the eggs develop like those of normal salamanders except that there is no final metamorphosis, the larval form being retained even when the animal becomes sexually mature. The eggs should be removed from the parents and the young reared in a separate container, as axolotls are voracious feeders and snap at anything moving. In fact, one of the difficulties of keeping even adult axolotls together is that when being fed they sometimes seize each other's limbs. The lost limbs are quickly regrown, and the deprived axolotls seem to suffer no material inconvenience in the meantime, but it spoils their appearance.

The largest of all living amphibians is the Giant Salamander, *Megalobatrachus*, with two species in Japan and China. It lives in cold mountain streams with a high oxygen content, and obtains much of its oxygen supply from the water by means of a highly vascularized skin. This poses difficulty in providing the right conditions in captivity, but the giant salamanders are otherwise quite hardy, and specimens have been kept in captivity for very long periods. A closely related member of the same family is the Hellbender, *Cryptobranchus alleghaniensis*, found in many rivers and streams in the eastern Unites States. Other permanently aquatic salamanders from North America are the Blind-eel, *Amphiuma means*, the Mudpuppies of the genus *Necturus*, and the sirens or 'Mud-eels' of the genera *Siren* and *Pseudobranchus*. All these have been successfully kept in captivity, but comparatively little research has been carried out on their breeding habits.

The aquatic salamanders about which least is known are undoubtedly those generally referred to as 'cave-salamanders' by reason of the fact that they inhabit underground lakes and streams, usually in limestone areas. Several species are known from the limestone regions of the southern United States, and it is possible that further species exist as yet undiscovered in that area. A similar-looking species belonging to an entirely different family occurs in a fairly limited area in South Europe. All these cave-salamanders have elongated, slender bodies with remarkably thin legs, and most retain the larval form with external gills throughout life. Eyes are either absent or vestigial and functionless, as might be expected of animals which live in constant darkness, and the salamanders are whitish or pinkish and their skins to some extent transparent. One of the American species, the Grotto Salamander or Ozark Cave Salamander, *Typhlotriton spelaeus*, is a partial exception in that the larvae live in mountain streams and appear and behave very much like the larvae of normal terrestrial species. They have functional eyes and external gills and are strongly pigmented, but as they approach adult size they swim upstream and enter caves, following which their colour fades to almost white and their eyes degenerate into functionless dark spots under the skin. In this particular species the external gills also disappear at about the same time.

The natural diet of the cave-salamanders appears to consist largely of small aquatic crustaceans of species which are also adapted to life in caves. In captivity they feed well on fresh-water shrimps, and most will take small earthworms and insect larvae. Most caves have a constant temperature of around 50°F (10°C), and it is doubtful whether any of the cave salamanders are subjected in their natural state to higher temperatures than this, though they may well have to contend with much lower ones at times, as many cave streams are fed by cold mountain torrents. Temperatures of 40–50°F (4·4–10°C) are suitable for them in captivity. They are, of course, quite at home in complete darkness, and strong light is probably best avoided. There is no direct evidence that moderate light does harm, but the tendency in most for their normally pale skins to become darker over a period if exposed to light suggests that the normal lack of pigment leaves them insufficiently protected against the effects of strong light.

Comparatively little is known about the breeding of cave-salamanders, as the only species which has been studied in captivity to any extent is the Olm, *Proteus anguinus*, from South Europe. It has been found that the optimum temperature for this species is around 50°F (10°C), but that a fairly wide range of temperature can be tolerated. However, if breeding in captivity is aimed at it would seem most important not to let the temperature of the water rise much above 50°F (10°C). Some confusion formerly existed as to whether the Olm laid eggs or gave birth to living young, but it would now seem that it is possible for either to take place. Under the right conditions, up to fifty or sixty eggs enter the oviducts, but all except one in each oviduct dissolve to form a liquid which nourishes the remaining egg. This egg eventually develops into a comparatively large young Olm, which is born alive (the young measure about 4 inches at birth, and the adults range between 8 and 12 inches). In this way, only two young are born at a time, or only one if the process fails to work in one oviduct, but if the temperature is too high all the eggs are laid as eggs and either fail to hatch or if they do hatch the small and immature young die shortly afterwards. Further laboratory research into the whole process would obviously be most valuable.

To avoid confusion, it should perhaps be emphasized that the term 'cave-salamanders' is used here to indicate only those aquatic forms which spend their lives in underground lakes and streams. Certain non-aquatic salamanders, such as some species of the genus *Hydromantes*, are often referred to as cave-salamanders through frequently inhabiting caves or cave entrances, but these should be treated as salamanders proper.

TAILLESS AMPHIBIANS

As with the tailed amphibians, it is convenient to divide the frogs and toads into three general groups for their requirements in captivity. These groups consist of the ground-living forms, the tree-frogs, and the permanently aquatic species.

The ground-living forms cover the vast bulk of the frogs and toads, the Common Frog, *Rana temporaria*, and the Common Toad, *Bufo bufo*, being typical examples. They live generally similar lives, spending much of their time on land, but resorting to water for breeding. The breeding season in the temperate zones is usually a given time of the year for each species, but in many tropical regions may be 'triggered off' by the onset of heavy rains. Mating involves a form of amplexus and external fertilization of the eggs. The normal pattern is for the eggs to be laid in water, in a mass, a long string, or even singly. The eggs hatch out into tadpoles, which eventually develop hind-legs and then front-legs. The gills and tail are absorbed and lungs develop at or a little before the time the creature leaves the water as a miniature frog or toad. There are, however, a number of exceptions to this general pattern, some involving what might be regarded as parental care of the eggs, or even of the young. The simplest form is perhaps that of the Midwife Toad, *Alytes obstetricans*, whose male wraps the fertilized eggs around his hind limbs and carries them about until the tadpoles are about to hatch, when he enters water to liberate them. Some tropical frogs, notably certain species in the family Rhacophoridae, lay their eggs on land in moist places. Hatching is usually delayed until heavy rain covers the eggs with water or washes them into a pool or stream, but in some cases the complete development and metamorphosis of the tadpole takes place inside the egg, and the young hatch out in the form of miniature adults. Other examples could be given of departure from the normal pattern of breeding, and it is obvious that in dealing with unusual frogs, particularly tropical ones, the breeding habits of the species concerned should be checked as far as possible to ascertain their requirements in captivity.

Frogs and toads which follow a conventional pattern of breeding cannot often be persuaded to breed under laboratory conditions. The main factors affecting them are likely to be limitation of space, incorrect temperature (especially temperature changes for those which breed seasonally), and suitable water conditions. Frogs and toads are generally more choosey than are the tailless amphibians in their choice of a breeding site. Vegetation and depth of water obviously play an important part with many species, and it is possible that the chemistry of the water is also a factor. Clean pond water is better than tap-water in most districts (though care should be taken to remove any carnivorous insects likely to attack the young tadpoles) and plenty of water-weed should also be included, even if only because it provides some cover for the adults in the water and may encourage them to stay in it. Once it is possible to persuade the adults to lay eggs, the main difficulty is over. The eggs may be laid in large clumps, long strings, or sometimes a few at a time or even singly. The eggs should be removed from the adults immediately, because their active movements could easily damage the eggs or young tadpoles, while some frogs which feed under water might even eat them. The safest method is to remove the whole water-container, replacing it with another for the adults. This avoids physical damage to the eggs or harmful results from a sudden change of temperature. The time taken for the eggs to hatch and the rate of development of the tadpoles is controlled by the temperature of the water, and either too high or too low a temperature may cause excessive mortality of eggs or larvae at some stage of development. Each species has its optimum temperature, and this can only be learned by experience, although tropical species generally require a higher temperature than those from temperate regions. Most tadpoles are vegetarian, although at a certain stage of development many of them become partly carnivorous by feeding on carrion. Most of them are equipped with horny lips with which they can scrape algae from water-weed or the sides of the tank, and the presence of such algae is therefore beneficial, particularly when the tadpoles are small. Later, the tadpoles will begin to nibble the vegetation, which may be supplemented with lettuce leaves if required. Small scraps of meat may be added to the diet of many species when the tadpoles are large enough. The tadpoles quickly find these by smell and devour them readily. Care should be taken to remove any uneaten meat or lettuce before they can pollute the water, and a few water-snails in the tank as scavengers will help to avoid pollution at this stage. When the tadpoles are about three-quarters grown is the time when the purity and oxygen content of the water are of the greatest importance. Later, as the lungs develop, the tadpoles are able to surface for air and depend less on the water as their source of oxygen.

As the tadpoles approach full size they develop hind-legs, then front-legs, and finally the tail starts to be absorbed. At this stage provision should be made to enable the young frogs or toads to leave the water, or they may drown. They are not strong swimmers at this time, and the absorption of the gills which accompanies the development of the lungs diminishes their supply of oxygen from the water. Some rocks protruding above the water or a piece of wood floating on the surface may enable them to leave the water, but to avoid casualties as far as possible, a gentle slope for them to crawl out of the water is best. Some mortality may have to be accepted, as occasional tadpoles somehow fail to make the switch from gill-breathing to lung-breathing and succumb when ready to leave the water or shortly afterwards.

The newly metamorphosed frogs should be moved to a container with damp soil and some vegetation such as moss or grass. Provided the soil is sprinkled from time to time to keep it moist, no water-container is necessary, and in fact, one could be a danger, as until their legs have grown a little more, the young amphibians are still poor swimmers and might easily drown. The main difficulty over the next period is the provision of suitable food, which naturally consists of very small insects. *Drosophila*, which can easily be cultivated in large numbers, are a good stand-by, and if sufficient greenfly are obtainable they are an excellent food. The container should be covered with fine-mesh wire, as even the wingless strains of *Drosophila* may escape, and the risk of escape by the young frogs themselves increases as they grow. They are soon able to take house-flies and later blow-flies, whose larvae may be obtained commercially. In general, almost anything which moves and is of suitable size may be used as food, but with some variation between species, e.g. toads readily eat ants, but most frogs will not do so. For practical purposes, those insects which can be obtained commercially or reared in large numbers in the laboratory are the most useful. In all cases the stimulus to feeding is movement of the prey, so that only live food can be given, and for the same reason large frogs and toads should not be kept together with small ones.

Housing requirements for most adult terrestrial frogs and toads are not particularly difficult to provide, the two main factors being temperature and humidity. Full activity, including feeding, can be maintained by species from temperate regions at a temperature range of about 50–65°F (10–18·3°C). Tropical species (except those which occur in the tropics but only at high altitude) find a temperature of 70–80°F (21·1–26·7°C) more suitable, and in some cases necessary. By far the most important consideration is humidity, and for most of the frogs high humidity is necessary. A fairly large water-container in the cage is advisable, but is not strictly required if a layer of moist soil is maintained, as amphibians generally imbibe water through the skin and do not drink in the proper sense. Some species like the Edible Frog, *Rana esculenta*, enjoy sun-bathing and can remain in full sunlight for long periods, but nevertheless, require access to moisture. Some frogs and toads are better adapted to drier conditions, and certain species in a wild state live in semi-desert regions. These forms avoid desiccation by burrowing (for which some are furnished with spade-like processes on the hind-feet) and emerge only at night or after rain. A deep layer of coarse sand and one or two large rocks or stones will provide them with the necessary facilities for burrowing, but it is as well to keep the sand slightly damp or even sometimes wet, particularly at high temperatures.

The nature of the soil helps to determine the kind of food which may be given, and the natural preferences of different species reflect this. The dry-soil types of toads eat large quantities of ants and other Hymenoptera, and many of these insects are ones not normally found in really damp habitats. The long and accurately aimed tongue of the toads is better designed to pick up small insects than the short, wide tongue of the frogs, which usually ignore very small prey. On the other hand, toads are as able as frogs to deal with large forms of prey, such as large earthworms, but these and other slimy creatures like slugs and snails cannot suitably be introduced into a container with dry sand or soil, as they quickly become coated and are then virtually inedible. Some of the largest frogs and toads, such as the Bullfrogs, *Rana catesbeiana* and *R. adspersa*, and the Marine Toad, *Bufo marinus*, will attack and devour vertebrate animals up to the size of a full-grown mouse. On the other hand, certain of the burrowing toads have a comparatively specialized diet, apparently living entirely on termites.

The container for frogs and toads should be as large as possible, and overcrowding should always be avoided. The more active frogs should be given plenty of room, as obviously a creature such as the Agile Frog, *Rana dalmatina*, which can leap a distance of several feet will come to grief if confined to a small cage. It should be remembered that the skin secretions of many frogs and toads are poisonous, and may prove fatal to other amphibians kept in contact with them. A notable example is the Pickerel Frog, *Rana palustris*, of North America, which should not be kept with other species.

The tree frogs in some respects require different treatment from the terrestrial frogs and toads. There are very many genera and species of them throughout the temperate and tropical areas of the world, although most are found within the tropics. The adults spend most of their time in trees or bushes and possess various adaptations for an arboreal existence, the most common being the provision of disc-shaped expansions of the ends of the digits, which act as 'suckers' and enable these frogs to climb and leap about safely in foliage, and even to walk up a vertical pane of glass. Most are nocturnal, spending the day-time pressed close to a branch or tucked away among leaves, and becoming quite active at night. The normal food is flies, moths, and spiders, which are found in trees, and in captivity they frequently ignore terrestrial creatures, such as mealworms and earthworms. They leap to catch their prey, and their eyes seem to be adapted to comparatively long-range vision, as they often appear to overlook insects at very close range. To accommodate their leaping and climbing activities, they should be provided with a roomy cage, which is tall as well as having a large base area. It should be furnished with one or two broad-leaved bushy plants such as small laurels, and it may be found more convenient to have them planted in pots rather than set in soil in the bottom of the cage. It is a wise precaution to include a shallow water-container, although this is not strictly necessary if the foliage is sprinkled regularly with water and the floor of the cage is covered with a fairly deep layer of moist soil. The cage should be covered, to prevent escape of both the frogs and the insects on which they feed. Blow-flies are a convenient basic food, or house-flies for the smaller frogs. They may be introduced either as adults or as larvae or pupae which will be consumed when they emerge as flies. A small hole in the top of the cage, closed by a well-fitting cork, is a very useful means of introducing food without opening the cage.

The breeding procedure of the tree-frogs varies considerably between species, most of them following the normal pattern of laying eggs in water which hatch out as tadpoles, others adopting protective devices for their eggs and young. Some build mud nests in shallow water in which the eggs hatch; others construct foam nests either in the water or among branches overhanging water, into which the tadpoles drop after emerging from the eggs. The female of certain South American tree-frogs carries the eggs on her back until they hatch out either as tadpoles or as fully formed froglets, and in the genus *Gastrotheca* the females develop dorsal pouches in which the eggs are carried until they have hatched and the tadpoles have completed part or all of their development. In a few tropical American species the male remains with the eggs, which are laid on land, until they hatch. The tadpoles attach themselves to his back or sides by suction and are carried around until they drop off in water.

Very few species of tree-frog have been successfully bred in captivity, for not only is it difficult to simulate their requirements but it is also essential to gain some knowledge of their life-history before such an attempt is made. The pouch-breeding species of *Gastrotheca* offer better chances of success than most, and at least one species, *G. marsupiata*, from the highlands of Ecuador has been bred with a certain amount of success in this country.

The final group to be considered is that of the *wholly aquatic frogs and toads*. Certain genera dotted about the warmer regions of the world spend virtually all their lives in water, and obviously have to be kept in water in captivity. The most widespread group is the family Pipidae, comprising three genera in

Africa and two in South America. These are all rather grotesque, flat-bodied toads with a number of adaptations for an aquatic existence, including powerful hind-limbs with long, fully webbed toes, lidless eyes set high on the head, and 'lateral-line' organs capable of detecting water-borne vibrations. The most practical container for them is a simple fish-tank. Probably because they lack eye-lids, they often prefer to shelter from bright light, and the tank should contain either a plentiful supply of water-weed or some sort of rock shelter under which they can retire if they wish.

The best-known species is the African Clawed Toad, *Xenopus laevis*, which because of its usefulness in biological tests has been widely bred in captivity. Its various sub-species are widely spread over most of the southerly half of Africa, and it inhabits areas of water of varying size, from small pools to the margins of large lakes. Although very much at a disadvantage when out of the water, it does on occasion travel overland from one stretch of water to another. Many of the pools in which it occurs dry up periodically, and it is able to lie dormant for long periods in the baked mud until the rains refill the pool and liberate it once more. Presumably because of this, breeding normally occurs immediately after the onset of heavy rains. Amplexus is inguinal (the male clasping the female around the groin), eggs are laid which hatch into tadpoles of a rather specialized type. During the first few days of its life the tadpole remains on the bottom, but later ascends to the surface and subsequently spends most of its larval life suspended head-downwards in the water, usually congregating in rather deeper water as the time for metamorphosis approaches. The tadpoles feed essentially on infusoria, which they strain from water passed in through the wide mouth and out through the gill-openings. Eventually, the legs and lungs develop and the tail is absorbed, following which the toadlet changes to a completely carnivorous diet. Small water-insects are accurately caught in the mouth by a sort of darting movement, and in addition the sensitive fingers are used to locate food in the bottom mud and transfer it into the mouth. As the toads increase in size they take much larger prey, and fully grown toads can satisfactorily dispose of quite large earthworms. In captivity, they also learn to take small strips of raw meat, and while quite voracious feeders when food is plentiful, they can fast for fairly long periods.

In breeding clawed toads the main difficulty is to find the right conditions for triggering off the process. Thundery weather tends to act as a stimulus, and imitating heavy rain by spraying water into the tank occasionally does the trick (Vallance, 1952). The most effective method is to transfer the toads at the right time of the year (June is the best month in this country) to another tank containing water some ten to twenty degrees colder than that from which they have been removed. If eggs are laid the toads should immediately be separated, or they will eat the eggs and probably the larvae. The latter commence to eat a few days after they are born, and the necessary infusoria can be supplied by adding green pond water. Useful supplementary foods are finely mashed liver and dried and ground nettle and lettuce leaves. Plenty of water-weed in the tank is advisable, particularly as the time of metamorphosis approaches, as the young toads can easily drown at this stage if not given facilities for supporting themselves near the surface of the water.

The South American members of the family are typified by the well-known Surinam Toad, *Pipa pipa*. The adults frequent forest pools and live rather similar lives to those of *Xenopus*, but their mode of breeding is different. As the eggs are laid, the male, while fertilizing them, spreads them over the back of the female, which develops a spongy nature at this time, and into which the eggs sink. Buried in this manner in the female's back, the eggs develop through all the larval stages until finally tiny replicas of the adult push their way out through the outer covering of skin and swim off. Although less able to withstand temperature ranges than *Xenopus*, these toads are fairly hardy in captivity, but comparatively little is known about their breeding requirements.

Other wholly aquatic frogs and toads are specialized members of the normally terrestrial families, and in superficial appearance tend to resemble the pipid toads. One or two Asian species have been studied to a slight extent, but these specialized forms offer a wide-open field for detailed research.

This generalized account of the amphibians is designed as a basic guide for those who find it necessary to keep and breed amphibians as laboratory animals for biological research. Some species which are highly interesting from a zoological point of view, such as the primitive members of the family Leiopelmidae, have not been dealt with because they are unlikely to be suitable for this purpose. All the species mentioned by name are ones which it should be possible to establish in the laboratory and on which detailed information is available in existing literature.

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REPTILES *

The reptiles living in the world today represent four orders, Rhynchocephalia, Crocodilia, Testudines, and Squamata. For the purpose of this chapter Rhynchocephalia and Crocodilia will not be considered. The order Squamata is divided into two sub-orders, Serpentes and Lacertilia, and these will be dealt with separately.

* The Editors regret that it has been necessary through lack of space to omit many families of Reptiles. It is hoped that those families omitted would not be important to the animal technician, and the editors sincerely hope that by this omission, the value of this important chapter has not been diminished.

Reference to the omitted families of Reptiles should be made to the author at 11 Churchill Road, St. Albans, Herts.

Order TESTUDINES

It is convenient to divide the testudines into three groups, which will be referred to as the tortoises, the terrapins, and the turtles. The tortoises are the species which live on land, the terrapins are those which frequent fresh water, and the turtles are the marine aquatic forms. To avoid confusion, it should be explained that in other countries these three terms do not always have precisely the same meaning. In America, for example, it is customary to refer to most testudines as 'turtles', the term 'tortoise' being reserved for certain specific land forms and 'terrapin' for a few fresh-water forms particularly esteemed for culinary purposes.

Tortoises

The entirely terrestrial testudines are a fairly distinctive group, although they are taxonomically separated into various genera, and some are more closely related to certain of the terrapins than to the other tortoises. They range in size from species with a maximum length of about 6 inches to the Giant Tortoises, which still survive on certain islands in the Indian Ocean and the Galapagos group, and which may reach a weight of several hundred pounds.

Different species vary to some extent in their requirements in captivity, and a knowledge of their natural habitats is an advantage. Some live in moderately temperate regions and hibernate in winter; it is possible to keep these outdoors throughout the year in this country provided they have somewhere to hibernate. Examples are the European Tortoise, *Testudo hermanni*, the so-called 'Greek' Tortoise or Mediterranean Spur-thighed Tortoise, *T. graeca*, and the Common Box Tortoise, *Terrapene carolina*, of North America. Most tortoises, however, come from tropical or semi-tropical regions and require temperatures in the range of 60–80°F (15·6–26·7°C) throughout the year. Subject to this, almost any kind of pen or cage is suitable, but as much room as possible should be allowed, as in spite of their fairly slow locomotion, tortoises like to move around and can cover a lot of ground during a day. In a pen the sides should be higher than the tortoise can reach when standing upright on its hind-legs, and wire netting should be avoided, as the tortoise might get its head and legs entangled. Many tortoises are good burrowers, and this should be remembered when designing the pen. Nearly all are diurnal, and like to tuck themselves well away at night. Both for this reason and to avoid too much exposure to rain, some sort of shelter is needed.

Many tortoises can go without water for long periods, but all drink copiously when thirsty. Drinking-water must therefore always be provided. Feeding does not present any particular problems, although there is some variation between species. All tortoises eat vegetable matter, some exclusively so. Colour and smell, which is well developed in the tortoises, play a part in the choice of food. The desert forms feed largely on the flowers and succulent leaves of cacti and other desert plants. The open-country species eat a wider range of tender leaves, flowers, berries, and other fruits. The forest tortoises prefer leaves, fruits, and certain types of fungus. In captivity, lettuce and dandelion are eaten readily by most tortoises, and a wide range of fruits, including apples, citrus fruits, tomatoes, and berries. The choice of food offered should be as varied as possible, as individual tortoises may have strong preferences, which may change from time to time. Some species include a good deal of animal matter in their diet also. The European Tortoise, *Testudo hermanni*, and the South American Tortoise, *T. denticulata*, readily eat carrion, which they can detect from quite a distance if it is sufficiently odorous, and both will also eat earthworms and various other invertebrates. These and some other species will eat raw meat in captivity, and it is probable that the occasional dead mouse, which they will tear to pieces before swallowing, helps to keep them healthy.

Tortoises often lay eggs in captivity, which with proper care and a little luck may hatch out. They should be kept at a temperature of around 75–80°F (23·9–26·7°C), but the greater difficulty is to maintain the correct degree of humidity. If kept too dry they will shrivel up, and if allowed to become too damp may be attacked by fungus. The correct humidity can really only be learned by experience, but the resultant humidity of a newspaper soaked in water and squeezed as dry as possible will be roughly that desired for the sand or soft loam in which the eggs should be buried. It is difficult at the temperature required for hatching the eggs to maintain the correct humidity of the packing material, and the best method is to place this and the eggs in a large earthenware flower-pot with a sheet of glass over the top. If the contents show signs of becoming too dry the outside of the pot should be wetted so that the moisture can soak through and restore the required humidity. The time the eggs take to hatch varies according to the species and the temperature, but is usually ten to twelve weeks in most cases.

If success in hatching the eggs is achieved there remains the difficult task of rearing the young. The main factors are temperature, which should not fall below about 70°F (21·1°C), and food. A varied diet containing considerable quantities of minerals is necessary to promote growth of the shell. The addition of a few drops of halibut-liver oil is beneficial, and there are some powdered calcium preparations on the market which will help to ensure proper shell development. Alternatively, powdered cuttlefish may be used, or a piece of the bone put into the cage for tortoises to gnaw. Failure to provide adequate minerals may result in irregular shell growth or even the death of the tortoise.

The main causes of illness in tortoises are respiratory infections, internal parasites, and eye troubles. The symptoms of what is commonly known as 'tortoise pneumonia' are wheezy breathing and a discharge from the nostrils. It appears to be contagious and can be fatal, but often reacts satisfactorily to treatment. This consists mainly of keeping the tortoise warm and free from draughts. Inhalations and antibiotics may be helpful in affecting a cure, but their use generally calls for an individual diagnosis in each case. Silver vitellin introduced into the mouth or nostrils is also reported to have been used successfully. Internal parasites, especially nematodes, are quite common in tortoises, and probably every tortoise in a wild state has its share. In captivity they sometimes increase greatly in numbers, and the obvious precautions are to keep the cage clean to avoid reinfection, and not to overcrowd. Tortoises seem to be prone to eye troubles of various kinds, and the most frequent symptoms are watering of the eyes and an inability to open one or both of them. The latter often arises in tortoises emerging from hibernation, though probably this arises simply from the eyelids having become stuck together, whereas in other cases the cause is more likely to be an infection. In all cases regular bathing of the eye with a weak solution of boracic is likely to be the best remedy.

Terrapins

These differ considerably from the tortoises in their habits and requirements in captivity, some rarely leaving the water, and others spending much of their time on land either basking or wandering around.

Their housing must take these habits into account, and for most species a large fish-tank is suitable divided into a water area and a land area. For the sun-loving species a lamp may be suspended above the land area, which may simply be a large flat rock placed in the water. Some species from temperate regions spend the winter in hibernation and will survive the winter outdoors in this country if given the right facilities. A few examples are the European Pond Tortoise, *Emys orbicularis*, the Spanish Terrapin, *Clemmys caspica leprosa*, Reeve's Terrapin, *Chinemys reevesi*, from Asia, and the Musk Terrapin, *Sternotherus odoratus*, from North America.

Terrapins in general are far more carnivorous than the tortoises, and some live entirely on an animal diet. According to the size of the terrapin, this may range over small water insects, worms, amphibians, and fish. The prey is seized in the mouth and either swallowed whole or torn to pieces with the claws of the powerful front limbs. In captivity natural food if available is best, but it may be supplemented by chopped raw meat or fish.

As in all reptiles, fertilization is internal, and in the wild state the females come ashore to lay their eggs in holes dug in soft soil, sand, or decaying vegetation. Most species have a definite time of year for laying their eggs, but there is some evidence to suggest that a few lay two or even three times a year. The period of incubation varies considerably according to the species, and is much affected by temperature and, possibly, by humidity, but usually takes two or three months, when the young, on hatching, make straight for the water. Any eggs laid in captivity should be treated in a similar way to tortoise eggs.

Food for the young should include as much calcium as possible, and the natural foods from which most of this is obtained are small fresh-water crustaceans, such as *Daphnia* and fresh-water shrimps. Woodlice seem to be an acceptable substitute, also mealworm and small earthworms. Some species will take a certain amount of vegetable matter such as lettuce right from the start. Raw meat should only be given to young terrapins very occasionally, but a fair amount can be included in the diet of larger terrapins, together with raw fish. Water-melon appears to be a particularly acceptable delicacy for the more herbivorous species. Several prepared 'turtle foods' on the market are expensive, harmless as a supplement, but inadequate as a staple diet. Given the right foods, even young terrapins should not need any vitamin supplement, but the need to supply calcium in growing terrapins is essential. Finely ground bonemeal may be mixed with meat or fish, and if pieces of cuttlefish bone are put into the water the terrapins will gnaw them. Chopped herring occasionally, or a few drops of halibut-liver oil mixed with the meat provide extra vitamins. Nearly all terrapins feed under water, and only a few are able to swallow their food on land. The food may be placed either in the water or on the land where the terrapins can take it into the water to eat it. They have good eye-sight and detect their food primarily by sight, but often use the nose to examine dead food before accepting it. Terrapins are certainly messy eaters, they tear to pieces with the claws anything too big to swallow whole, and this may result in rapid fouling of the water. To avoid having to change the water too often, large pieces of meat and fish are best chopped up to a small enough size to be swallowed. An alternative method is to have a separate small feeding tank in which the terrapins are placed at mealtime.

The main illness of terrapins in captivity, and one which particularly affects the young, is a condition known as 'soft-shell'. The shell becomes soft and pliable and the terrapin becomes lethargic, its eyes close, and it ceases to feed. Unless the condition can be cured, the terrapin dies in a week or two, probably from starvation. Evidence suggests that this condition results from an inadequate diet, and the only hope of a cure is to get the animal feeding again on the right foods. This can be done if the condition is tackled at an early stage by increasing the temperature of the water to 85–90°F (29·4–32·2°C) and doing everything possible to persuade the terrapin to feed. Penicillin and some other antibiotics can help by alleviating the condition temporarily, but will not by themselves effect a permanent cure. What appears to be a separate form of illness, with comparable symptoms, is one in which the eyes become very swollen and cannot be opened. Recent investigation suggests that this is a condition affecting the entire lymphatic system, spreading into most organs of the body. The cause is obscure, and so far no definite cure is known. These are both essentially diseases of captivity, rarely occurring in the wild, and lack of natural sunlight may be at least a factor. Some benefit seems to be derived from exposure for 5–10 minutes daily to the rays of a small ultra-violet lamp at a distance of about 2 feet. Proper diet is, however, undoubtedly the major requirement for the prevention of these diseases, as many terrapins have been successfully reared in captivity without any access to natural sunlight or ultra-violet light, and some terrapins, such as the Snapper, *Chelydra serpentina*, which are predominantly aquatic and do not have the basking habit, can also succumb to 'soft-shell' in captivity if not given proper treatment.

Sub-order LACERTILIA

There are some 2,500 species of lizards known to exist in the world today, and they vary tremendously in bodily form, habits, and habitat. This can therefore be only a general treatise, and will follow the simple form of listing some of the more important families and outlining their main characteristics and requirements.

Family Agamidae

Over thirty genera and about 300 species are included in this family, ranging from Africa and South Europe through South Asia to Polynesia and Australia. They are mostly strongly built lizards with well-developed limbs, some of them reaching a moderately large size. They are active, alert, and diurnal.

Most are terrestrial, though a few are largely arboreal. Special mention must be made of the South Asian genus *Draco*, which includes some twenty or more species of mainly arboreal lizards with long, slender tails and five or six posterior ribs greatly prolonged, movable laterally, and connected by membranous sheets of skin which enable the lizards to make gliding leaps from branch to branch or even from tree to tree. Another unusual member of the family is the Moloch Lizard or 'Thorny Devil', *Moloch horridus*, which is extremely adapted to its habitat in the desert and semi-desert regions of parts of Australia. Its food appears to consist entirely of ants. A number of the agamid lizards include a certain amount of vegetable matter in their diets, and a few, such as the various species of the genus *Uromastix* from Central and Western Asia and extreme North-East Africa, are almost entirely herbivorous. Otherwise, these lizards feed mainly on insects.

Most of the agamids can be successfully kept in captivity.

Family Amphisbaenidae

Individual specimens of various species have been kept alive in captivity for fairly long periods. In general, the best medium seems to be a mixture of soft sand and leaf-mould, kept just damp enough not to crumble. The lizards make regular runs, and surface only occasionally at night, although in a wild state they sometimes appear after heavy rains. Proper observation is extremely difficult under these circumstances, but a specimen of *Amphisibaena alba* kept by the writer for some months is still going strong, having been offered no food other than earthworms, which certainly disappear regularly. It seems almost certain that the water-uptake is through the skin, as this particular specimen has received no water other than what can be obtained from the slightly damp soil.

Family Anguidae

The best-known species in this country is the limbless Slowworm, *Anguis fragilis*, which has a wide range covering most of Europe, including the British Isles (but NOT Ireland), parts of North Africa, and South-West Asia as far as North Persia. In Scandinavia it is found as far north as Latitude 65°. It is hardy in captivity, and can withstand a wide range of temperature, being most active at around 80°F (26·7°C), but feeding (at least as far as specimens from the more northerly part of the range are concerned) at temperatures as low as 50°F (10°C). It spends much of its time burrowing in soft soil or under stones or logs, but occasionally emerges to sun-bathe (particularly in early spring, or gravid females in early summer) and frequently after rain. It prefers a slightly moist habitat, and should be provided with a layer of loam or leaf-mould, which should be sprinkled with water to prevent its becoming too dry. This is important with the young, which quickly become dehydrated if exposed to dry heat. The Slowworm will lap up drops of water with its broad tongue. Favourite foods are worms and slugs, particularly the small, white slug often found in gardens.

Another legless species often imported into this country is the so-called 'Glass-Snake' or Sheltopusic, *Ophisaurus apodus*, from the Balkans, South Russia, Asia Minor, and Morocco. It is much larger than the Slowworm, reaching a length of 4 ft and a diameter of 1½ in. It is also hardy in captivity and becomes quite tame. It lives in rocky, bushy areas, and feeds on insects, eggs, small birds and mammals, small snakes and other lizards.

With all the anguids it is as well to remember that the tail is extremely brittle and will be discarded if the lizard is roughly handled. A new tail will grow in due course, but the appearance of the lizard may be permanently spoiled.

Family Chamaeleontidae

The true chamaeleons (not to be confused with a few species, such as some of the members of the American iguanid genus *Anolis*, which are often popularly, but erroneously, referred to as 'chamaeleons' because of their ability to change colour to some extent) are found in Africa, Madagascar, and parts of South Asia, with one North African species overlapping into South Spain. They are very distinctive in appearance, with high and rather narrow bodies, opposible digits, prehensile tails, independently movable eyes, and extremely protrusible tongues. Most are high arboreal, spending most of their time in trees and bushes, though a few live more on the ground. Some species are furnished with up to as many as three 'horns' placed anteriorly on top of the head. There are about eighty species, of which the majority belong to the genus *Chamaeleo*.

Their food consists entirely of insects, which are stalked and caught on the tip of the very long, sticky tongue. This organ can be extruded very rapidly to a considerable distance, and the ability to aim it extremely accurately is undoubtedly assisted by the binocular vision made possible by the turret-like form and the remarkable mobility of the eyes.

The requirements of chamaeleons in captivity still require a lot of clarification, and most of them do not normally survive for more than a few months. In many ways, the most suitable are some of the smaller species commonly known as 'dwarf chamaeleons', which have frequently been kept in captivity for fairly long periods and bred with a fair measure of success. The reasons for this are probably several—their small size (rarely exceeding 5 in.) makes it easier to provide them with sufficient food, their young are born alive, whereas most chamaeleons are oviparous, and they can withstand much lower temperatures. The background to this latter ability is that either, like some South African species of the genus *Microsaura*, they live at a considerable distance from the Equator or, although living near the Equator, they occur only at high altitude like the Two-lined Chamaeleon, *Chamaeleo bitaeniatus*, and Marshall's Dwarf Chamaeleon, *C. marshalli*. These dwarf chamaeleons are most active in the daytime at temperatures of around 70–75°F (21·1–23·9°C)—higher temperatures should be avoided if possible—but suffer no harm if the temperature falls at night to as low as 45°F or even 40°F (4·4–7·2°C). With most other chamaeleons it is necessary to keep to a much closer temperature range, around 80°F (26·7°C).

Spacious housing accommodation is almost a necessity, and the cage should be at least 2 or 3 ft high and even much larger if possible, furnished with bushes or branches in which the chamaeleons can climb. The size of the cage is particularly important if several chamaeleons are kept together, as they have a well-developed territorial instinct, and each specimen will choose a small 'roost' for sleeping and as a base from which to operate in search of food. Any attempt by another chamaeleon to usurp or even enter on to the 'roost' will be opposed. In too small a cage this instinctive behaviour pattern is disrupted, and the chamaeleons may fail to settle down to a natural routine.

Chamaeleons require regular and plentiful food, and in a wild state their diet is somewhat varied. These requirements are not always easy to satisfy in captivity, and this is probably one of the reasons why captive chamaeleons so often fail to thrive. Flies and moths, some species of which can be bred in quantity, are useful foods, but even better are crickets and grasshoppers or, for some of the larger species, locusts. Chamaeleons drink only from rain-drops or dewdrops, and seem incapable of learning to drink from any kind of vessel. The branches or vegetation in the cage should therefore be sprinkled with water every two or three days. This has, of course, the effect of increasing air humidity, and although many chamaeleons come from rain-forest areas, they do not seem able to withstand humid conditions in captivity. Whatever the reason, good ventilation of the cage is necessary. Most chamaeleons like to bask on occasion, and unless sufficient sunlight is available, a suitable lamp should be mounted in the roof of the cage.

Apart from the dwarf species, which give birth to living young, very little success has attended efforts to breed chamaeleons in captivity. The females of most species descend to the ground to lay their eggs in shallow holes which they dig in soft soil or leaf-mould. Any attempt to breed the oviparous species therefore calls for a layer of suitable soil in the bottom of the cage. The eggs or the young should be moved to a separate cage and the young, particularly of the smaller species, need plentiful supplies of quite small insects, such as *Aphis* or *Drosophila*.

Family Lacertidae

The 'true lizards' constitute a large family, with a score or more of genera and some 150 species. The main home of the family is Africa (though it does not exist in Madagascar), and a number of species also occur in Europe, where it is the dominant form of lizard, and in Asia.

The largest genus is *Lacerta*, with some thirty species in Europe, Asia, and Africa north of the Equator. Two of the three British lizards belong to this genus, the Viviparous Lizard, *L. vivipara*, and the Sand Lizard, *L. agilis*. The largest of the European lizards is the handsome Eyed Lizard, *L. lepida*, from South France, the Iberian Peninsula, and North-West Africa. The males frequently reach a length of 2 feet. Many of the Wall lizards of Europe also belong to this genus, and *Lacerta erhardii*, for example, has no less than thirty-one recognized sub-species in the south-east corner of Europe, most of them among the Greek islands.

Most of the lacertid lizards are happy in captivity with a bed of sand and a few rocks. They are mostly tolerant of fairly wide temperature fluctuations, but naturally, those from Africa require more warmth than those from farther north. They are essentially diurnal, sun-loving lizards and require a lamp in the cage in the absence of adequate sunlight. A water-dish should be provided for drinking. Food consists of a wider range of invertebrates, particularly moths, spiders, and flies. Large species, such as the Eyed Lizard, will also eat other lizards, small mammals, nestlings, eggs, succulent fruit, and occasionally tender vegetation, and many of the smaller species also eat soft fruit, such as berries. Most take mealworms in captivity.

The young of a few species are born alive: the Vivparous Lizard being an example. But most of the members of this family lay eggs which are buried in sand or soil. They are not particularly difficult to rear in captivity, provided the young are given sufficient warmth and food. Otherwise there is a tendency for the bones, especially the jaws and spine, to grow badly. This may be due to vitamin deficiency, as short periods of exposure to ultra-violet rays seem to have a beneficial effect.

Family Teiidae

Around forty genera and 150 species of teiid lizards live in the New World, and of the smaller species, probably the genus *Cnemidophorous*, with some two dozen species covering an area from the United States to South America and the West Indies, is fairly typical. It includes the Race-runners and the Whiptails, fast and active terrestrial lizards of dry, and usually, sandy places. They are not particularly easy to keep in captivity unless given spacious accommodation. They are oviparous, laying thin-shelled eggs in the sand. Food consists of insects of all kinds. A closely related genus which reaches as far north as Mexico is *Ameiva*, which includes a number of attractive, striped species, slender with long tails, some of them reaching a foot or more in length. They are also very active, and the larger species eat eggs and small vertebrates as well as insects.

Sub-order SERPENTES

Snakes lend themselves to being kept in captivity, and certainly some species should make satisfactory laboratory animals. It is necessary to emphasize the great variety of the snakes, of which some 2,500 species are known to science. These vary from tiny, worm-like snakes 6 or 7 in. long when full-grown to the Reticulated Python, *Python reticulatus*, and the Anaconda, *Eunectes murinus*, both of which have been credited with attaining a length of 30 ft.

The different species of snake are as versatile in habit as they are in form. They represent the most successful evolutionary radiation the reptiles have achieved since they declined from their period of dominance on earth, and variously occupy a wide range of habitats. There are terrestrial snakes, burrowing snakes, tree snakes, freshwater snakes, and sea snakes. In addition, there are harmless snakes and dangerous snakes, the latter including the more virulent of the poisonous snakes, as well as the larger constrictors.

Feeding

All snakes are carnivorous and feed on whole animals. These are usually caught alive, though dead animals are frequently eaten by snakes. Occasional reports of vegetarianism among snakes have yet to be proved, and certainly the snakes are designed for feeding on other animals. The long, sharp teeth are admirably suited for holding struggling prey, the jaws are capable of considerable extension, and the gastric juices are powerful enough to digest flesh, bones, and sometimes feathers and fur. Different species of snake eat different kinds of animals, and it is, of course, necessary in each case to know what is the right food to offer. The larger constricting snakes largely eat warm-blooded animals; many smaller snakes, such as the Common Adder, *Vipera berus*, eat small rodents, lizards, and sometimes young birds; others, such as the Grass Snake, *Natrix natrix*, feed mainly on amphibians; many aquatic and semi-aquatic snakes are mainly fish-eaters; many smaller snakes, such as the

American Ring-neck Snakes (*Diadophis*) and Ground Snakes (*Storeria*), consume earthworms as part at least of their diet; and other small snakes eat grubs and insects. With occasional unpredictable exceptions, each species keeps to its own particular diet, and it is no use offering a Grass Snake only mice or an Adder only fish. Some snakes feed mainly by day, others mainly by night. Food that is not eaten fairly quickly when it is put into a cage should be removed. This particularly applies to live rodents, which may easily harm or even kill a snake too sluggish or otherwise unwilling to feed. If more than one snake is kept in the same cage there is always the danger if food is left in the cage that two snakes may both seize the same morsel. Snakes often continue to swallow whatever is in their mouths, so that one snake may even swallow the other in this way. This does not apply, of course, if the food requirements of the snakes differ, but is a real danger with snakes which eat the same kind of food. Snakes react to food by both movement and smell. A live animal may be quickly seized because it is moving, and a dead or motionless animal detected by smell. Many snakes feed in darkness, on rodents in their burrows, and they rely on smell, using, in addition to the nose proper, the forked tongue to carry scent particles to Jacobson's organ, a sort of nose in the roof of the mouth.

Many snakes simply seize their prey and swallow it without further ado. The Grass Snake is an example. Some, like the Smooth Snake, *Coronella austriaca*, when feeding on lizards will throw a coil or two around the lizard to subdue its struggles. The constricting snakes, such as the Pythons, the Boas, and the Rat-snakes (*Elaphe*), kill their prey by constriction, usually seizing it in the mouth and throwing two or three coils around it or even pressing it against a solid object. When the animal is dead the coils are relaxed and swallowing commences.

The fangs of the poisonous snakes are primarily designed to kill or subdue prey, and play only a secondary defensive role. There are three groups of such snakes, in each of which the fangs operate differently. The group known as the 'back-fangs' has short, rigid poison fangs in the rear of the upper jaw. These snakes seize their prey and chew it and bring the poison fangs into play, holding on until the poison takes effect. Most of these snakes have weak venom, and some time may elapse before the prey ceases to struggle. The cobras and their allies have similar fangs in the front of the jaw, with which they can strike their prey, though in a somewhat clumsy manner compared with the viper group, in which the fangs are much longer and mounted on a movable bone so that they can be erected when the mouth is opened and quickly driven into the prey and withdrawn. Generally with these two groups, and invariably with the vipers except when small animals are being eaten, the animal so struck runs off and dies after perhaps a few minutes. The snake then tracks it down by means of the tongue.

A specialized group of vipers known as the 'Pit-vipers', which includes among others the rattlesnakes, have a pit roughly between the eye and the nostril which acts as a sensitive thermoreceptor, capable of detecting heat radiations from a warm body. The pythons have similar but smaller organs in the plates of the upper lips. If dead food is offered to these snakes they are sometimes likely to accept it if it has been warmed. Some rodent-eaters can be persuaded to eat if a mouse or rat is placed in a small box with an opening on one side just big enough for the snake to get its head through. Presumably this corresponds with the conditions in a rodent's burrow.

Housing

For most snakes, a simple, unadorned cage is best. Normal requirements are a retreat of some sort, as most snakes like to hide away when sleeping, and a water-dish or bowl. Many snakes like to bathe from time to time, and a large enough bowl is an advantage for this, but even for the water-snake it is not necessary. The Mud Snake, *Farancia abacura*, and one or two others need to be kept in a damp environment, but as a general rule the cage should be kept quite dry apart from the water-container. Most of the diurnal snakes like to bask, and sunlight or a lamp should be used. However, the temperature range of each species needs to be carefully checked, as too high a temperature can be fatal for snakes, and it should be possible for the snakes to get away from the source of warmth to a cooler part of the cage. Much of a snake's life is taken up with moving about to regulate temperature, and they will do this automatically if given the chance. Heaters should be guarded if necessary so that the snakes do not burn themselves.

For the tree snakes, which rarely descend to the ground, branches or a small bush are a practical necessity. Burrowing snakes require sand or soil as the case may be, and the permanently aquatic snakes must naturally be kept in water.

Dangerous snakes

Obviously some special precautions need to be taken when dealing with these. The larger constrictors should be handled carefully, as apart from their constricting powers, they can give a nasty bite. Individual snakes may, of course, become tame; but the fewer risks taken the better. The cage in which these powerful snakes are kept must also be quite strong, as they can exert a considerable pressure in a confined space.

With the dangerously poisonous snakes the golden rule is to handle them as little as possible. In any case, the use of tongs or other instruments for holding the snakes always runs the risk of causing some damage unless carefully used. The ideal is to so arrange the cage that all the necessary jobs of feeding and cleaning can be carried out, as it were, at a distance. Food can be introduced through a small hole high up in the cage, with a well-fitting door or lid. Cleaning and the removal of uneaten food is possible with long-handled instruments which can also be operated through a small port. The best arrangement for a water-container is a sunken pool with a drain-pipe attached to a flexible hose outside the cage. By raising or lowering the free end of the hose, it can be used for both filling and emptying the pool. A cock, operated from outside the cage, will avoid any possibility of even small enough snakes escaping through the pipe. A few species of cobra known as the 'Spitting cobras' are capable of ejecting their venom accurately over a distance of several feet, and it can be dangerous if it gets into the eyes. If there is any chance of this happening, goggles should be worn. The treatment of snake-bite cannot be dealt with in this article, but obviously the correct methods should be learned and the proper sera in respect of the specimens kept should be held in readiness. If adequate precautions are taken they should never be needed, but accidents can happen.

Sloughing

All snakes slough or cast their old skins at regular intervals, usually every two or three months. Up to a week prior to sloughing the snake becomes lethargic and ceases to feed, the colours become drab, and the eyes take on a milky appearance. Both sight and the sense of smell become impaired. A day or two before the skin is cast the milkiness largely disappears from the eyes, as a result of a lubricating fluid being secreted between the old and new skins. Eventually, the snake seeks out some rough object, such as a stone or branch, on which it rubs the sides of its head until it has lifted the old skin away from the lips. The skin is pushed back beyond the head and the snake then proceeds to crawl out of the old skin, which it can do easily if it is able to entangle the skin in branches or vegetation. If the snake is healthy the skin should come off entire, including the covering of the eyes. It sometimes happens, particularly with snakes in poor health, that patches of old skin remain on the body, or even that the snake fails to cast its skin altogether when the time comes. If this happens, the old skin will dry on to the body, usually shrinking a little in the process. It must then be removed by hand, which is made easier if the skin is first soaked in water or preferably liquid paraffin. If the covering of the eyes does not come off when the snake skins it may also fail to do so at the next sloughing, and thus several coverings can build up on the eye, with the result in extreme cases that the snake may not be able to see. These coverings must be removed with forceps, which is a tricky operation. Many snakes like to immerse themselves in water for long periods as the time for sloughing approaches, and it is a good thing to have a large enough bowl of water in the cage at this time.

Diseases and pests

Snakes in captivity can suffer from a number of well-recognized diseases, and at least one external parasite can be very troublesome. Fortunately, all these complaints are fairly amenable to treatment and can be prevented by proper care. The most difficult disease to deal with is a respiratory infection commonly known as 'snake pneumonia'. The symptoms are lethargy, loss of appetite, and in severe cases a watery discharge from the mouth or nostrils. If a snake suffering from this disease is examined the mouth and throat will usually be found to be full of thick mucus, the presence of which may impair breathing and cause the snake to 'wheeze' noticeably. The extent to which the disease is infectious is not altogether clear, and it seems probable that it is acquired only by snakes already in poor health. It often makes its appearance in snakes which have been subjected to extremes of temperature, and there is more than a suspicion that it can be transferred by the Snake Mite, which will be discussed below. An infected snake should be quarantined and kept free from draughts but with good ventilation at a regular temperature of around 75–85°F (23·9–29·4°C) or perhaps slightly higher for some tropical species. Sulphanilimide powder dropped on to the floor of the snake's mouth frequently clears up the infection in a matter of days, repeating the treatment every other day until the symptoms have disappeared. The amount administered should be only enough to dust the inside of the mouth lightly, as overdoses of sulphanilimide taken into the system may have detrimental effects. Silver vitellin is also reported to have been used with some success in cases of pneumococcal pneumonia in reptiles, a few drops of a 15 per cent solution being administered into the mouth or nostrils; so also is Achromycin, giving about 50 mg for each 3 ft of snake, in tablet form.

Another disease affecting the mouths of snakes is known as 'mouth-rot' or 'mouth-canker'. It is evidenced by a whitish, cheesy deposit inside the mouth, particularly in the groove along each side of the lower jaw-bone. If unchecked, erosion of the flesh and even of the bones of the jaw can take place. The manner in which the complaint is acquired is not altogether clear, but healthy snakes in clean surroundings are unlikely to acquire it. If treated early the condition can be cleared up by removing the deposit and lightly dusting the affected parts with sulphanilimide powder or painting them with dilute tannic acid. The general health of the snake must be considered if a recurrence is to be avoided.

Snakes kept in damp or dirty surroundings easily acquire skin sores, consisting of small pustules between the scales filled with a watery or cheesy deposit. The condition can become extensive in some cases, practically covering the snake's body. Treatment consists of dusting the sores with sulphanilimide powder, but the main necessity is to rectify the housing conditions, and sometimes this is in itself sufficient to bring about an improvement. The disease is likely to arise in overcrowded conditions, and probably comes from contact with faecal matter. When damp, this gives off a certain amount of ammonia, which seems to be the irritant that may start the complaint. Small snakes in particular are very susceptible to such dirty conditions, and may even die quite quickly if exposed to them. For this reason, snakes packed together in bags or boxes for transport should be carefully examined on arrival and immediately washed clean of any faecal matter. If the sores can be cleared up the scales may grow into shape again over a period, and each successive sloughing will result in a better appearance.

Intestinal disorders are common in snakes in captivity, and almost certainly arise from bad feeding or housing. The practice of feeding snakes on raw meat must be deplored in this respect, as it easily gives rise to impaction of the faeces. This may sometimes be resolved by anal irrigation with warm water or castor oil, aided if necessary by gentle massage, but in severe cases is very difficult to remove. Cages should also be kept clean enough to ensure that food does not become smeared with faecal matter before ingestion. Regurgitation of food may be a sign of either stomach trouble or too high a temperature.

Intestinal parasites are unusual in snakes, while the most troublesome external parasite of snakes is undoubtedly the Snake Mite, *Ophionyssus natricis*, which under certain circumstances build up rapidly to very large numbers among captive snakes. First indications may be the appearance of tiny white spots on the snake's body, and closer examination will show the mites embedded between the scales or moving about on the skin. A magnifying glass may be needed to detect the smaller ones, though when gorged they are easily visible. In any infested snake there are almost certain to be some embedded in the rim of the eye. They irritate the snakes, lower their condition, and probably act as vectors for some snake diseases. The eggs are laid in cracks and crannies in the cage, or in debris, such as pieces of cast skin. The mite can be kept under control provided consistent precautions are taken. Newly arrived snakes should be examined and quarantined to ensure that the mite is not introduced with them. Facilities for bathing may help the snakes in ridding themselves of the mite, which can drown if immersed for long enough, and many mites can be removed or killed if the snake is 'rubbed down' with liquid paraffin. Some insect powders are very effective if sprinkled in the cage, though care must be taken to avoid any containing DDT. 'Pulvex' can be recommended. The powders should not be allowed to get into the drinking-water or come into contact with the snakes' food, and they are best used either inside the snakes' sleeping box or under sheets of thick paper laid on the floor. A recent development in America is the use of the Sorptive Dust SG67, a silica aerogel causing desiccation of the mites, but shown under test to be harmless to snakes. It is now being marked in the United States for this purpose under the trade name DRI-DIE 67.

A word should be said about forced feeding. Almost any complaint from which snakes can suffer is likely to lead to loss of appetite, and starvation may even precede the illness in causing death. Newly captured snakes, particularly of the more nervous species, often refuse to feed until they have become used to captivity. Although snakes are able to go without food for long periods, lack of food brings about a lowering condition, making the snake more vulnerable to disease, in addition to the risk of eventual starvation. Although forced feeding is not normally to be recommended, it may sometimes enable a snake to be kept going under such circumstances until normal feeding is resumed. To avoid physical damage or shock to the snake, care and patience are necessary for this operation. In some cases it is sufficient to open the mouth of the snake slightly and insert the food, and the snake will automatically proceed to swallow it. In other cases the piece of food can be gently pushed into the throat and massaged into the gullet until it is swallowed by the snake. If these methods fail it is probably best to administer a liquid mixture of beaten egg and perhaps some finely chopped raw meat by means of a rubber and glass syringe of the eye-dropper variety. All this involves opening the snake's mouth to start with, and this must be done with extreme care to avoid shock and damage to the jaws or teeth. The snake should be held firmly but gently behind the head with one hand, and the other used to open the mouth with a smooth wooden spatula. Some dexterity in introducing the food or syringe will then be needed, and an assistant may be necessary.

FISH

The fish commonly kept in the laboratory may be divided, according to their natural habitat, into two main types—marine and fresh-water. Each of these groups contains both cold-water and tropical fish. Freshwater fish are more popular because they are easier to maintain than the salt-water types.

HOUSING

Fish are usually kept in glass aquaria, but aquaria may be constructed of any material which is waterproof, is not easily corroded by water, and is not poisonous to fish.

All-glass aquaria may be undesirable because they are of uneven thickness and are therefore liable to crack and cannot be repaired. Furthermore, the uneven glass distorts the appearance of the fish and makes observation of them difficult. Tanks are usually made from sheets of glass held in position in a metal frame with putty. Aquarium putty (sold under various trade-names) is specially designed for its purpose; it remains soft, and so allows for the different rates of expansion and contraction of glass and metal, and, unlike ordinary putty, is not toxic to fish. For tanks of up to 1 cu ft in volume (5 gallons capacity or 18 in. × 10 in. × 10 in. deep) glass may be used and the frame may be of pressed steel. For larger tanks of up to 20 gallons capacity, brass angle $\frac{3}{8}$ in. is suitable for posts and top frame, and base frame $\frac{3}{4}$-in. material. From 25 to 40 gallons, $\frac{3}{4}$-in. post and top frame with 1-in. bottom frame. Beyond that, 1-in. angle iron throughout. The base itself should be of slate or reinforced plate glass.

Aquaria are covered to minimize the loss of water by evaporation, to reduce temperature variations, to keep out dust, and to prevent some species (e.g. Xiphophorus) from jumping from the tank. Lids are usually made from non-ferrous metal. The electric-light bulbs commonly used to illuminate the tank may be supported in the lid. A sheet of glass may be used as a lid, but in this case the metal edge of the tank must be protected with rubber strips to preserve it from the corrosion which would otherwise occur due to condensation of water between the lid and the frame. Any lid must be so shaped and positioned on the tank that the water which condenses on its underside drips back into the tank and does not run away to the exterior.

To find the capacity in gallons of any rectangular container, multiply the length, height, and width in inches together, and divide by 231, e.g. an aquarium 20 in. × 16 in. × 15 in. would be

$$\frac{20 \times 16 \times 15}{231} = 20{\cdot}7 \text{ gallons}$$

WATER

Water, the environment of fish, is all important. Water may be hard or soft, acid or alkaline. Fortunately, the common species will tolerate considerable variations in the constitution of the water, although they are happiest with neutral conditions. Most tap waters are safe to use after they have been left standing for two or three days. This period of 'maturation' permits the escape of dissolved gas and allows the growth of organisms, which the fish may eat, in the water. Before it is used, the water should be tested on litmus paper, which will turn red in acid, blue in alkaline, or remain unchanged in neutral conditions. Acidity may be reduced by adding sodium bicarbonate to the water, and alkalinity by adding tannic acid or a filtrate of peat moss. Hard water may be softened by diluting it with rain water or with ice formed from condensed water round the ice box of a refrigerator (NOT water from ice trays) or with distilled water to which had been added sea salt in the proportion of three level teaspoonfuls to the gallon. The water may become hard if soluble, chalky or alkaline rocks (e.g. limestone, marble) are placed in the aquarium. If rocks are used, smooth, naturally worn rocks or well-scrubbed coal or slate are suitable; but they should be free from cracks in which food particles could collect and decay. It is not necessary for the fish, to cover the floor of the aquarium with gravel. The floor of an aquarium may be covered with gravel if some medium in which plants may root is needed.

HEATING

Tropical fish must have a warm environment. Heat may be applied from small gas jets placed under the tank, which is protected by asbestos, but electrical heating is more commonly used. A heater may be made from a coil of wire placed in a hard-glass test-tube which can be made water tight with a rubber bung through which passed insulated wire leading to a simple thermostat. One such heater of about 100 watts submerged in an average 10–12-gallon tank would provide sufficient heat, though often two heaters are fitted in case one should break down. The average water temperature for tropical fish is 75°F (23·9°C). It should be remembered that, though most fish will tolerate large, *gradual* temperature changes (often of ±20°F (11°C)), the bacterial count of the water increases and the oxygen content decreases as the temperature rises, with obviously harmful results.

LIGHTING

Lighting is not essential for fish, but it is essential if the fish are to be seen. If an aquarium contains plants these plants will require light. Two light bulbs of 40 or 60 watts each switched on for 12–15 hours daily is usually recommended, but the situation and contents of an aquarium determine the amount of artificial light that is needed. Algae develop when there is too much light; plants die when there is too little. Experience soon indicates the amount of light needed. As a guide, 'very poor' light is a position where a newspaper cannot be read easily by daylight.

PLANTS

It is now accepted that plants are not essential for keeping healthily stocked aquaria. All the necessary interchange of gases occurs quite naturally at the water-surface. It is therefore important that tanks should expose a large surface area of water to the air. Each inch of fish (tail excluded) should have 24 sq. in. of surface area of water; thus it comes about that a deep tank will not necessarily hold more fish than a shallower one of the same width and breadth.

Plants are useful in forming a refuge for fry (immature fish) and some types of eggs. Aquatic plants are grouped roughly as floating, rooted, or bunched, each group needing a different treatment. The floating plants, e.g. crystalwort (Riccia), fairy moss (Azolla), and the common duckweed (Lemna) need no planting; they float on the surface, giving excellent hiding-places for fry, and acting as a light filter which is often a better method of preventing the growth of algae than is altering the amount of light.

The rooted plants have definite root bases similar to common terrestrial plants. They reproduce by strawberry-like runners and, when planted, the gravel or compost should cover the roots but not the crown or junction of the roots and leaves. Examples of rooted varieties are the many types of Cryptocorynes—The Vallisneria (eel grass, tape grass) and Sagittaria (arrowhead) species both resemble wide-bladed grasses, and the various types of Echinodorus, of which the popular amazon sword plant is one.

Bunched plants, as their name implies, are planted in bunches and have scarcely any roots. At times adventitious roots grow from leaf nodes but these are few and are far from essential. The common Canadian pondweed (Anacharis, formerly Elodea) is one of the best known, and others are fanwort (Cabomba) and milfoil (Myriophyllum). Some plants, usually the rooted types, appear to die off when transplanted, but they almost invariably recover in time. Dead leaves, along with other debris, should be removed from the aquarium regularly. Many water plants flower.

AERATION

Aeration is obtained by a small electric pump which forces the air through a tube to the bottom of the aquarium, sending up a spray of small bubbles. These bubbles contain oxygen which is absorbed by the water mostly at the surface. Aeration is particularly valuable to turn on at night in aquaria partly depending in the daytime on the oxygen from plants. The plants make no oxygen at night, but give off carbon dioxide, which is not wanted in the fish tank. Aeration is also valuable in hot weather when the natural oxygen content of water is low. When overcrowding of fish is unavoidable the ordinary capacity of the aquarium may be doubled by the use of aeration.

Air is composed approximately of 4 parts of nitrogen to 1 part of oxygen. When water dissolves air into itself it does not take the two gases in that proportion, but 2 parts of nitrogen to 1 part oxygen, a ratio of oxygen which is twice as much as we breathe.

The amount of oxygen as a part of air which pure water is capable of absorbing by volume at different temperatures is shown in the table below:

Water at 50°F (10·0°C) 7·8 parts per 1,000 by volume
Water at 60°F (15·6°C) 6·9 parts per 1,000 by volume
Water at 70°F (21·1°C) 6·3 parts per 1,000 by volume
Water at 80°F (26·7°C) 5·7 parts per 1,000 by volume
Water at 90°F (32·2°C) 5·0 parts per 1,000 by volume

It will be seen how rapidly the oxygen content diminishes as the temperature increases.

FILTRATION

Filtration is a method of removing unwanted dirt and organisms from the aquarium water. The action of the filter also keeps the water circulating, and thus speeds up the interchange of oxygen or carbon dioxide at the surface as does normal aeration.

Most types of aquarium filters have to be attached to a pump, which forces air down a tube into the aquarium water, the air bubbles rise up a second tube taking small amounts of water with them, the water then falls into a perforated container which holds the filter medium, usually glass wool. Activated carbon is often added to the medium to further purify the water; the clear water then drips through the filter's perforations back into the aquarium.

SNAILS

Water-snails eat algae, but they excrete much waste material in the tank. They are therefore of doubtful value, and may well be excluded from the tank, with the possible exception of the Japanese burrowing snail (Melainia), which aerates the compost, and the very large apple snail (Ampullaria), which rapidly produces infusoria invaluable when rearing fry. Infusoria is any animal organism that is of suitable size to feed to young fish before they are old enough to tackle small Daphnia.

FOOD

It is generally conceded that living foods produce the best results, but dried prepared foods are easily bought and stored, and most fish will enjoy the change. Crushed snails, chopped earthworms (Annelida), and finely chopped or scraped raw liver of chicken or beef are excellent foods. Dried foods should be fed in small pinches and should be eaten in 5 minutes. Any uneaten food must be removed from the tank before it begins to decompose.

Daphnia (water flea) is an acceptable food, as also are white worm (Enchytraeus), brine-shrimps, and micro-worm (Anguillula). Cultures of white worm may be bred on a bread-and-milk medium.

Brine-shrimp eggs are sprinkled on the surface of a jam-jar of water in which one teaspoonful of common salt has been dissolved. The jar is then floated in the aquarium, and the eggs hatch in 24–36 hours. The shrimps should be washed free of salt before they are used as food.

Some fish need more vegetables than they can get from the vegetation in the tank, and lettuce and boiled spinach can be supplied in these cases.

BREEDING

Most cold-water fish need a lot of space for breeding, but some smaller varieties, such as goldfish (Carassius), can be bred in an aquarium. The tropical fish either bear live young (Ovo-viviparous) or lay eggs (oviparous).

The famous Guppy (Lebistes), the Platy, and Swordtail (Xiphophorus) are examples of the family Poeciliidae which are live-bearers. At sexual maturity in these fish the anal fin (which is just posterior to the anus or vent) of the male develops into an intromittent organ of reproduction, the gonopodium. The sperm or milt of the fish pass along a groove in this organ, which is placed touching, but not into, the female. The fertilized eggs are retained in the female for about four weeks, the period varying slightly with temperatures and from species to species. The young fish hatching from the eggs are from $\frac{1}{8}$ to $\frac{1}{4}$ in. long. They do not require microscopic food, but can be fed immediately on dust-fine powder food, micro-worms and brine-shrimps (Artemia).

The egg-laying or oviparous fish are far more numerous, and are classified according to the position taken up by the eggs. The main groups are: (i) the demersal eggs, which are heavier than water and sink; (ii) the sticky eggs, which adhere to plants or gravel; and (iii) the non-sticky eggs.

The Zebra-fish (Brachydanio) is an example of the non-sticky, demersal type of egg. When ready to breed the female appears more rounded when viewed from above or below, and should be placed in a breeding tank with the male in the evening; spawning should take place next morning. Like many fish, the Zebra is an avid egg-eater. To prevent egg-eating the water should be about 3–4 in. deep, and the bottom of the tank should be covered with small marbles or glass rods between which the eggs may fall and be hidden. The parents should be removed after spawning, and the eggs should hatch in 48 hours. Like most fry, they hang, by suction, to the glass sides of the tank for a few days. No food need be given until they are free swimming, as they exist on their yolk-sac until that time.

At this stage the fry is fed microscopic food, or 'infusions'. Infusions are cultures of tiny infusoria which can be made by placing boiled hay, banana skins, manure, or dead leaves in water for a few days, when these protozoa and other organisms will appear. These cultures can easily 'go bad'. Alternative foods are the yolk of a hard-boiled egg made into a 'mist' by stirring it in water, and the ampullaria snail. As the fry grow they can be changed to a diet of micro-worms and brine-shrimps. Fry should be fed small amounts at frequent intervals, not less than four feeds per day.

The Tiger Barb (Puntius) and the goldfish are examples of the sticky-egg type. Sexing the barbs is the same as for zebras (i.e. there is a yellowish cast over the female, particularly on the tail fin), but the male is more highly coloured. The male goldfish develops small white tubercles on the gill-plates in the breeding season. Pairs may be mated as described for zebras, but clumps of bush plants, such as milfoil, should be placed in the tank to receive the eggs. Since gravel can be a hindrance, the plants can be tied with cotton and weighted down with a stone. The pairs should be removed after spawning and the eggs and fry treated in the same manner as the zebras.

Most common fish can be bred in this way. Sexing some species is impossible without internal examination, but the plumpness of females and the colour of the male is indicative of the sex.

The Anabantids have an air-breathing apparatus called a labyrinth organ. They blow nests of bubbles, into which the fertilized eggs are blown and guarded by the male. The most dangerous time for anabantids is at about three weeks of age, when the fry go to the surface to fill their air-breathing organ. At this time great care must be taken to ensure that the air is at the same temperature as the water. Many die at this time, and a glass cover fitting tightly over the tank will greatly help the survival of the young fish.

HANDLING

Fish are covered by a protective film, and should never be handled unless it is essential to do so, as once the film is broken the fish are more susceptible to infections. If fish must be picked up, use a soft-net, keep it wet, and hold the fish to stop it falling or jumping, and, perhaps, injuring itself.

CHAPTER TWENTY

Animal Genetics

Offspring resemble their parents to a greater or lesser degree. These resemblances are due to the inheritance of parental characters by the offspring. Inheritance is not a simple process: sometimes the characters of one parent are noticeably predominant in an offspring, sometimes those of the other. Moreover, the inherited characters can be modified by external factors over which the animal has no control. For example, body size may be determined as much by the availability of food during growth as by inheritance. There are therefore two main factors which determine the growth and development of an organism, the inherited or *genetic* factors, and the external or *environmental* factors. The science of inheritance is called *genetics*.

GENES AND CHROMOSOMES

Inherited characters are controlled by *genes*. The gene can be considered as the basic unit of inheritance, one gene controlling one character, though this is an oversimplification as will be shown later. Each animal possesses a considerable number of genes. The genetic make-up of an animal, which determines its inheritance, is called the *genotype*. The genotype is then conditioned by the environment to form the animal's adult characteristics, which is called the *phenotype*.

Genes occur in linear order along the *chromosomes*. Chromosomes occur in the nuclei of all cells, but are only visible when the cell undergoes division. A group of mouse chromosomes is shown in Fig. 1. The number of chromosomes in each cell is usually constant within a species, but differs between species. For example, the number in the mouse is 40, in man 46, and in the Chinese hamster 22. This characteristic number is called the *diploid* number of chromosomes and is given the symbol $2n$.

Two types of cell are exceptional in that they do not possess the diploid number of chromosomes. These are the gametes, i.e. the egg and the spermatozoon. Instead the gametes possess one-half of the diploid number, which is known as the *haploid* number of chromosomes ($1n$). Thus, in the mouse the spermatozoa and eggs carry the haploid complement of twenty chromosomes, each chromosome being different from the others. At fertilization the union between the gametes restores the diploid number of forty chromosomes in the embryo. But for each chromosome in the egg there is a similar one in the spermatozoon. In the mouse embryo after fertilization there are thus twenty pairs of chromosomes, and in the human embryo twenty-three pairs. The two members of each pair are called *homologous* chromosomes. The cells of the embryo divide in a manner that results in all cells retaining the diploid chromosome complement, hence all adult cells are diploid. This type of cell division is called *mitosis*. The special type of cell division which occurs just

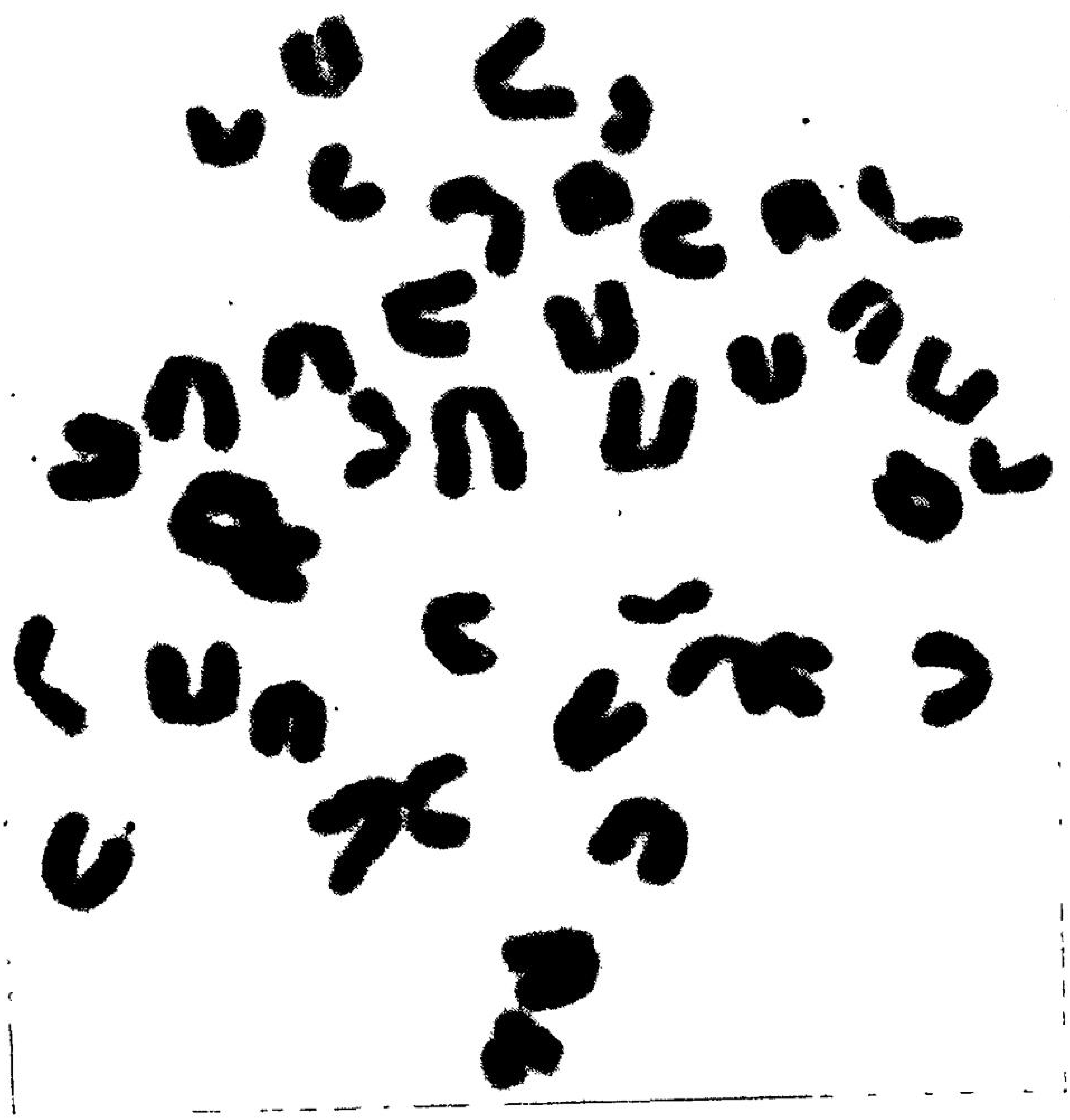

FIG. 1. Diploid set of mouse chromosomes. This mitosis is at metaphase. Each chromosome is split into two chromatids, which are attached at the centromere.The mitotic spindle has been removed chemically. $2n = 40$.

before the gametes are formed, and which results in the eggs and spermatozoa having the haploid complement of chromosomes, is called *meiosis*. We will discuss these types of cell division later.

Both the haploid egg and the haploid spermatozoon carry a complete set of genes. After fertilization the embryo thus contains two sets of chromosomes on which are carried two sets of genes, one set paternal and the other set maternal. Each character in an animal is therefore controlled by two genes, one paternal and the other maternal. The two genes then interact in determining the expression of the character they control, and the type of interaction can be very varied.

To take an example, consider coat colour in rabbits. An offspring may receive the gene for *Himalayan* from the mother and a similar gene from the father. An individual with two identical genes is said to be *homozygous* for that character. On the other hand, the father may have donated the gene for *Himalayan* and the mother the gene for *albino*; the offspring would thus have two different genes for coat colour and be *heterozygous* for that character. Heterozygous animals, or *heterozygotes*, have two different genes for the one character. Which of the two genes will predominate over the other in expression depends on which is *dominant* and which *recessive*. As the gene for *Himalayan* is dominant to albino, the heterozygote will be *Himalayan* and the recessive gene *albino* will not be apparent in the individual. Genes which control the same character, e.g. *Himalayan* and *albino* in the above example, and which occur at the same point on a chromosome (see below), are called *allelomorphs* or *alleles*. Many but not all allelic genes show dominance or recessivity. Sometimes both genes can interact and produce an intermediate or unusual expression, and others may dominate on different parts of the body. The character we have chosen is also influenced by the environment, for a low temperature during development can influence the expression of the gene *Himalayan*.

Genes which govern a particular character always occur at the same place on the chromosome carrying them. It is often necessary to define this place on the chromosome, and the term *locus* is used. Alleles thus occur at the same locus. Genes for other characters will occur at different loci. Three or more allelic genes may occur at a particular locus, e.g. the genes controlling *Agouti* coat colour in mice, or the *ABO* blood groups in man. Except in unusual circumstances, any particular individual can, of course, possess only two of these genes, for most individuals are diploid. Examples of unusual inheritance will be given later.

We must now study what happens to the chromosomes during mitosis or meiosis. From what has been said above, it is clear that a knowledge of the behaviour of chromosomes during cell division is needed for our understanding of inheritance.

CHROMOSOMES AND CELL DIVISION

Normal cell division–*Mitosis*

Mitosis occurs in cells throughout the body, and each daughter cell inherits a diploid complement of chromosomes similar to those of the mother cell. The events of mitosis are represented diagrammatically in Fig. 2. The non-dividing cell consists of a nucleus and cytoplasm. In the first stage of cell division (called *prophase*) chromosomes appear in the nucleus. Each chromosome is long and thread-like, and is composed of two identical halves called *chromatids*. During the second stage, *metaphase*, the chromatids shorten, and remain attached to each other at a particular point known as the *centromere* (Figs. 1 and 8). The chromosomes then become arranged at the mid-point of a structure called the *mitotic spindle* (Fig. 3). The nuclear membrane has by now disappeared. In the succeeding stage, *anaphase*, the centromeres divide and the chromatids move apart along the spindle to form two identical groups of chromatids at each end of the mitotic spindle (Fig. 3). During the last stage, *telophase*, the spindle disappears, each group of chromatids become included in a nuclear membrane, and two daughter cells are formed by division of the cytoplasm. The chromatids then duplicate themselves in the nucleus. Each daughter cell thus possesses a chromosome complement identical with that of the mother cell.

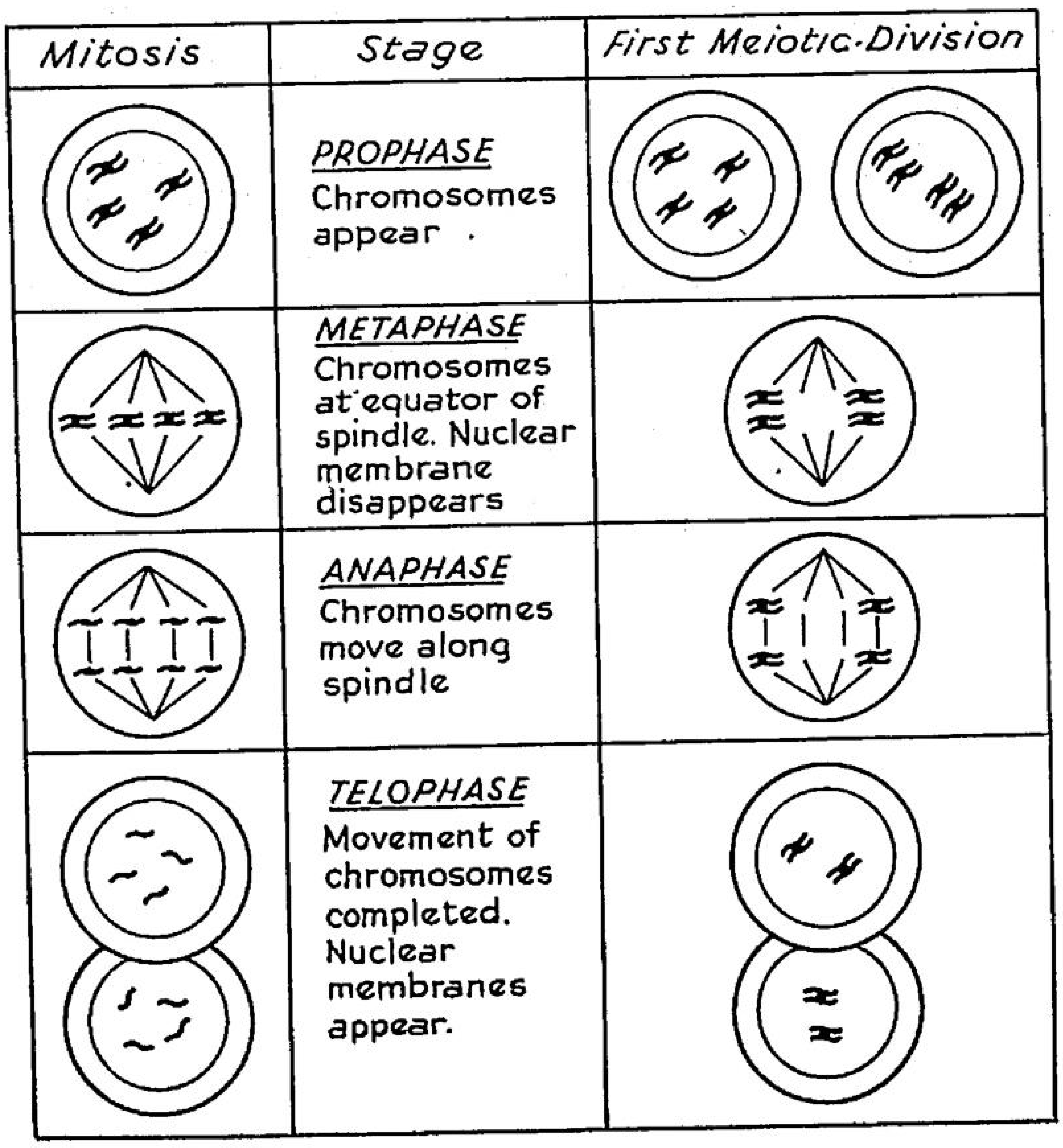

FIG. 2. Diagrammatic representation of meiosis and mitosis.

Reduction division–*Meiosis*

If the diploid number of chromosomes is to remain constant in a species the two gametes which contribute equally to the new generation must carry the haploid number of chromosomes. The reduction in the number of chromosomes takes place during the two meiotic divisions which immediately precede the formation of the gametes in the testis or ovary. Meiosis is divided into the same stages as mitosis (Fig. 2). During prophase of the first meiotic division a series of events takes place which are very different from events during mitosis. Each chromosome becomes paired with its corresponding homologous chromosome (Fig. 4). There are thus four chromatids, i.e. two pairs, alongside each other (Fig. 6). Some of these chromatids may exchange a segment with one of the chromatids of the homologous chromosome (Fig. 6). This i

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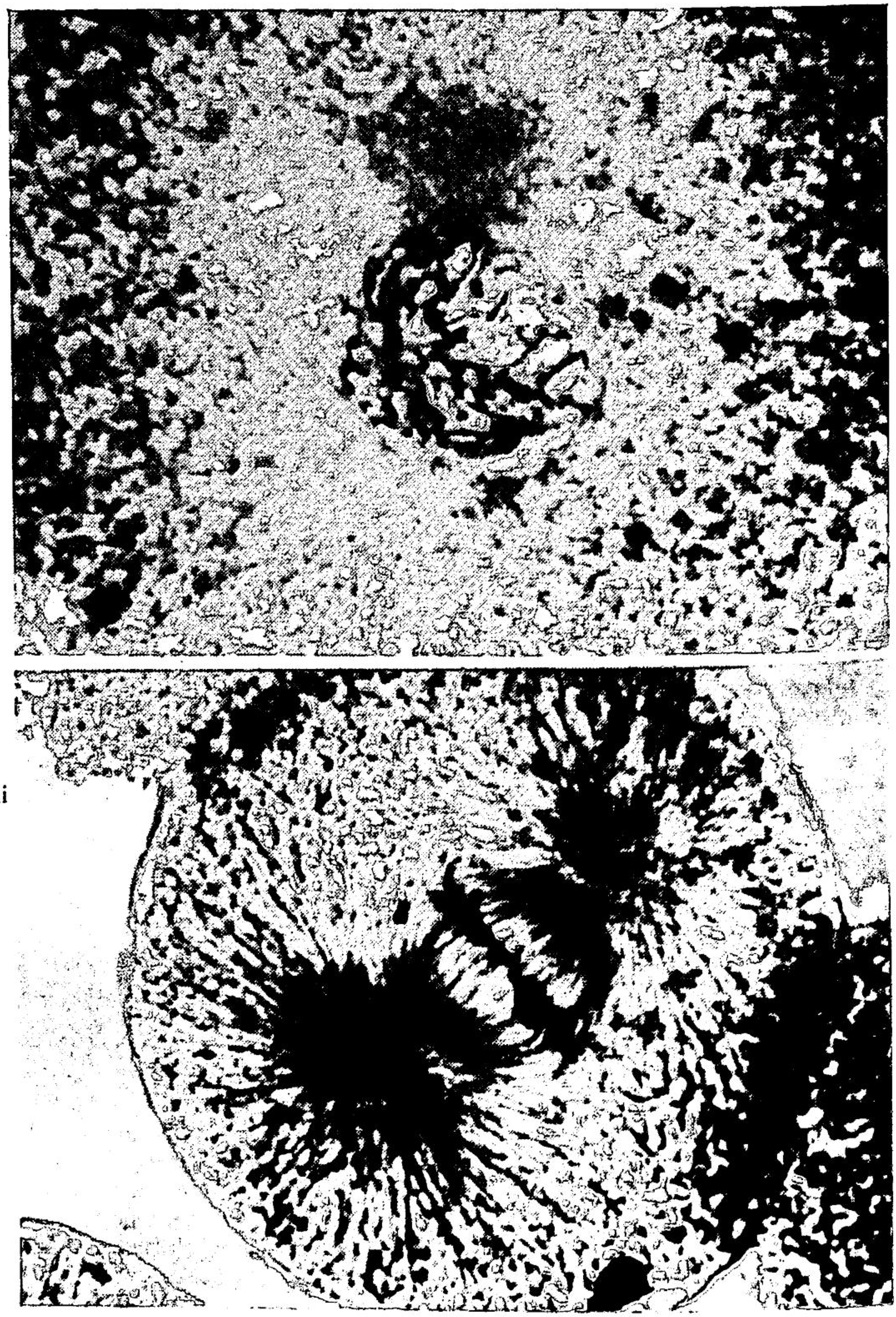
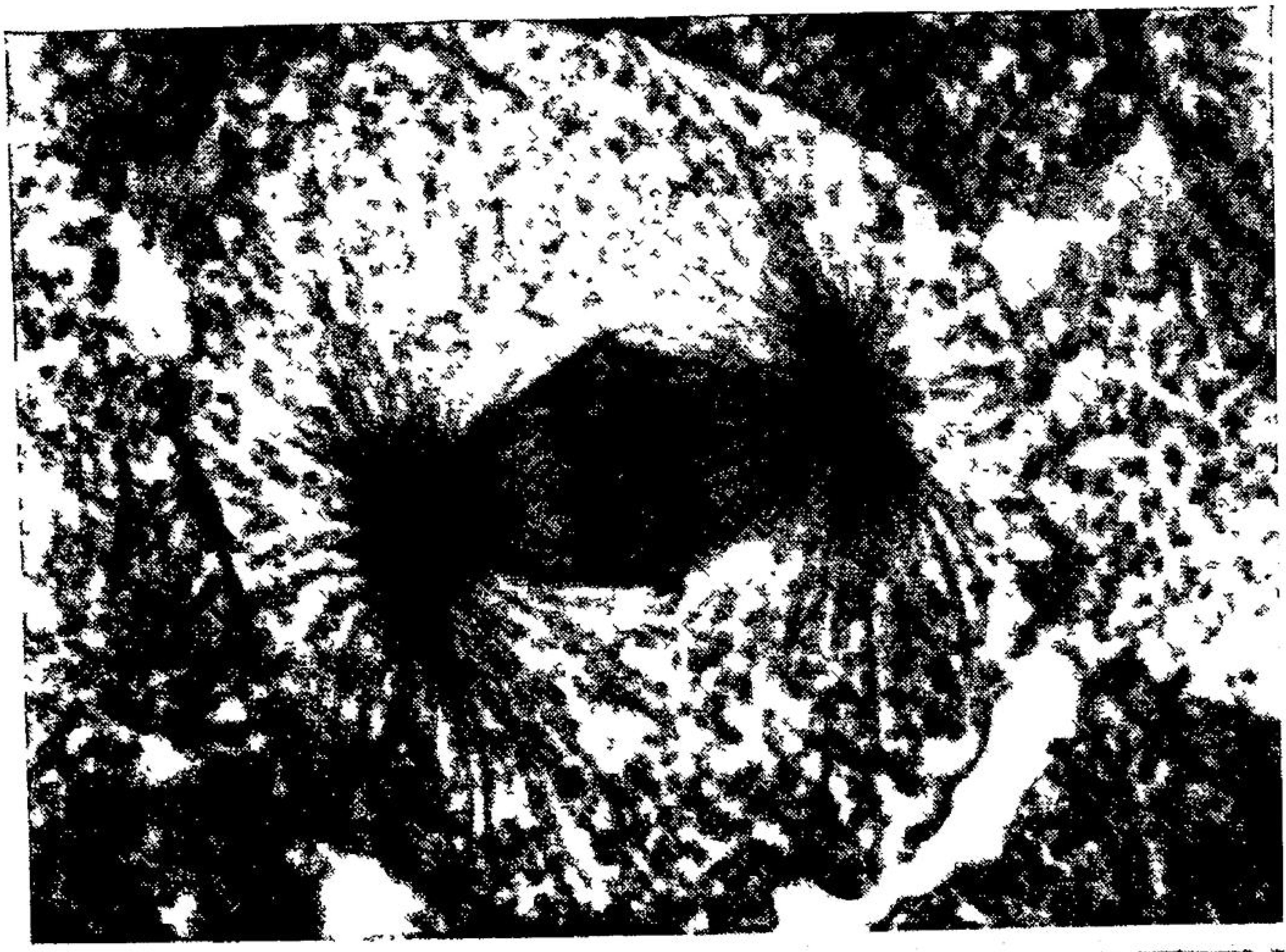


Fig. 3(*a*). Successive sequencies of the stages of mitosis.

(*a*) Mitosis in fixed cells of the whitefish blastula.

(i) Prophase, the chromosomes are becoming distinguishable. (ii) Metaphase, the chromosomes are shortened and attached to the mitotic spindle. (iii) Early



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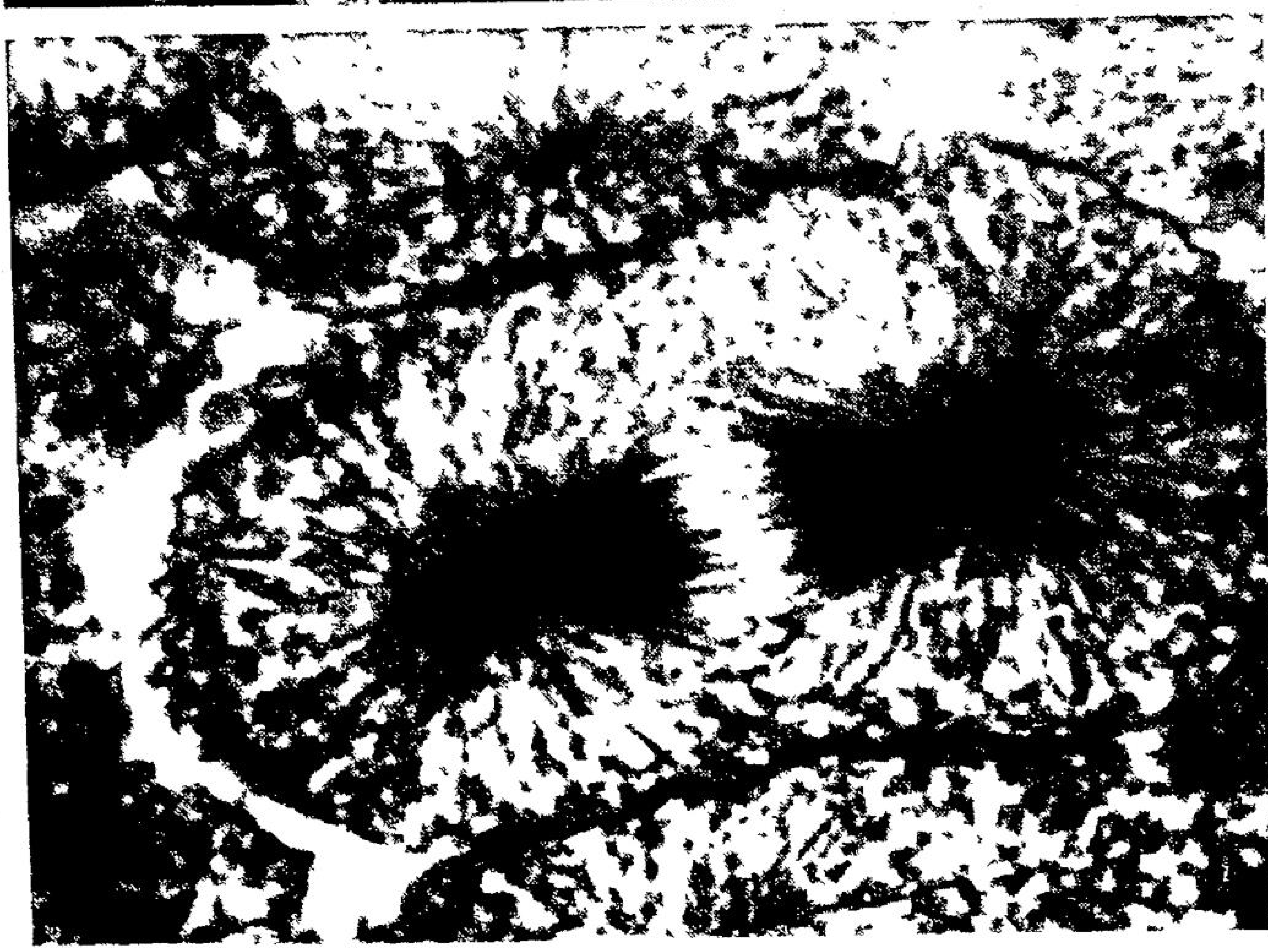

iv

FIG. 3(*a*) iii and iv

anaphase, the chromatids are just beginning to move apart along the spindle. (iv) Late anaphase, the chromosomes are at the end of the spindle. (v) Telophase, the spindle is breaking down and the cell has almost divided into two. (vi) Two v

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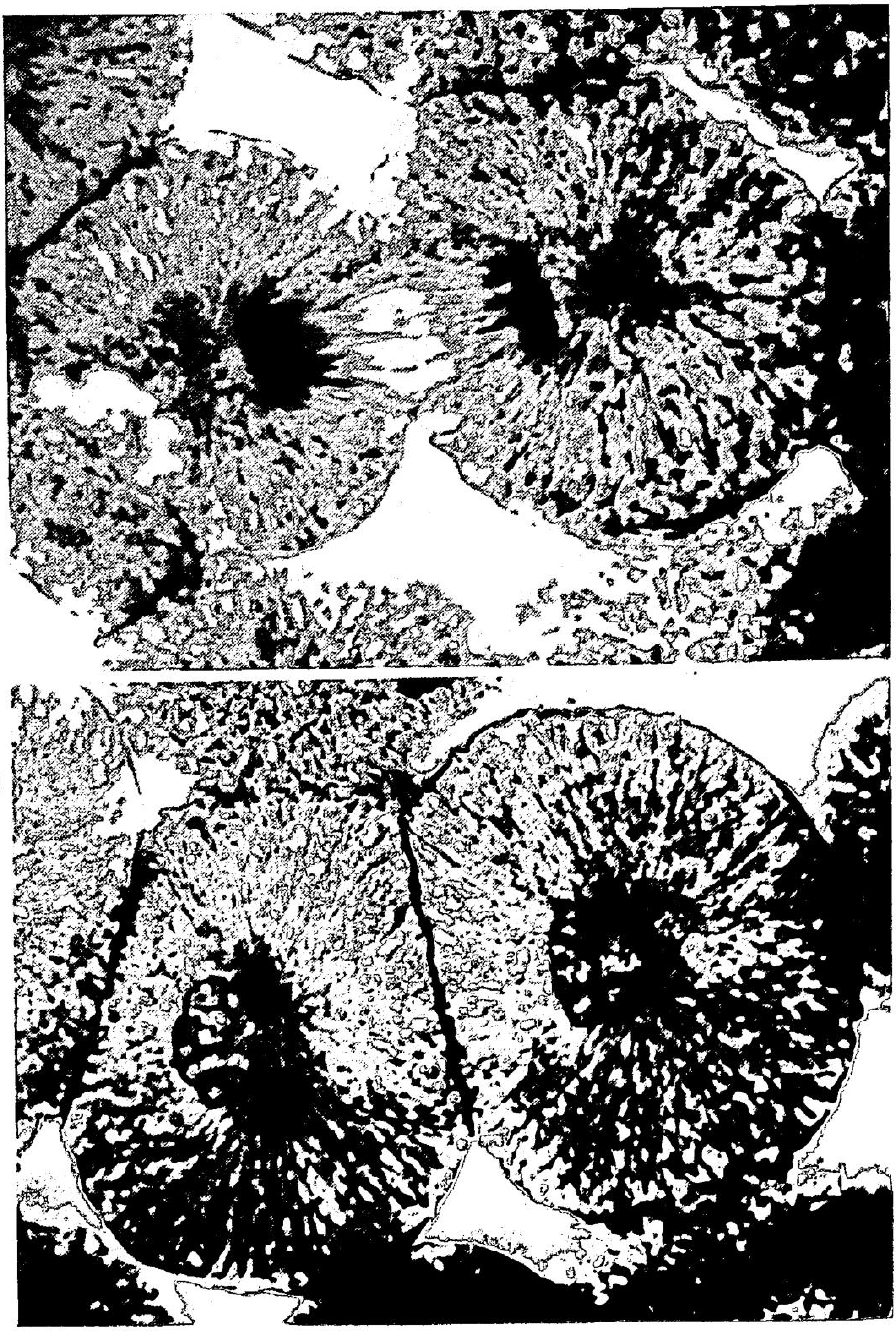


FIG. 3(*a*) v and vi

daughter cells have formed, the spindle has almost disappeared, and a nucleus has been reconstituted in each daughter cell. These pictures are published by courtesy of Mr M. R. Young.

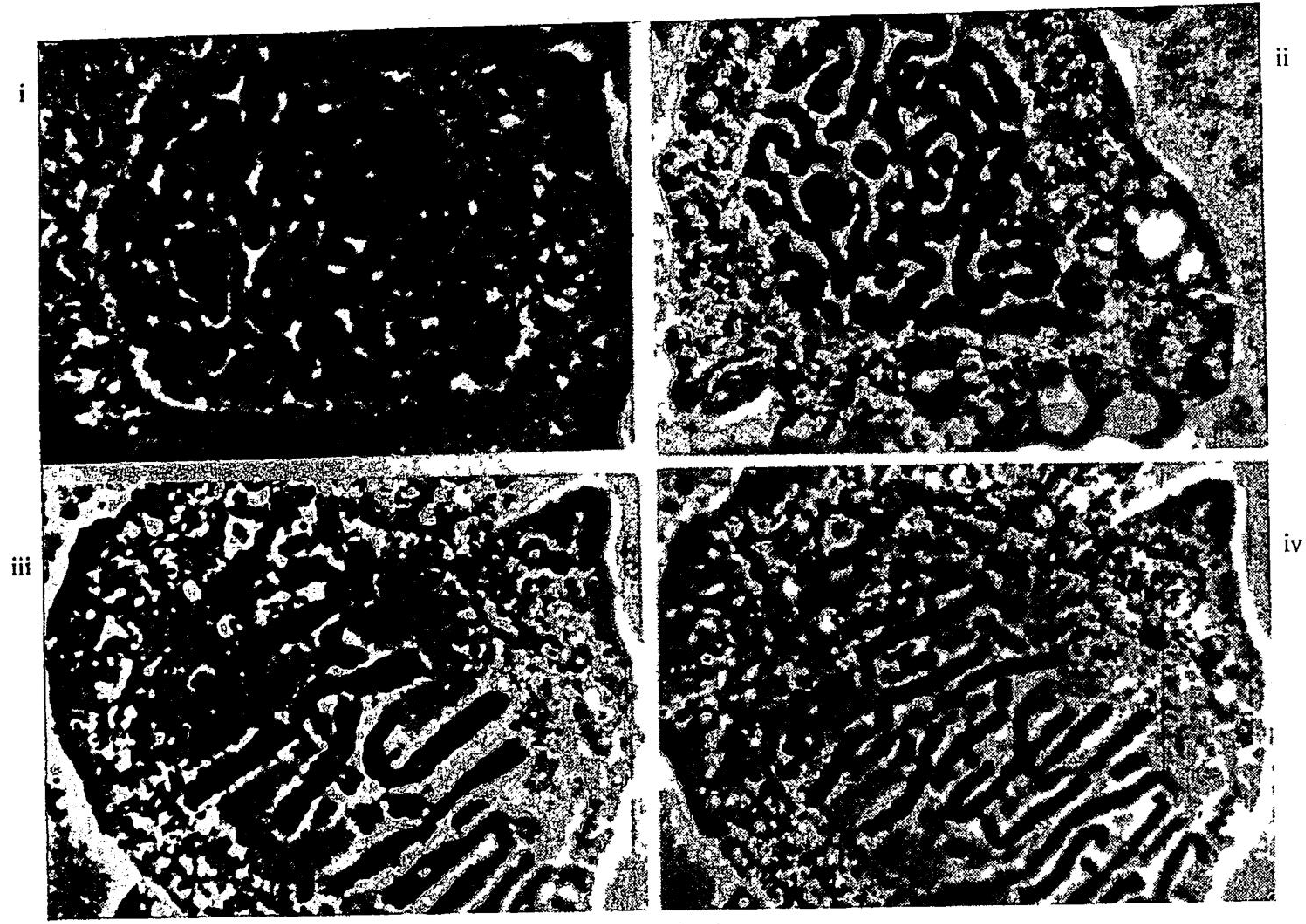
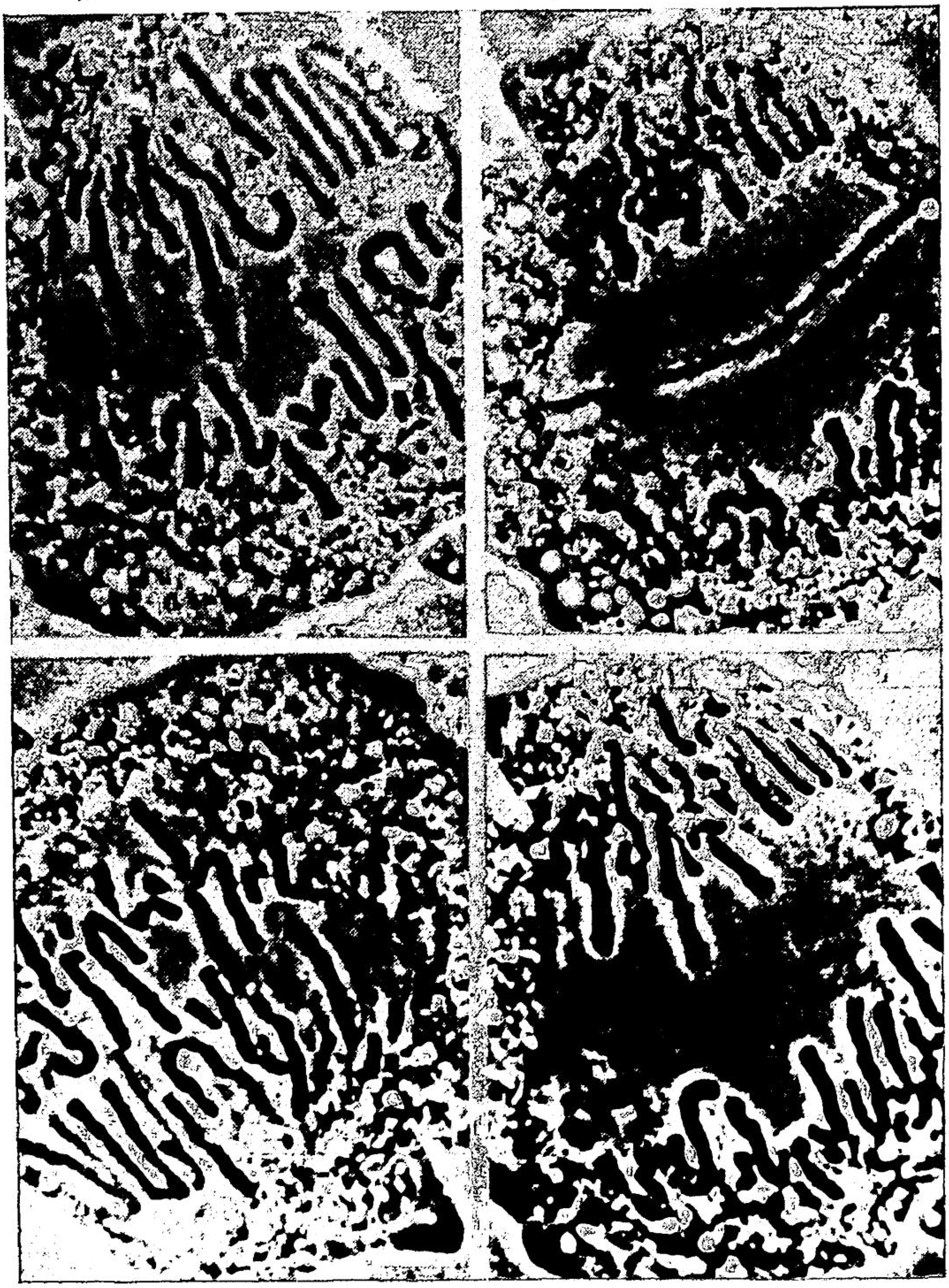


FIG. 3(*b*) i – iv

(*b*) Mitosis in a living endosperm nucleus.
(i) and (ii) Prophase. (iii) Metaphase. (iv) to (vii) Anaphase. (viii) Telophase. Note that at metaphase each chromosome is composed of two chromatids. These pictures were taken by time-lapse photography, and are published by courtesy of Dr A. Bajer and Films of Poland.

vi viii



v vii

FIG. 3(*b*) v – viii

exchange of material between two chromatids is most important genetically, for it results in a reciprocal exchange of genes between two of the chromatids.

The two chromosomes remain in close association during metaphase. In anaphase another event occurs which has no counterpart in mitosis: the centromeres do not divide and the homologous chromosomes, each consisting of two chromatids, move apart to the opposite poles of the spindle. The failure of the centromeres to divide thus results in the two daughter cells each possessing one-half of the number of chromosomes present in the mother cell. In other words, the daughter cells are haploid, though each chromosome is composed of two daughter chromatids.

The second meiotic division follows immediately after the first. This is like a mitosis: the daughter chromatids which had formed during the first meiotic division separate at anaphase during the second division. Each of the two cells produced after the second meiotic division thus contains the haploid number of chromatids, and these haploid cells then differentiate into eggs or spermatozoa. Meiosis therefore halves the number of chromosomes in the gametes, and fertilization restores the diploid number in the embryo. Meiosis and fertilization are thus complementary, and their relation in the chromosome cycle is shown diagrammatically in Fig. 5.

THE INHERITANCE OF CHARACTERS

We can now use our knowledge of chromosome behaviour in studying the inheritance of genes. It is necessary to know the types of offspring, and the ratios of these types, produced from various crosses, i.e. homozygote × homozygote, homozygote × heterozygote, heterozygote × heterozygote. We will first study the inheritance of a single gene in such crosses, then two genes, and finally more complex situations.

(*a*) INHERITANCE OF CHARACTERS CONTROLLED BY A SINGLE GENE

The laws governing the inheritance of a single gene were laid down by Mendel, and are often referred to as *Mendelian*.

For simplicity, genes are given a symbol, usually one or a few letters. The initial letter of a dominant gene is usually written in capitals. A gene that causes waving of the coat in mice is called *Rex*, symbol *Re*. Homozygous *Rex* animals are *ReRe*, homozygous *non-Rex* are *rere*, and heterozygotes are *Rere*. How are the parental genes distributed among the offspring when two animals are crossed?

Suppose we cross two animals, a male mouse homozygous for *Rex* and a female homozygous for *non-Rex*, i.e. *ReRe* × *rere*. Both paternal chromosomes carry *Re*, hence all spermatozoa will carry the gene *Re*. Likewise, all of the mother's eggs must contain *re*. At fertilization all of the offspring will

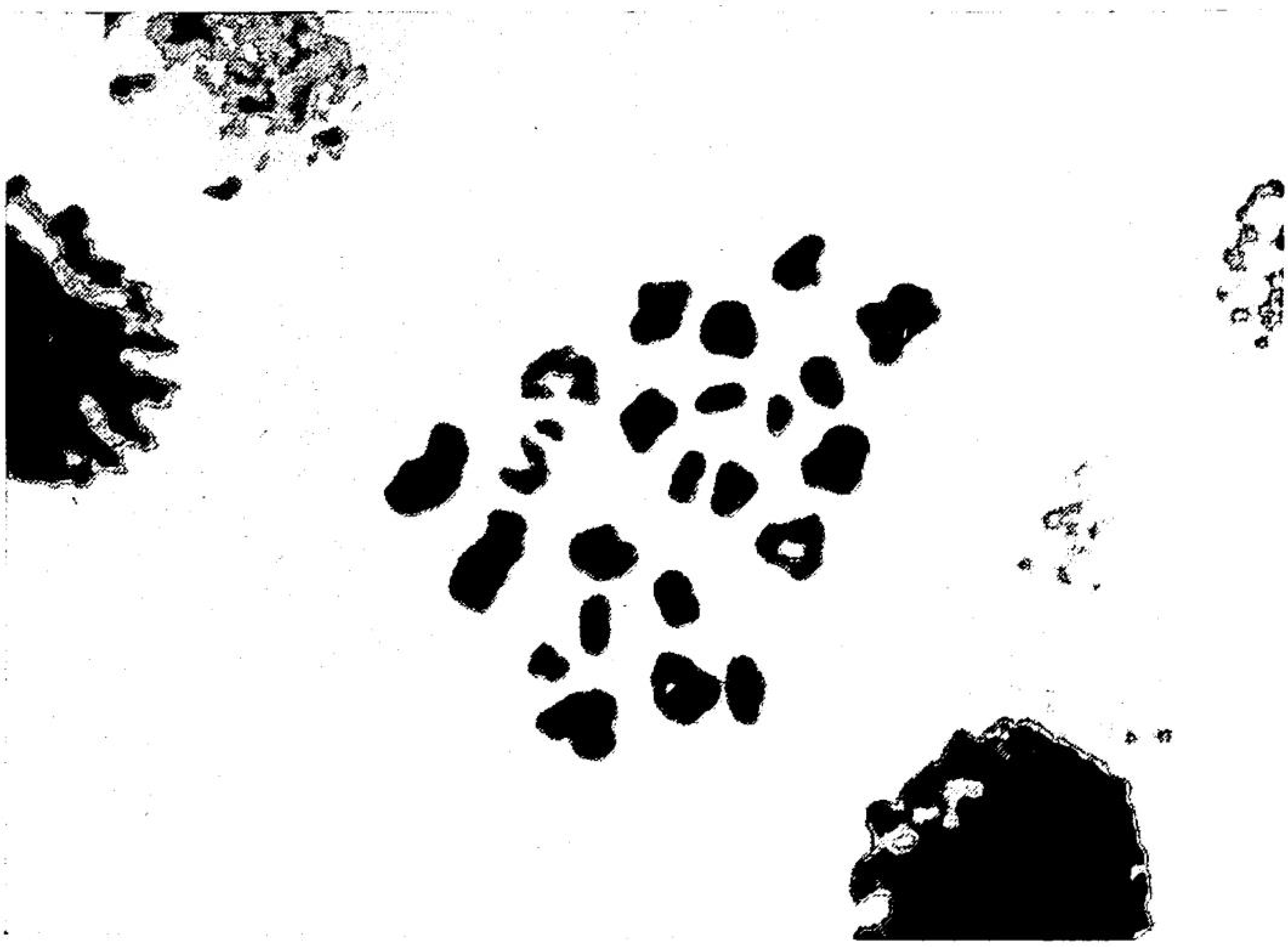


FIG. 4. Meiotic prophase in the human spermatocyte. The diploid number of chromosomes in man is 46, hence there are 23 pairs of prophase chromosomes during meiosis. Published by the courtesy of Dr C. E. Ford.

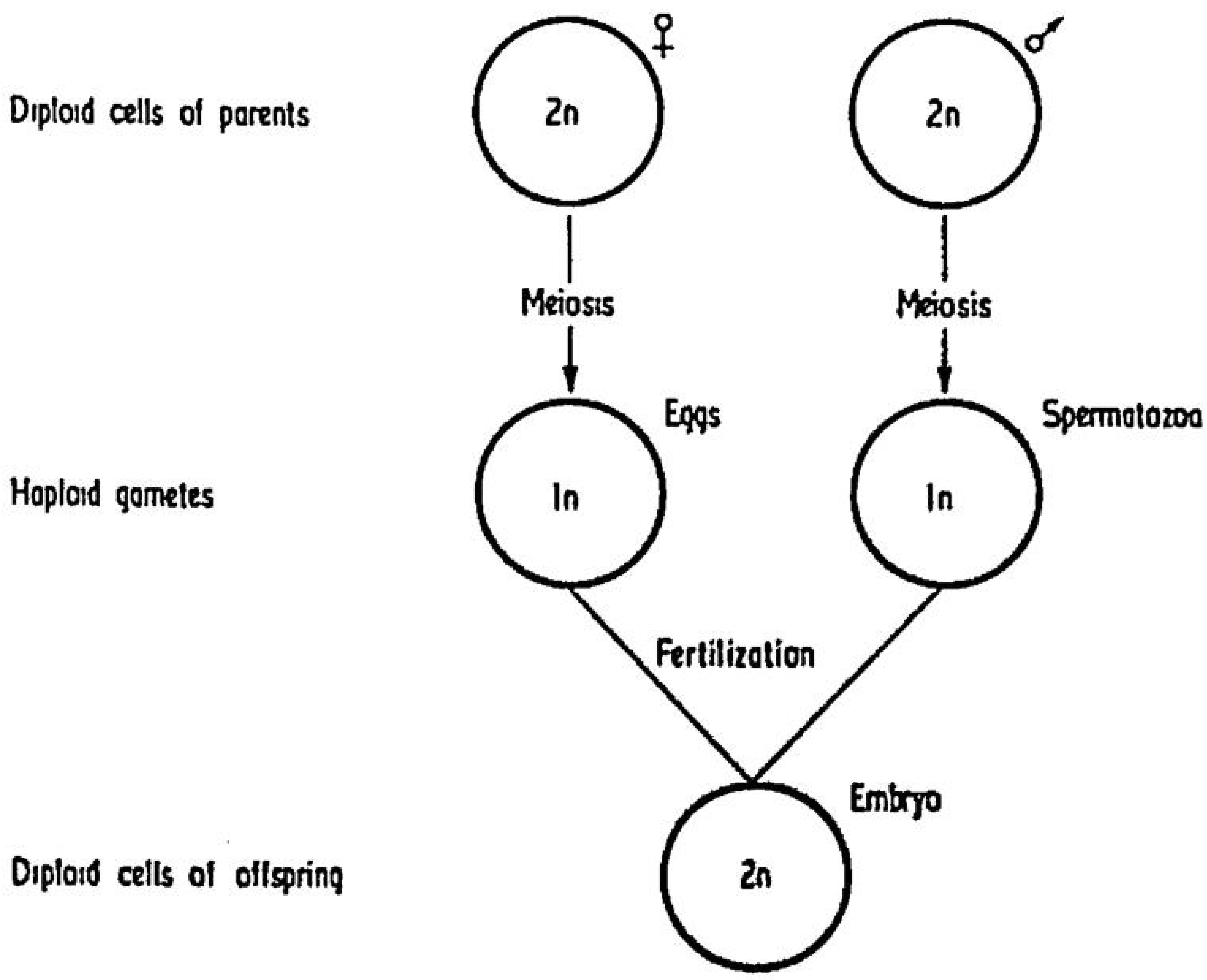


Fig. 5. Relationship between meiosis and fertilization, and the constancy of the chromosome complement.

receive a gene *Re* from the father and *re* from the mother. We can forecast the types of offspring produced with the aid of a table:

rere ♀ × *ReRe* ♂

Types of spermatozoa

		Re	*Re*
Types of eggs	*re*	*Rere*	*Rere*
	re	*Rere*	*Rere*

The genotype of the offspring is shown in the squares.

Now *Re* is dominant to *re*, hence all of the offspring, which are heterozygotes of genotype *Rere*, will be *Rex*. The offspring from such a parental cross is called the F_1 *generation*, or simply the F_1. Crossing of two dissimilar homozygotes thus results in all F_1 offspring being heterozygous. We would have obtained the same result if the male had been *non-Rex* and the female *Rex*, i.e. if we had made the *reciprocal cross*. The types of offspring from all crosses we shall describe are not determined by the paternal or maternal origin of the genes unless they are carried on the *sex chromosomes*.

Suppose we had crossed two similar homozygotes, e.g. *ReRe* × *ReRe*. All sperm and eggs will carry *Re*, and all offspring will be homozygous *ReRe*. Likewise, the cross *rere* × *rere* will produce all *rere* offspring. The crossing of two similar homozygotes thus results in all offspring being similar to the parents.

Suppose we cross two heterozygotes, i.e. *Rere* × *Rere*. The gametes, being haploid, will contain either *Re* or *re*. In the great majority of systems studied one-half of the spermatozoa and of the eggs contain *Re* and the other half *re*; there is no selective loss of either gene during gametogenesis. A heterozygote therefore produces dissimilar gametes in contrast to a homozygote, which produces similar gametes. The separation of parental alleles during the formation of the gametes is called *gene segregation*. An egg carrying *Re* or *re* can then be fertilized by a spermatozoon carrying *Re* or *re* giving rise to *ReRe*, *Re re* or *rere* embryos, as shown in the following table:

Rere ♀ × *Rere* ♂

Types of spermatozoa

		Re	*re*
Types of eggs	*Re*	*ReRe*	*Rere*
	re	*Rere*	*rere*

The genotype of the offspring is shown in the squares.

In the majority of such systems studied there is no selective fertilization, i.e. spermatozoa carrying *Re* have an equal chance of fertilizing eggs carrying *Re* or *re*. There is thus random fertilization, and the four types of F_1 offspring shown in the diagram occur with equal frequency.

Now, *Re* is dominant to *re*. In the cross outlined above the expected ratio of offspring will be 1 *ReRe* : 2 *Rere* : 1 *rere*. But phenotypically *ReRe* is indistinguishable from *Rere*. The F_1 ratio expected will thus be 3 *Rex* : 1 *non-Rex*, though two of the *Rex* mice will be heterozygous for *non-Rex*. Sufficient offspring must be classified to establish a 3 : 1 ratio, for small numbers can lead to mistaken conclusions. An example of a 3 : 1 ratio is given in Table 1. It is most important to note that a cross between two *Rex* animals has produced some non-*Rex* offspring. Another important fact to note is that genes maintain their integrity between generations, even when recessive and not visible. Thus *re* segregates normally in the F_1, though absent phenotypically in either parent.

TABLE 1. 3:1 SEGREGATION IN THE MOUSE

Two animals heterozygous for the gene *pink-eye, p*, were crossed. The 216 offspring were classified and the results were:

Pp ♀ × *Pp* ♂

Types of offspring expected	1*PP*	:	2 *Pp*	:	1 *pp*
	3			:	1
Numbers of offspring expected	162			:	54
Numbers of offspring found	161			:	55

(Data from CARTER, T. C. and FALCONER, D. S., *J. Genet.*, 50, 399, 1952)

The remaining crosses can be dealt with briefly. The offspring ratio for crosses between homozygotes and heterozygotes depends on whether the dominant or recessive gene is homozygous:

Rere ♀ × *ReRe* ♂

Types of spermatozoa

		Re	*Re*
Types of eggs	*Re*	*ReRe*	*ReRe*
	re	*Rere*	*Rere*

Rere ♀ × *rere* ♂

Types of spermatozoa

		re	*re*
Types of eggs	*Re*	*Rere*	*Rere*
	re	*rere*	*rere*

The cross *ReRe* × *Rere* results in all offspring being phenotypically *Rex*, whereas *rere* × *Rere* results in one-half *Rex* and one-half *non-Rex*. In contrast to matings between heterozygotes, no new genotypes are found in the F_1 of these crosses. We can thus summarize the types of offspring and the frequency of these types resulting from various crosses. Crosses between similar homozygotes result in offspring identical with the parents, whereas those between dissimilar homozygotes result in heterozygous offspring. Heterozygous pairs produce offspring in the ratio 3 dominant : 1 recessive, two of the dominants being heterozygous. Lastly, crosses between homozygotes and heterozygotes result in two genotypes in the offspring, these genotypes being similar to those of the parents.

Mendelian ratios can be used to determine whether animals are homozygous or heterozygous for particular genes. For example, if a homozygous *Rex* male

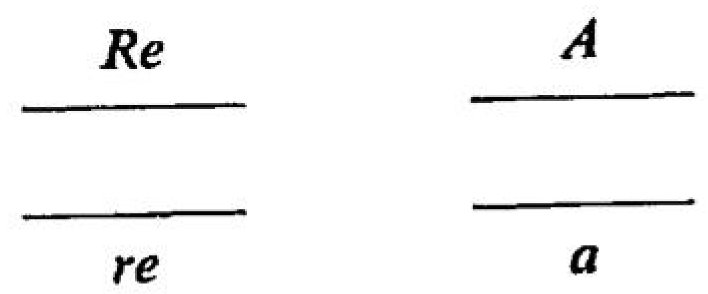
(*ReRe*) is mated to a homozygous non-*Rex* female (*rere*), all offspring will be heterozygous *Rex* (*Rere*). If the male was heterozygous, the cross would be *Rere* × *rere* and one-half of the offspring would be non-*Rex*. Likewise, if an animal was heterozygous for a recessive gene, e.g. albino, *Cc*, matings to an *albino* homozygote, *cc*, would result in one-half of the offspring being *albino*. Mendelian ratios are also used to test whether a character is controlled by a single gene. If an *albino* homozygote is crossed to a *non-albino* homozygote the cross would be *cc* × *CC* if *albino* is controlled by a single gene. All offspring will thus be heterozygotes *Cc*. The heterozygotes are now crossed back to the recessive parent, i.e. *Cc* × *cc*, and one-half of the offspring will be *albino*. This type of cross back to one of the parents is called a *backcross*, and backcrosses should always give rise to a 1 : 1 ratio if the character is determined by a single recessive gene.

(*b*) INHERITANCE OF TWO CHARACTERS

The inheritance of two characters depends on the relationship between the two genes and their position on the chromosomes. The two genes could be either on different chromosomes or at different loci on the same chromosome. When on the same chromosome, genes are known as *linked* genes. We will first study inheritance of two characters in the F_1 when the genes are on separate chromosomes.

(i) Segregation of genes carried on separate chromosomes

Consider two genes in the mouse, *Agouti* coat colour, *A*, which is dominant to *non-Agouti a*, and *Rex*, *Re*, a coat-waving gene and its recessive allele *re*. A male heterozygous at both loci will be *Aa Rere*. What kinds of spermatozoa will be produced by the male? If we ignore all the chromosomes except those carrying these two genes the chromosome constitution of the animal can be represented as follows:



After meiosis each spermatozoon will carry the haploid number of chromosomes, and hence contain one of the genes at each locus. Suppose a spermatozoon carried *Re*: it will also have an equal chance of containing *A*, and be *Re A*, or *a* and be *Re a*. Likewise a spermatozoon carrying *re* could also carry *A* or *a*, and be *re A* or *re a* respectively. Thus the double heterozygote *Rere Aa* can produce four types of spermatozoa *A Re*, *A re*, *a Re*, *a re*. Moreover, the four types of spermatozoa are produced in equal numbers. Likewise, a female of identical genotype will produce four types of eggs in equal numbers. How can we forecast the types of offspring in the F_1 from a mating of two such individuals?

The simplest way is to construct a table in the same way as for the inheritance of a single gene. As before, the four types of spermatozoa are written along the top of the table, and the four types of eggs down the side.

The expected genotypes can then be filled in by combining the different types of gametes:

Aa Rere ♀ × *Aa Rere* ♂

		Types of spermatozoa			
		A Re	*A re*	*a Re*	*a re*
Types of eggs	*A Re*	*AA ReRe*	*AA Rere*	*Aa ReRe*	*Aa Rere*
	A re	*AA Rere*	*AA rere*	*Aa Rere*	*Aa rere*
	a Re	*Aa ReRe*	*Aa Rere*	*aa ReRe*	*aa Rere*
	a re	*Aa Rere*	*Aa rere*	*aa Rere*	*aa rere*

Each type of spermatozoon and egg is produced in equal numbers, and each spermatozoon has an equal chance of fertilizing any egg. Each genotype in the above table will thus be produced with equal frequency. Some of the genotypes in the squares are similar, and can therefore be grouped together. When this is done the following genotypes and phenotypes occur:

GENOTYPES	No.	PHENOTYPES	No.
AA ReRe	1	*Agouti Rex*	9
Aa ReRe	2		
Aa Rere	4		
AA Rere	2		
AA rere	1	*Agouti non-Rex*	3
Aa rere	2		
aa ReRe	1	*non-Agouti Rex*	3
aa Rere	2		
aa rere	1	*non-Agouti non-Rex*	1

The phenotypic types resulting from a cross between two double heterozygotes thus appear in a 9 : 3 : 3 : 1 ratio. An example is shown in Table 2.

TABLE 2. 9:3:3:1 SEGREGATION IN THE MOUSE

Two animals, both double heterozygotes for the genes *a*, *non-agouti*, and c^{ch}, *chinchilla*, were crossed. The 361 offspring were classified, and the results were:

Aa Cc^{ch} ♀ × *Aa* Cc^{ch} ♂

Phenotype of offspring	*AC*	Ac^{ch}	*aC*	ac^{ch}
Expected ratio of offspring	9 :	3 :	3 :	1
Expected numbers of offspring	202·5	67·5	67·5	22·5
Numbers of offspring found	219	60	62	20

(Data from CARTER, T. C. and FALCONER, D. S., *J. Genet.*, 50, 399, 1952)

If one of the two genes was homozygous in the above cross, e.g. *ReRe*, then the cross would be *ReRe Aa* × *ReRe Aa*. The Re locus would thus be fixed, and the genes *Aa* would segregate in the 3 : 1 ratio described earlier for a single gene segregation.

The results of all of the crosses described so far, and the following deductions drawn from them, were discovered by Mendel, who worked with the garden pea. From these Mendelian ratios we can deduce that genes retain their individuality from generation to generation, and that they are not contaminated by other genes. Secondly, we know that allelic genes segregate away from each other during the formation of the gametes and freely recombine at fertilization. In the following parts of this chapter, however, we will describe circumstances which cannot be explained in Mendelian terms.

(ii) Segregation of two genes carried on the same chromosome

Genes that occur on different parts of the same chromosome are known as *linked* genes, and they segregate quite differently from those carried on different chromosomes. Suppose we have a male which is heterozygous for two genes, *Aa Bb*, both genes being carried on the same chromosome. Also, suppose that the two dominant genes came from his father and the two recessives from his mother. At meiosis this particular pair of homologous chromosomes will thus be:

A B

———

———

a b

When the chromosomes separate at meiosis, most spermatozoa will carry either *AB* or *ab*. The types *Ab* and *aB* will be formed only if there is an exchange of genetic material between homologous chromosomes during meiotic prophase. This process is called *crossing-over* and is expressed diagrammatically in Fig. 6, showing a crossing-over between the locus *A* and *B*. After a single crossing-over two of the four chromatids have a new association of genes: *A* is linked to *b* on one chromatid, and *a* is linked to *B* on another. The other two chromatids, *AB* and *ab*, are similar to the original parental chromosomes. The frequency of these new associations of genes will clearly depend on the frequency of crossing-over.

Ab and *aB* are called the *crossover classes* or *recombinants*. Suppose that a crossing-over had occurred in approximately 10 per cent of spermatozoa in a male. Since the recombinants are produced in approximately equal numbers, 5 per cent of the spermatozoa will be *Ab*, and 5 per cent *aB*. The remaining 90 per cent will be parental types, i.e. 45 per cent *AB* and 45 per cent *ab*. If this male was backcrossed to a female that was homozygous for both recessive genes, i.e. *aa bb* (in which crossing-over would not affect the ratio of the gametes, all of them being *ab*), then the ratio of the offspring would be: 45 per cent *Aa Bb*, 45 per cent *aa bb*, 5 per cent *Aa bb*, 5 per cent *aa Bb*. An example is shown in Table 3.

The frequency of crossing-over is approximately proportional to the distance between two genes. A low percentage of recombinants indicates strong linkage, i.e. the genes are close together. An example is the close linkage between *pink-eye* (*p*) and *short-ear* (*se*) in the mouse. Genes which are a great distance apart on the same chromosome will show little or no linkage; if the chromosome is a long one the genes might appear to be unlinked because two or more crossovers may occur. If two genes are present on the same chromosome, e.g. *A* and *B* in the diagram above, they are said to be *linked in coupling*, and they will tend to associate together until separated by a crossover. If *A* and *B* are on different chromosomes they are *linked in repulsion*, and will tend to segregate away from each other. The distance between linked genes is usually

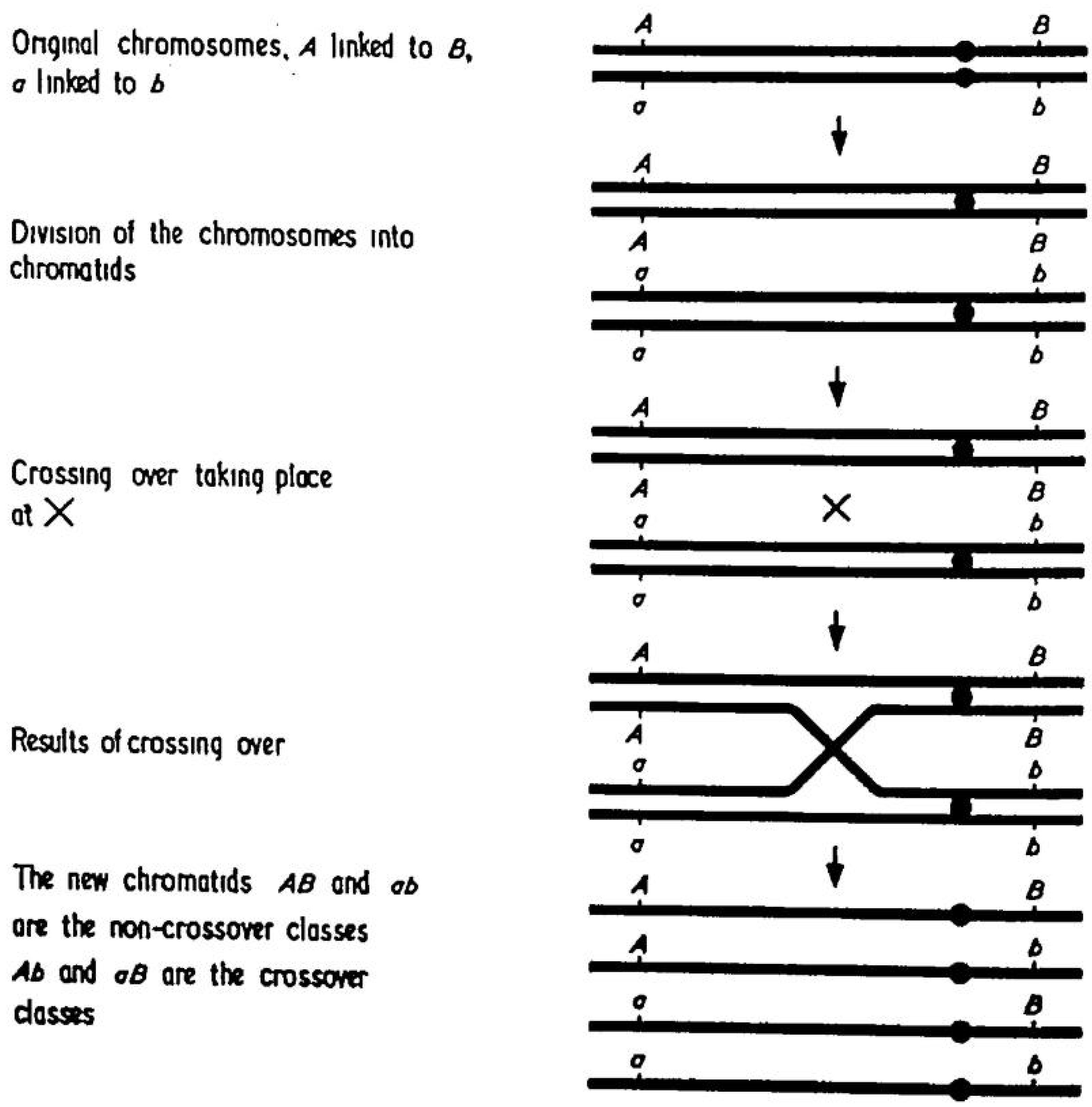


FIG. 6. Diagrammatic representation of crossing over between two loci.

measured in units of crossing-over, one unit being 1 per cent of crossing-over. Linkage analysis of hundreds of pairs of genes in the fruit fly *Drosophila melanogaster* and other species has been a major tool in demonstrating the arrangement of genes in linear order along the chromosomes.

TABLE 3. LINKAGE BETWEEN TWO GENES IN THE MOUSE

The two genes are *a*, *non-agouti*, and *we*, *wellhaarig*, which causes waving of the coat. A double homozygous female was crossed to a double heterozygous male, and the offspring were classified. The results were:

aa wewe ♀ × *Aa Wewe* ♂

Type of eggs	*a we*			
Types of spermatozoa	*A We*	*A we*	*a We*	*a we*
	Aa Wewe	*Aa wewe*	*aa Wewe*	*aa wewe*
Offspring classified	332	32	44	317

Total number of offspring = 725

Conclusion: *A* is linked to *We*, and *a* to *we*

(Data from HERTWIG, P., *ZIAV*, 80, 220, 1942)

(c) SEX CHROMOSOMES, SEX DETERMINATION, AND SEX LINKAGE

The sex of an animal is determined by a pair of chromosomes called the *sex chromosomes*, the remaining chromosomes being called the *autosomes*. There are two types of sex chromosome: the *X* and *Y*. One sex has an identical chromosome pair, *XX*, and the other has dissimilar chromosomes, *XY*. In mammals the male sex has an *XY* sex-chromosome complement and the female *XX*. All eggs produced by the female will have one *X* chromosome in addition to the autosomes, and is therefore called the *homogametic sex*. The male produces equal number of spermatozoa containing an *X* or a *Y* chromosome in addition to the autosomes, and is therefore called the *heterogametic* sex. If an egg is fertilized by a spermatozoon carrying an *X* chromosome the embryo will have two *X* chromosomes and will be a female. An egg fertilized by a *Y*-bearing spermatozoon will be *XY* and develop into a male. The expected ratio of male to female offspring will thus be 1 : 1, although this ratio may be modified by other factors, e.g. differential mortality of the two sexes during embryonic development.

Two kinds of character are associated with the inheritance of sex. The first of these, known as *sex-limited characters*, are caused by genes which can only have an effect in one sex because of a physiological difference between the two sexes, e.g. genes affecting milk yield. The second type are *sex-linked characters*. Sex-linked genes lie on the *X* chromosome. Much of the *Y* chromosome is inert genetically and has no genes, so that sex-linked genes situated on the *X* chromosome have no counterpart on the *Y* chromosome. This condition, where the male has necessarily only one gene for a character, is called *hemizygosity*. Many sex-linked genes are known, e.g. two in man are red-green colour blindness and haemophilia. Recessive sex-linked genes will appear as dominants in the male, since they are hemizygous and have no allele on the *Y*-chromosome. The frequency of sex-linked recessive conditions in a population is thus much higher in males than in females, where the recessive is hidden by the dominant allele. There is obviously less chance of a female being homozygous for a recessive gene than there is of a male being hemizygous.

Sex-linked characters exhibit a type of inheritance not displayed by genes borne on the autosomes. Suppose a male mouse with the dominant gene *Bent-tail*, *Bn*, is crossed to a female with the homozygous recessive, *bn*. Representing the cross diagrammatically, with *x* and *y* as the sex chromosomes:

$bn_x bn_x$ ♀ × $Bn_x{-}_y$ ♂ x

Types of Spermatozoa

		Bn_x	$-_y$
Types of eggs	bn_x	$Bn_x bn_x$	$bn_x{-}_y$
	bn_x	$Bn_x bn_x$	$bn_x{-}_y$

The offspring will be 2 *Bnbn* females, 2 *bn* males. The female offspring have inherited the characteristics of their father through the inheritance of his

X-chromosome, and the male offspring have inherited the recessive characteristics of their mother because there is no allele on the Y-chromosome. This phenomenon is called *criss-cross inheritance*, and is characteristic of sex-linked characters.

We have assumed that no genes are carried on the Y chromosome. This is not strictly true: in some species a portion of the Y chromosome contains genes and behaves with the corresponding part of the X chromosome in a manner similar to relationship between two autosomes. The similar portions of the X and Y chromosomes are called the *pairing segments* of the sex chromosomes. Genes on the pairing segments follow the normal rules of Mendelian inheritance, whereas those on the *non-pairing segment* of the X chromosome follow the laws of sex-linked inheritance described above. Very rarely, genes occur on the non-pairing segment of the Y-chromosome. One of the few known genes of this type is *bobbed* in *Drosophila*, and at least one is known in man. Such genes are obviously confined to males and are transmitted from father to son.

INTERACTION OF GENES: GENE MUTATION

In Mendel's original experiments the allelomorphic genes that he studied were either dominant or recessive. This relationship does not always hold, and all gradations are known between cases which show complete dominance and those where the heterozygote is strictly intermediate, e.g. the pink F_1 hybrid from a cross between red and white snapdragons. Other types of relationship occur. One of the loci governing coat colour in mice is the *Agouti* locus. The gene A, *Agouti*, which produces the coat colour typical of wild mice, is dominant on the back of the animal. An allele a^t, *tan*, is dominant white on the belly but recessive to the gene *Agouti* on the back. Heterozygotes Aa^t are thus *Agouti* on their back and white on their belly. A third allele at this locus, a, *non-Agouti*, is recessive to both A and a^t. In cattle the gene R is incompletely dominant to r. Thus RR cattle are red, rr white, and Rr roan. Incomplete dominance of this type will upset the expected 3 : 1 Mendelian ratios when two heterozygotes are crossed, and will instead give a 1 : 2 : 1 segregation of red : roan : white respectively.

Genes at different loci, i.e. non-allelic genes, also interact with each other, and such interactions can also lead to modifications of the expected segregation ratios. A well-known example of this is the gene *albino*, c, which when homozygous completely masks the action of other genes affecting skin and hair colour. The masking effect of one gene by another gene which is not an allele is called *epistasis*. But since *albino* is recessive, the coat colour in mice heterozygous for albino, Cc, will depend on the other genes controlling coat colour. Epistasis will also modify Mendelian ratios. For example, if two heterozygotes $AaCc$, *Agouti non-albino*, are crossed the segregation in the F_1 should be 9 *Agouti non-albino*, 3 *Agouti albino*, 3 *non-Agouti non-albino*, 1 *non-Agouti albino*. Since albino is epistatic, however, the ratio will actually be 9 *Agouti non-albino*; 3 *non-Agouti non-albino*, 4 *albino*.

Some characters are dependent on two or more genes acting together. In fowls the presence of the two non-allelic dominant genes for rose comb (R) and pea comb (P) results in a new type of comb called the walnut comb. Two or more non-allelic genes which interact to produce a character not formed by either of them alone are called *complementary factors*. Genes which have no known effect unless they occur with other genes are known as *modifiers*. The extent of white as opposed to coloured hair in roan cattle is due to modifying genes. It is probable that many phenotypic characters are the result of the interaction between several or many genes. The coat-colour genes in rabbits and rodents can be taken as examples. In mice three alleles at the *agouti* locus, A, a^t, and a confer agouti or non-agouti coat colour with a white belly; at the *black* locus B and b confer a black or brown coat respectively; and *albino*, c, is epistatic to both loci. A mouse of genotype *AA BB* is agouti black, *AA bb* is agouti brown and lighter in colour, *aa bb* produces a deep chocolate brown, and *aa BB* is black. In addition to these, other genes act by making the coat slightly lighter, spotted, or striped. Some of these genes, all non-allelic, are *pink eye*, *p*, *dilute*, *d*, which are both diluting genes, *pied*, *s*, a spotting gene, *Tabby*, *T*, which causes striping, and many others.

It is also probable that most if not all genes influence several characters, although their effect on one particular character may be most evident. When a gene causes changes in two or more characters the gene is called *pleiotropic*. Thus, the dwarfing gene in mouse, *dw*, also affects the development of the gonads. Most genes probably also exert effects on such fundamental characters as viability and fertility.

Genes which have marked effects on viability can clearly lead to a disturbance in the Mendelian ratios, the most extreme examples being those which cause the death of the embryo. The dominant gene A^y, which causes yellow coat colour and obesity in mice when heterozygous is lethal to the embryo when homozygous. Thus, matings between two heterozygous yellow mice produce yellow : non-yellow offspring in the ratio 2 : 1 because the homozygous yellows die before birth. Lethal genes often have some effect in the heterozygote, although not always so. In man *sickle cell anaemia*, a disease of the red blood cell, is caused by a gene which is lethal when homozygous but which has only slight effects in the heterozygote.

GENE MUTATION

Occasionally the nature of the gene becomes changed, and this process is called *gene mutation*. The mutated gene then reproduces itself in its new form, and the new and old forms are then allelic. Mutation occurs very infrequently, though some genes have a higher mutation rate than others. Most mutations are recessive and may differ from the original gene in various ways: morphological, physiological, or biochemical. Many mutants produce striking alterations in the phenotype, e.g. *white eye* in *Drosophila* and *Yellow* in mice, while others may have only small effects. Estimation of mutation rates is difficult; dominant mutations can be scored easily, but recessives can only be identified when homozygous. Recessive mutations can thus segregate for many generations in heterozygotes before being detected in a homozygote. The process of mutation is generally reversible, and *back mutation* occurs when a mutant gene reverts back to its original form. The rates of forward and back mutation are usually different.

Many if not all of the allelic genes which occur in animal populations have arisen by mutation. With many genes it is obviously impossible to know which is the normal and which is the mutant allele. Usually the dominant is referred to as the normal or *wild-type*, and the recessives as mutants. The term mutant is thus used loosely. Mutation can be induced by various experimental means, e.g. X-rays, ultra-violet light, and various chemicals, and these techniques have been used to produce new genotypes. The effect of such agents is usually non-directed, i.e. mutations of various sorts occur randomly at various loci. Ionizing radiations induce many dominant lethals; indeed, the incidence of induced dominant lethals is often used as an indication of the mutagenicity of an agent.

CHROMOSOMAL ABERRATIONS

Another type of genetic change which can profoundly influence the phenotype of an animal is a change in the chromosome number or structure. Most animals are diploid ($2n$), i.e. they contain two complete sets of chromosomes. Occasionally, one or more extra sets occur, and this phenomenon is called *polyploidy*. Polyploidy is commonest among plants, and many cultivated plants are tetraploid ($4n$) and larger than the diploid variety. Recently a boy was found to have many triploid ($3n$) cells, but polyploid mammals seldom develop to birth. Mouse, rat, and human triploids have been found in the early and middle stages of gestation, but are usually retarded or dying.

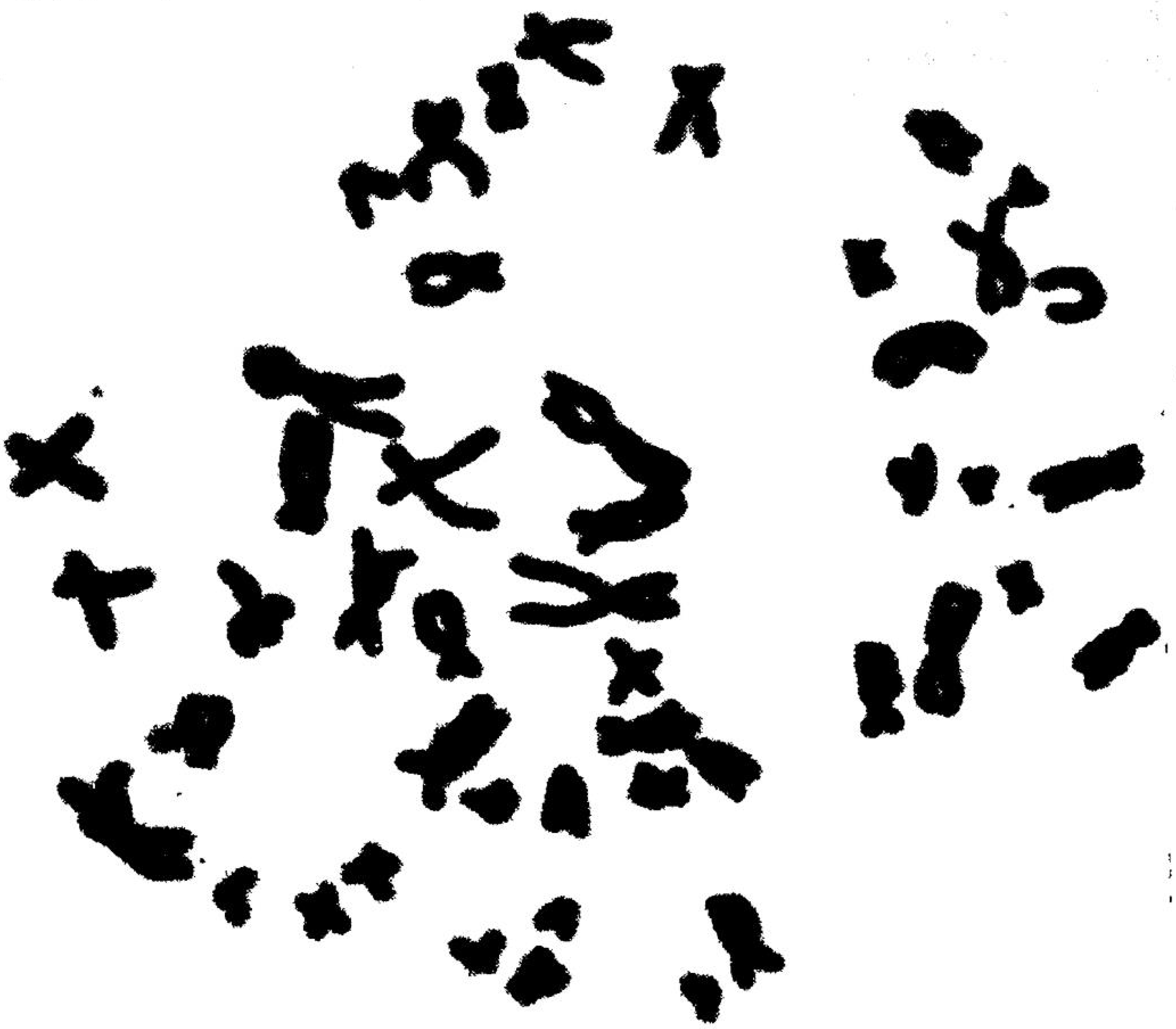
The loss of one chromosome from a set can also occur, and such an individual is called a *monosomic*, i.e. $2n - 1$. An individual with one extra chromosome is called *trisomic*, i.e. $2n + 1$ (Fig. 7). Until recently monosomic or trisomic mammals were unknown. Now it is clear that various intersex states in humans are the result of an anomalous sex chromosome constitution through the loss or gain of a sex chromosome. Moreover, trisomics other than those involving the sex chromosomes have been found in man, and the extra chromosome may play a significant role in idiocy or other mental defects (Fig. 7).

Changes may also occur within a chromosome. Part of a chromosome can be lost, and this is called a *deficiency* or a *deletion*. A classical example is *Notch* in *Drosophila*, which causes a notch in the wing margin. It is inherited as a sex-linked dominant in the female, but is lethal in the male. Deficiencies of various sorts have recently been found in humans, and also in certain cancerous cells. X-rays or other irradiations can induce deficiencies. Conversely, a chromosome may have an extra small piece in it, and this is called a *duplication*. A piece of a chromosome may break from its normal place and become attached to an unrelated chromosome. This type of rearrangement is known as a *translocation*. Like deficiencies and duplications, translocations can be inherited, and the extra piece can function like an extra chromosome.

QUANTITATIVE INHERITANCE AND THE SCIENCE OF ANIMAL BREEDING

Mendelian genetics was clearly sufficient to explain clear-cut qualitative differences such as those we have already described, but was more difficult to reconcile with the *quantitative* differences between animals in which all gradations occur between two extremes, e.g. height, weight, intelligence,

(*a*)



(*b*)

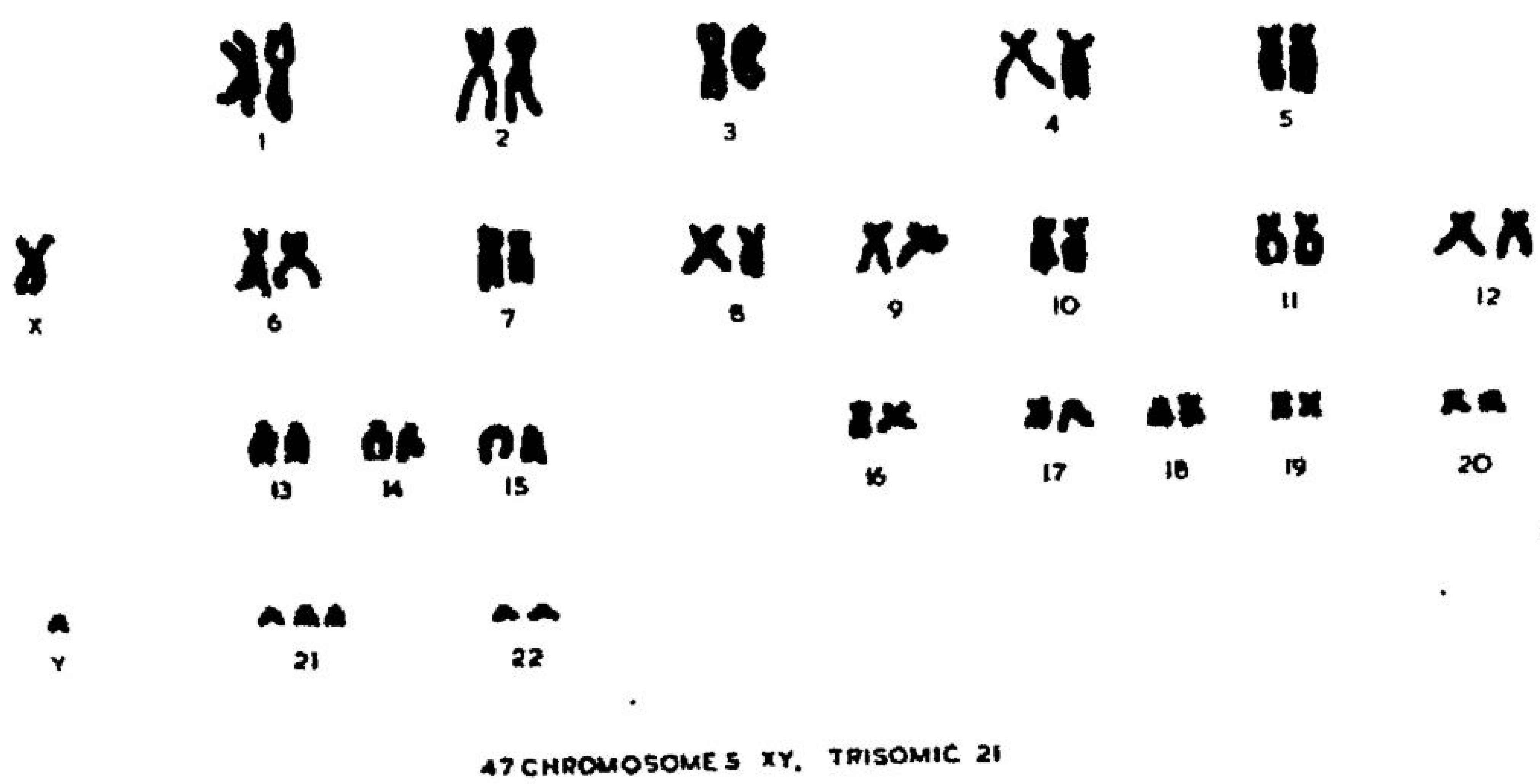


FIG. 7. (*a*) Metaphase chromosomes obtained from a cell obtained from a Mongoloid idiot.

(*b*) When the chromosomes are arranged in homologous pairs, an extra chromosome, no. 21, is found. This person thus possessed 47 chromosomes instead of the diploid 46. Published by the courtesy of Dr C. E. Ford.

fertility, etc. But it was shown that the inheritance of quantitative characters could be accounted for in Mendelian terms because the continuous variation of these characters was due to the joint action of many genes, each having a small effect but acting cumulatively. One of the first examples to be interpreted in this way was the colour of grain in wheat which appeared to be influenced by three genes, each acting additively. In a cross between the race with three dominants (*AA BB CC*) and that with the three recessive (*aa bb cc*) the F_1 were all intermediate (*Aa Bb Cc*). The parental forms appeared again in the F_2, together with five intermediates between these types. The F_2 was much more variable than the F_1, and the three genes were clearly segregating to give a wide variety of phenotypes in the F_2. When characters are controlled by many genes the classification of the different genotypes becomes extremely difficult or impossible, for the variation within a population is almost continuous. In such complex situations alternative methods of approach are needed, as it may be impossible to identify more than a few of the genes involved. Various methods have been evolved for the study of such *quantitative* characters.

A type of breeding now used extensively is *inbreeding*. This involves crossing two closely related animals, e.g. father × daughter, brother × sister, etc. Father–daughter or brother–sister crosses are perhaps the most used in inbreeding. After several generations of inbreeding the inbred offspring closely resemble each other and certain characters are fixed in the population. For example, a skin graft from one animal to another will only be accepted by the recipient if the donor is of similar genotype, i.e. an identical twin or a member of the same inbred line. Why do inbred animals of the same line resemble each other so closely? Consider one locus, e.g. *A*, in a mating system of brother × sister. If the brother and sister are both *AA* all the offspring will be *AA*, and this locus will be *fixed*, i.e. all members of the inbred line will be *AA*, as long as brother–sister mating continues. Mutation of *A* to *a* will interfere with this system, but gene mutation occurs very rarely, and we can neglect it here. Alternatively, the brother and sister might have been homozygous *aa*. Then *a* would become fixed in the inbred line. If both brother and sister were heterozygous *Aa* the offspring would occur in the ratio 1*AA*: 2*Aa* : 1*aa*. With continued brother × sister mating we will ultimately select either *AA* or *aa* animals for crossing, and one of the two genes will be fixed. Thus, inbreeding increases homozygosity in a population. With continued inbreeding, homozygosity occurs at more loci, and the more predictable is the genotype of the offspring. After many generations of inbreeding each inbred line possesses its own characteristics, i.e. those that have been fixed.

But if there are any harmful genes in a stock which become homozygous by inbreeding, then there will clearly be a deterioration of the stock, and this is known as *inbreeding depression*. Such harmful genes are sufficiently frequent to make continuous inbreeding difficult: many inbred lines become homozygous for deleterious or lethal genes and may become undersized, infertile, or die out completely. Many inbred lines have been successfully established, however, presumably free of such deleterious genes. In some cases it may well be that a residue of heterozygosity persists in an inbred line, and that this small amount of heterozygosity is necessary for survival.

If two inbred lines are crossed the resulting hybrid is superior in vigour to either of the two parental lines. This type of cross is known as *outcrossing*, and the superior quality of the offspring is known as *heterosis* or *hybrid vigour*. Heterosis is probably due to the increased heterozygosity of the hybrid as compared with the homozygosity of either parental line. The two parental inbred lines are unlikely to be homozygous for the same harmful recessive genes, and the presence of such genes in the hybrid will thus be masked by the normal alleles from the other line. For example, if one inbred line has a recessive gene *a* and the other has *b*, both genes causing poor growth, then the cross between the two lines will be $aa\ BB \times AA\ bb$, and all F_1 offspring will be $Aa\ Bb$. If the F_1 offspring are crossed the F_2 and successive generations show a decline in heterosis. This would be expected if heterozygosity was the cause of heterosis, since breeding the F_1 would result in segregation at the heterozygous loci restoring homozygosity in the F_2 and later offspring. Heterosis has been applied especially to maize breeding, where the crossing of two inbred lines produces an F_1 which is extremely sturdy and productive. Recent evidence has also indicated that hybrids are superior to their inbred parents for various types of assay.

Identical twins are of considerable value in studying the expression of quantitative characters. The two members of a pair are genetically identical, since they arise from the same fertilized egg. Identical twins are thus invaluable in studies where animals of the same genotype are required, and can be used experimentally in the same way as inbred animals. Unlike inbred animals, however, twins can possess many heterozygous loci. Human twins who were separated early in life are yielding valuable information on the expression of genetic traits in different environments. Because they are of identical genotype, differences between separated twins in intelligence, aptitude, etc., yield much information on the effects of environment on these characters.

ANIMAL BREEDING

The foremost genetical approach to animal breeding is by *selection* of the breeding colony for the desired characters. Genetics is of use in animal breeding only so far as these characters are heritable, and the aim of the geneticist is to increase the frequency of the desired genes in a population. In turn, increasing the frequency of these genes depends on the efficiency with which individuals carrying a favourable genotype can be selected. The well-known breeds of farm animals have resulted partly from the selection of breeding types over many generations and partly from the improved control of environmental conditions, e.g. feeding, hygiene, etc.

One of the most used methods at the present time is the grading up of livestock by the selection of one parent. The male is clearly the best parent to select, since he can be mated to many females, especially by techniques of artificial insemination. Many beef herds, especially in the United States, have been graded up by the use of selected sires. When breeding animals for beef the phenotype can be used as an indication of the genotype of a bull. But selection of the male on his phenotype is obviously of little use in upgrading dairy cattle, and a good estimate of his genotype is obviously required. Examination of the pedigree is helpful, but at best such information can be only an approximate guide. Even with beef breeds the phenotype often gives little indication of the genotype due to effects of dominance, epistasis, and other phenomena. The best method of determining an animal's genotype is to test it by mating the male to large numbers of females in widely differing environments, and then measuring the performance of his daughters. This technique is known as *progeny testing*. By use of statistical techniques the character being studied, e.g. milk yield, can then be analysed in terms of sire, dam, and environment, and the most suitable sires can be selected. Progeny testing is being widely used in agriculture at present.

Other techniques of animal improvement are being widely practised, and are mentioned briefly. Some breeds have been upgraded by crossing to other breeds. The import of a few sires into a country is used to improve the native breeds, the crossing being usually accompanied by a rigorous selection programme. For some characters, selection through both parents is practised, e.g. increasing the egg production of hens. Wide crosses, e.g. horse × donkey, are invaluable in certain areas of the world.

Another use of genetics in animal breeding is the elimination of undesirable genes from populations. Selection against an undesirable dominant is simple, and these genes can be removed in one generation. Recessive genes are much more difficult to eradicate, since heterozygotes cannot be detected without test-mating and phenotypic selection will remove only the homozygotes.

CHAPTER TWENTY-ONE

Mammalian Reproduction

Production of germ cells

The ability to produce new living individuals is a basic characteristic of all animals that increase their numbers by sexual reproduction, in which new individuals develop from germ cells produced by the parents.

Male germ cells (sperms or spermatozoa) are produced in series of compartments or tubules of the testes; they travel through small ducts (vasa efferentia) to a larger duct (vas deferens) which leads directly to the exterior of the body through a copulatory organ (penis). In some animals the lower end of the vas deferens is enlarged for sperm storage (ampulla). The prostate, seminal vesicles, and bulbo-urethral glands provide secretions to suspend and activate the sperms.

Female germ cells (eggs or ova) are produced as individual cells in the ovary. Each ovum develops within a Graafian follicle which enlarges as the egg matures and finally ruptures to release it (ovulation). The release of ova occurs periodically, and generally at a definite time in relation to the internal physiological rhythm known as the oestrous cycle. After release the ova travel down the oviduct or Fallopian tube into a larger organ, the uterus where the young animal develops; the terminal part of the female tract is the vagina.

Embryonic development

The spermatozoon unites with the egg (ovum) to form the zygote, which becomes divided up into a vast number of smaller cells during the ensuing embryonic development. The fertilized egg passes into the uterus, embeds itself in the uterine wall, and starts growing, becoming first the embryo and later the foetus; it is nourished through a complex organ, the placenta, which is partly derived from the uterus and partly from the embryo. The placenta serves the embryo with a supply of oxygen and food materials which are suspended in the maternal blood, and it disposes of the carbon dioxide and other waste products deriving from the embryo.

Oestrous cycles

Certain species of mammal have clearly defined periods of heat or oestrus of short duration which recur throughout the year at regular intervals; that is to say in a cyclic manner. The laboratory rat, whose oestrous cycle has been most carefully studied, is one such species, and it is described as being polyoestrous (having many cycles).

Certain other mammals, whether they become pregnant or not, have only one period of heat per year, i.e. the British fox. The vixen (female) comes into oestrus and ovulates spontaneously in January, and whether mated or not she remains in anoestrus (out of heat) until the following year. Such animals are known as monoestrous (having one oestrus per breeding season).

Table 1 is a detailed study of the oestrous cycle in the rat as published by Long and Evans, 1922. The separation of these five stages may be defined by the examination of the vaginal content. To carry out this examination it is necessary to take a smear from the vagina, transfer it to a slide, and examine it under a microscope. There are several methods of taking vaginal smears: (1) a drop of saline is pipetted into and out of the vagina, and then transferred to a slide; (2) a small sterile cotton-wool plug is moistened with saline, inserted into the vagina with forceps, and then wiped on a slide; (3) a nickel–chromium wire loop, previously heated in a flame and cooled in saline, is used to remove some cells from the vaginal wall by gentle scraping.

Ovulation

The shedding of mature eggs or ova from the ovary is known as ovulation, and may either be spontaneous or induced. Spontaneous ovulation occurs when the Graafian follicle ruptures independently of mating; it takes place at each oestrus, and so the time between ovulations is related to the length of the oestrous cycle. Induced ovulation takes place only after the stimulus of mating, the act of copulation causing the ovaries to release mature eggs. In some rodents evidence of mating may be detected by the presence of a cornified plug in the vagina. In others a vaginal smear will reveal the presence of spermatozoa. Mature eggs may remain in the ovaries for anything up to ten days and then degenerate as the next crop matures.

In many animals seasonal changes in the length of daylight determine the time of onset and the duration of the breeding season. Ovulation generally occurs at night-time. The effect of light upon the activity of the ovary is mediated through the pituitary gland. Male animals, too, often show a distinct breeding season related to the season of the year.

Gestation

Gestation or pregnancy is the term applied to the period occupied by the development and growth of the young animal within the uterus of the mother, i.e. from the fertilization of the ovum to the birth of the young animal.

During this period enlargement of the uterus takes place and there is considerable development of the mammary glands.

After fertilization and implantation of the ovum the unborn animal (foetus) lies within a sac formed by the amnion and chorion; these sacs contain a considerable body of fluid which protects the foetus from shock and injury.

Towards the end of pregnancy the unborn animal in the uterus usually takes up a position with its head towards the mother's posterior. The birth of the young animal is brought about by strong contractions of the uterine muscle assisted by voluntary contractions of the abdominal muscles. Later the membraneous sac containing the foetus enters the vagina, after which the sac ruptures and part of the amniotic fluid escapes.

For some time the newly born animal remains attached to the uterus by the

TABLE 1

INDICATIONS IN THE OESTROUS CYCLE OF THE RAT

Stage	Superficial genitalia	Vaginal smear	Uterus	Ovary	Length	Remarks
I	Lips slightly swollen Vagina dry	Epithelial cells only	Increased distention fluid	Ovarian growth Follicle enlargement	*c.* 12 hours	Heat may occur
II	Lips swollen Vagina dry	Cornified cells only	Maximum distension Early regression	Large follicles Maturation of egg	*c.* 12 hours	Heat Copulated
III	Lips still swollen regressing Cheesy mass in vagina	Cornified cells only	Epithelium degenerating	Ovulation	*c.* 15–18 hours	Not in heat
IV	No swelling Mucosa moist	Cornified cells and leukocytes	Begins regeneration	Eggs in oviduct	*c.* 6 hours	
V	No swelling Mucosa moist	Leukocytes and epithelial cells	Epithelium regenerated	Copora lutea formed	*c.* 57–60 hours	Oestrus

Copulation may occur in late stage I, in stage II, and occasionally in early stage III. Ovulation may occur in late stage II and later. Copulation time usually precedes the ovulation, the limits so far found being 6–30 hours. The copulation plug forms 3–8 hours after copulation, and is not by itself an adequate criterion of timing.

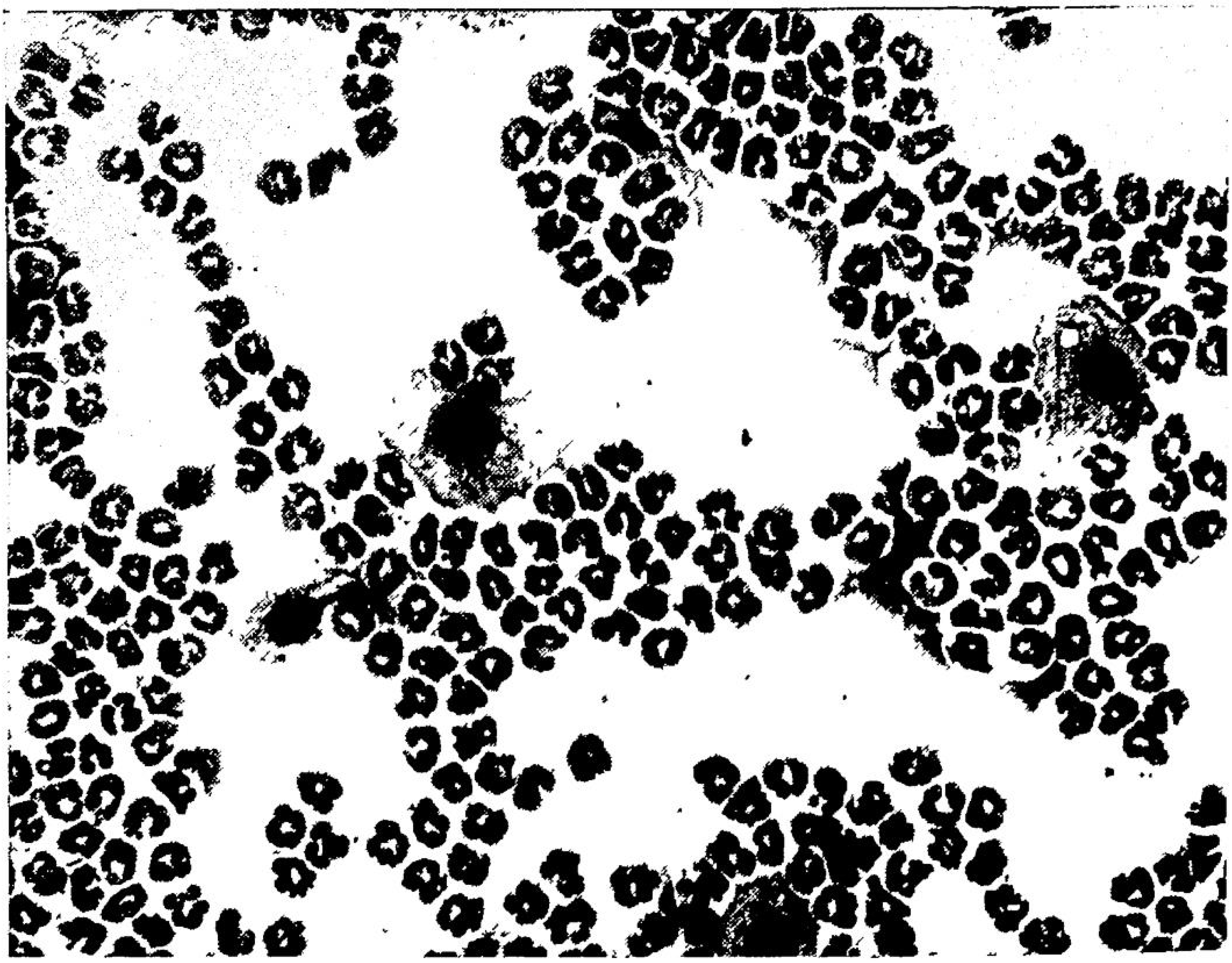


FIG. 1

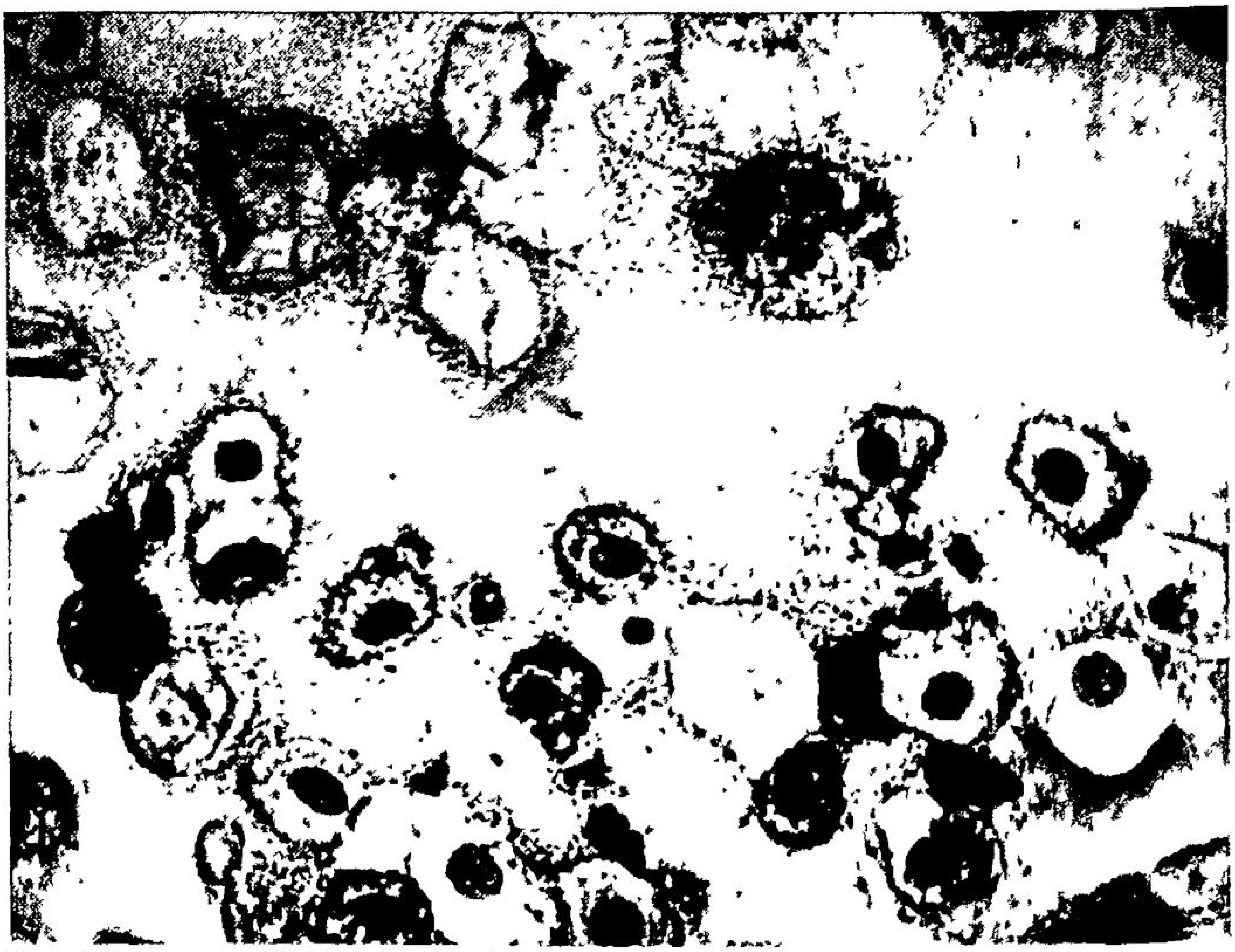


FIG. 2 (see page 290 for captions)

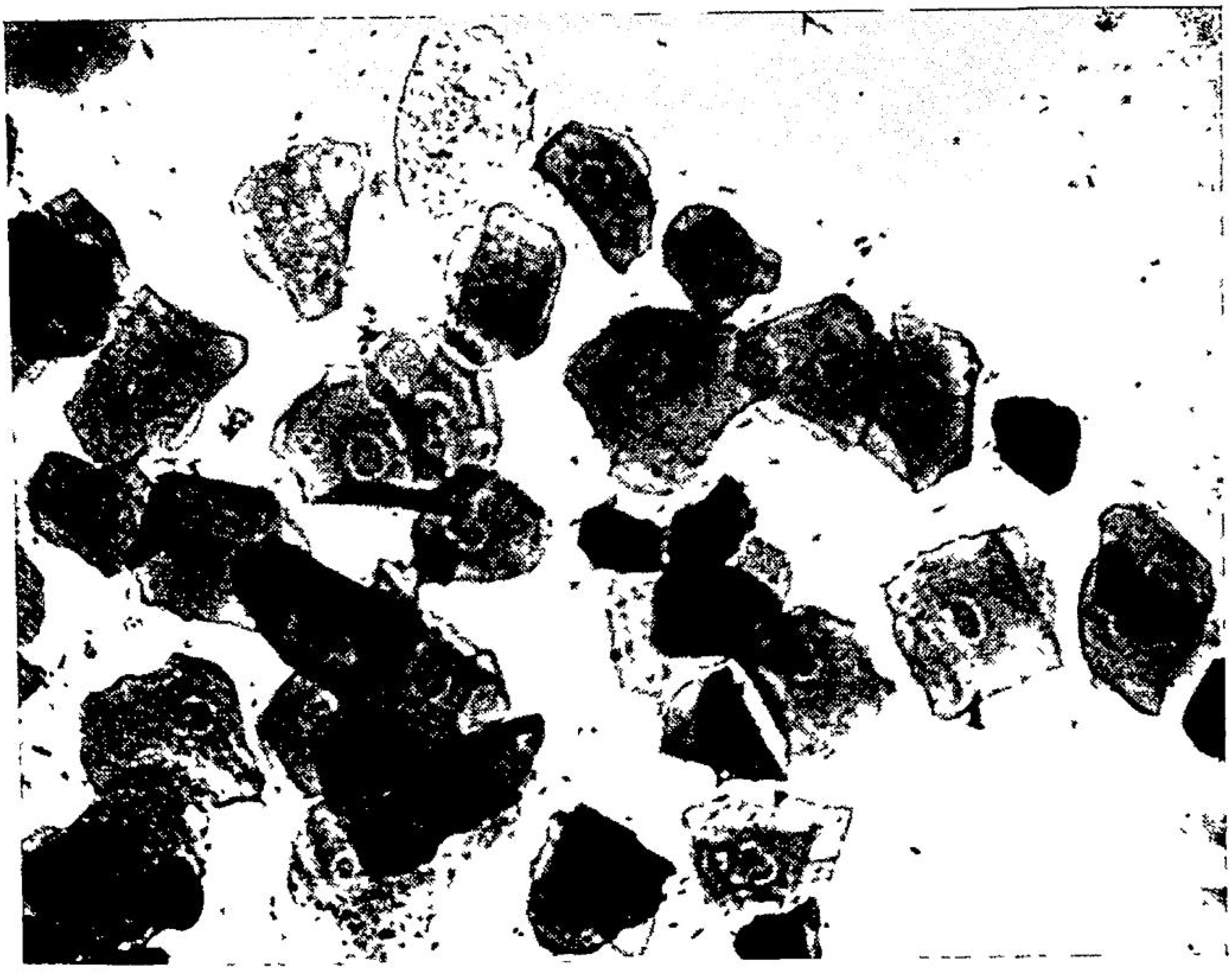


FIG. 3

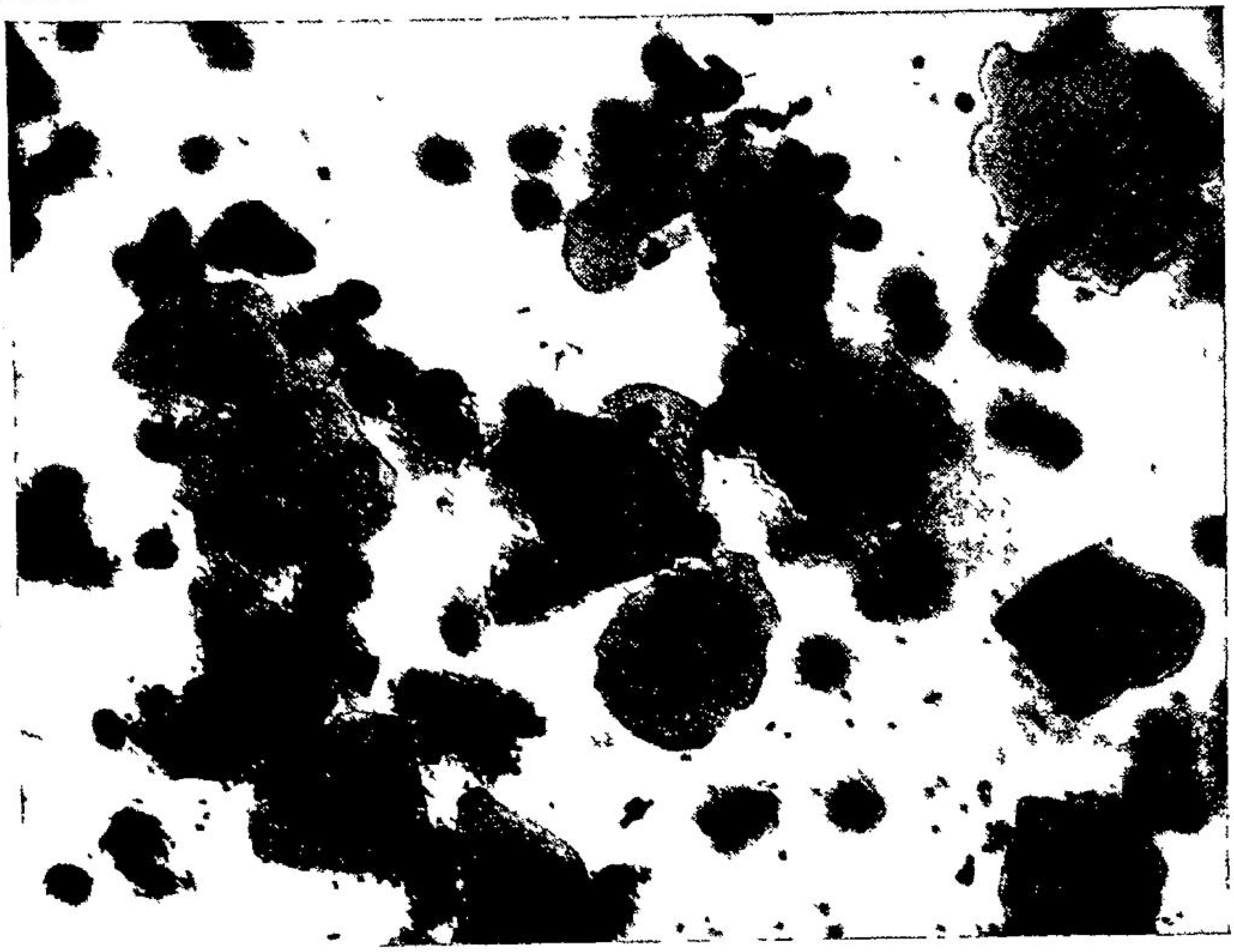


FIG. 4 (see page 290 for captions)

umbilical cord and the placenta. This cord is normally severed by the mother, and she may eat the placenta and the membraneous sac while cleaning the young animal. Parturition is the term applied to the emptying of the uterus and the termination of pregnancy.

The gestation period varies considerably with different species, and the young are born at varying degrees of development, i.e. guinea pigs are born fully furred, with eyes open and quite mobile, rats are born naked and blind.

In the rat a slight discharge of blood may be observed 10–12 days before parturition, see Table 3, 'Synopsis of Reproduction in Laboratory Animals' and Table 2, 'Synopsis of Reproduction in Less Common Species'.

Hormonal regulation

The growth of the accessory sex organs and the development of the sex characteristics depends on hormones secreted by the gonads, i.e. androgen by the testes, oestrogen and progesterone from the ovaries. Progesterone is derived from the corpus luteum, which develops out of the ovarian follicle after the ripe ovum is shed. Progesterone prepares the uterus for the reception of the fertilized egg and maintains pregnancy. The placenta also secretes progesterone, the loss of the source of progesterone at parturition allows a post-partum oestrus to occur in some species. The development of the mammary glands and lactation is also dependent on ovarian hormones. Finally the secretion of all testicular and ovarian hormones, as well as the production of sperms and ova, depends on the action of gonadotrophins (hormones secreted by the pituitary gland).

THE OESTRUS CYCLE OF THE MOUSE

In textbooks 'pro-oestrus' leucocytes and cornified cells should not be shown together, but in practice they are often seen at the same time; in fact all three types of cell may be seen in the same picture.

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1. LEUCOCYTES: Present at all stages of the cycle except when the female is on heat
2. At the approach of oestrus the leucocytes disappear and the smear consists mainly of EPITHELIAL CELLS with marked nuclei
3. OESTRUS: The leucocytes have disappeared. The smear consists of CORNIFIED CELLS ONLY. The female is on heat
4. POST-OESTRUS: The return of the leucocytes among the cornified cells. The female is no longer on heat

TABLE 2

SYNOPSIS OF REPRODUCTION IN LESS COMMON SPECIES

Animal	Breeding season	No. of oestrus periods	Mechanism of ovulation	Intervals	Length of gestation	Average litter size	Recurrence of oestrus after parturition	Age to wean young
Hedgehog	April–May July–August	Repeated	Spontaneous	—	35–40 days	5	Next breeding season	40 days
Wood rat	No definite season	Repeated	Spontaneous	7 days	32–33 days	2	End of lactation	21 days
Bank vole	No definite season	Repeated	Spontaneous	5 days	20 days	4	Post-partum	21 days
Field vole	No definite season	Repeated	Spontaneous	5 days	20–21 days	3–4	Post-partum	14 days
Orkney vole	No definite season	Repeated	Spontaneous	5 days	20–21 days	3–4	Post-partum	14 days
Swamp rice rat	No definite season	Repeated	Spontaneous	7 days	27 days	4–8	Post-partum End of lactation	19 days
Chinese hamster	No definite season	Repeated	Spontaneous	4 days	20 days	3–7	Post-partum *	24 days
Root vole	No definite season	Repeated	Spontaneous	5 days	20–21 days	4	Post-partum	19 days

* Less regularly than in mice. Only the gentlest of females will permit mating at the post-partum oestrus. (Personal communication, G. Yerganian, Children's Cancer Research Foundation Inc., Boston, Mass.)

TABLE

SYNOPSIS OF REPRODUCTION

By Dr A. S. Parkes, F.R.S., and

Revised

Animal	Breeding season	No. of oestrus periods in a breeding season	Duration of oestrus	Mechanism of ovulation
Monkey (Macaque)	Probably no definite season	Repeated	—	Spontaneous
Cat	No definite season	Repeated	7–10 days	Sometimes spontaneous but generally after mating
Dog	Two seasons a year	One	7–13 days	Spontaneous
Ferret	March to July	—	Very prolonged in absence of male	Only after mating
Rabbit	No definite season	—	Very prolonged in absence of male	Only after mating
Guinea pig	,, ,,	Repeated	6–12 hours	Spontaneous
Rat	,, ,,	,,	10–20 hours	,,
Mouse	,, ,,	,,	10–20 hours	,,
Cotton rat	,, ,,	,,	2–3 days	,,
Golden Hamster	February to October	,,	12 hours	,,
Horse	February to August	Repeated	3–15 days	Spontaneous
Cow	No definite season	,,	12–30 hours (in summer) 6–8 hours (in winter)	,,
Sheep	August to March (varies with locality)	,,	30–36 hours	,,
Goat	September to February	,,	2–3 days	,,
Pig	No definite season	,,	3–4 days	,,

Notes

Breeding season. In some animals there is a definite restricted season out of which breeding never occurs. Slight seasonal fluctuation in reproductive activity, as seen in the rat and mouse, does not amount to a true restricted breeding season.

Oestrus and ovulation. The period at which an animal will mate, and at which ovulation

3

IN LABORATORY ANIMALS

Professor E. C. Amoroso, F.R.S.

1961

Interval between ovulations in unmated animal during breeding season	Length of pseudo-pregnancy	Length of pregnancy	Usual size of litter	Recurrence of oestrus after young born
28 days	No special pseudo-pregnancy	24 weeks	1	2–3 months
15–21 days	No special pseudo-pregnancy	64–66 days	3–6	4th week of lactation
—	60 days	60 days	3–6	Next breeding season
—	42 days	42 days	6–10	End of lactation or next breeding season
—	14–16 days	30 days	5–10	3rd week of lactation
14–16 days	No special pseudo-pregnancy	62 days	3–5	Post-partum oestrus, then regular cycle
5 days	12 days (after sterile mating)	22 days	6–10	Post-partum oestrus, then not till end of normal lactation
5 days	,, ,,	19 days	6–10	,, ,,
7 days	?	27 days	4–8	,, ,,
4 days	9–10 days (after sterile mating)	16 days	7–9	End of lactation
20–25 days	No special pseudo-pregnancy	11 months	1	Early in lactation
3 weeks	,, ,,	9 months	1	,, ,,
16–17 days	,, ,,	5 months	1–2	Next breeding season
2–3 weeks	,, ,,	5 months	1–2	,, ,,
3 weeks	,, ,,	4 months	8–15	End of lactation

the shedding of eggs from the ovary into the top of the female reproductive tract, normally takes place.

Pseudo-pregnancy. **If pregnancy does not occur after oestrus some animals have a period of pseudo-pregnancy, during which changes similar to those seen in pregnancy occur in the uterus and mammary gland.**

CHAPTER TWENTY-TWO

Breeding of Common Laboratory Animals

INTRODUCTION

Any system for breeding laboratory animals must take into account the habits and nature of the species concerned; for example, does oestrus occur seasonally or is it a feature of a regular cycle; can males and females be confined in one cage or pen without fighting; is it safe for an adult male to be left in the cage together with its unweaned offspring?

Among laboratory animals, the female will permit mating only when she is in oestrus (or 'on heat', or 'in season'). In some species the male as well as the female exhibits a breeding season, e.g. ferrets; in other species the male remains fertile and capable of mating throughout the year, though the female comes into oestrus and permits mating only for a short period once or twice a year, e.g. dogs; in other species the male remains fertile throughout the year and the female exhibits a continuously recurring oestrus cycle, e.g. rats.

Some animals are content to live and breed peaceably together in a pen or cage, e.g. mice and guinea pigs, while others must be housed separately once they are adult and have not been reared together, or they will fight among themselves, e.g. adult male mice and Chinese hamsters.

When a female of a species having a continuously recurring breeding cycle becomes pregnant the oestrus cycle is suppressed and does not recur until towards the end of lactation, except in those species in which there is a *post-partum oestrus*, which occurs within a few hours of parturition. Females having a post-partum oestrus will permit mating at this time if the male is present. When such matings occur, the females are pregnant while still suckling a litter. In guinea pigs it is desirable that they are permitted to mate at the post-partum oestrus to avoid the long delay which intervenes before the restoration of the regular breeding cycle at the end of lactation. Mice also mate at the post-partum oestrus, and when this has occurred care must be taken to wean the previous litter at nineteen days of age so that the female may be free to care for the new litter.

THE ESTABLISHMENT OF A COLONY

In the beginning, clean, vigorous stock animals must be obtained, and it is customary to import such stock from existing, flourishing colonies. If good-quality stock animals are not obtainable, then such stock as can be had must be improved. Stock may be improved slowly by the same methods as must be applied to the maintenance of a colony—namely the careful selection of breeding stock and the rigorous application of hygienic measures. The careful selection of breeding stock is an inescapable duty in any colony at any time, and more will be said on this point later.

Germ-free colonies

The obvious way to obtain 'clean' animals is to take the young by Caesarian section (the uterine contents are normally sterile) under aseptic conditions and to rear them under aseptic conditions. The original animals have to be hand-reared, but once they have attained maturity they can be mated to produce new stock, and to foster young, taken by Caesarian section, of the same or other compatible species. Such animals have to be kept in 'isolaters' and can never be handled in the normal manner. It follows that these colonies are both difficult and expensive to run. They are therefore kept only for special purposes and to supply a nucleus of animals for Specific Pathogen Free colonies.

Specific Pathogen Free colonies (S.P.F. colonies)

These colonies are not completely germ-free, but are free from certain known pathogens and are maintained in very clean conditions. The colonies are housed in buildings from which germs are excluded as nearly as is possible. The buildings are sealed and ventilated with sterilized air; food, bedding, and equipment are sterilized as they enter the buildings; personnel must change, take a shower-bath, and redress in sterilized, protective clothing, including head covering and mask. The buildings must constitute self-contained-units having their own stores, cleaning plant, laundry, offices, and staff rest rooms. These arrangements will not keep ALL the bacteria and viruses from entering the buildings, but the number of organisms entering will be reduced to a minimum, and, thus, the chance of introducing pathogenic organisms is very small indeed. Colonies maintained in this manner may only be said to be 'super clean'; they are not sterile in the bacteriological sense. While colonies have to be maintained by human beings who have to enter the clean buildings, the chance of introducing infective organisms remains. The continuance of the clean condition depends entirely on the conscientious performance of their duties by the personnel. A breakdown in the hygienic precautions means that the colony, or a section of it, must be restarted. The nucleus stock for such a colony should be obtained from a germ-free or a specific pathogen-free (S.P.F.) stock. Otherwise, in the first instance, animals must be taken by Caesarian section and hand reared.

'BACTERIOLOGICAL SCREENING'

If new stock cannot be obtained from existing S.P.F. or germ-free colonies and if it is not practicable to set up such a colony, then the existing stock must be examined carefully for the presence of pathogenic organisms. Such a procedure is known as bacteriological screening. It is a highly skilled job, and can only be undertaken by persons trained in the necessary techniques. Faecal and throat swabs and blood samples are taken from the animals, and the organisms from the specimens are cultured and typed. The investigation reveals the

Breeding stock is never taken from the first litter from any female. This litter is often smaller, both in number and size, than the second litter, which is usually the best litter from any female. Further, delaying the task of selecting the parents of the next generation until a female throws a second litter provides an opportunity to consider the progress which has been made by the members of the first litter.

The selection of breeding stock begins in a negative manner by the rejection of obviously unsuitable litters or of apparently suitable litters thrown by unsuitable parents. The grounds for eliminating litters are:

(1) Visible physical abnormalities or deformities.

Note should be made of any litter exhibiting any deformity, and a careful watch kept among related litters for the appearance of the defect.

(2) Litters which are—

(i) small in number and/or size;
(ii) comprised of *one sex only*;
(iii) 'uneven', that is, comprised of animals of widely different sizes and body weights.

(3) Litters, either of whose parents have developed some abnormality, e.g. a tumour; or, when brothers or sisters, in a previous litter, have developed some abnormality.

The grounds for eliminating a female are:

(i) showing any sign of viciousness, such as unwillingness to permit her litter to be handled by a technician with whom the animal was familiar;
(ii) producing litters showing any of the signs listed above;
(iii) the maiming of any of the litter by biting; any neglect of the litter, such as a habitual failure to return straying young to the nest or failure to keep the young clean.

In fairness to the animals it must be remembered that females who neglect their litters usually do so because they are unable to suckle the young, and failure in lactation may arise from inadequate nutrition of the mother.

The process of elimination outlined above cannot be done without reference to records which have been kept in some detail.

The second step is to choose new breeding stock from the remaining litters, and here the experience of hand and eye is needed. The animals should appear clean, no 'gummy' eyes, running noses, dirty paws or tails, and no sign of diarrhoea. The fur should be thick, sleek, and glossy, not 'staring'. The animals should appear alert, active, and inquisitive; and, when handled, should feel to be firmly fleshed, not thin and fragile.

The number of animals set aside as new stock should be in excess of the number thought to be needed. This allows for losses from accidents and from animals which prove to be infertile. Furthermore, if there is an unexpected increase in the demand for animals there will be sufficient stock available to help meet that demand. Excess stock can be killed later, but it is a wise precaution to carry as large a stock as possible through to rearing a first litter.

Where several lines, or sub-strains, are carried in one colony care must be taken to allow space and cages for new stock from each line. If this is not done a line may inadvertently be squeezed out of existence merely because there is no accommodation for it. On the other hand, there is little point, in an ordinary breeding programme, wasting time, space, and trouble encouraging a weak line to thrive when its place could be better filled by the expansion of a more vigorous line.

RECORDING

Breeding-stock animals must be marked individually in such a way that there can be no doubt as to any animal's identity, and there must be no duplication of numbers within any one generation of any one strain in any one room. Though it is desirable that no identity number should ever be duplicated among living animals, this is possible only in slow-growing animals big enough to be tattooed with long numbers. In a large colony of small, fast-growing animals, such as mice, this is next to impossible.

The information recorded is determined by the breeding system in use and the requirements of the establishment, but some recorded information is essential for the conduct of a successful breeding programme. Each animal must be so recorded that its ancestry may be traced back to the origin of the colony.

The minimum of information needed about any breeding female is:

(i) its identity, parentage, and date of birth;
(ii) date of mating and identity of male used at each mating;
(iii) dates of birth of litters, and the number and sex of the offspring;
(iv) the fate of the litters (e.g. to breeding stock or issued for experiments);
(v) the cause of death (post-mortem record cards should be attached to the animal's stock card).

And about any breeding male is:

(i) its identity, parentage, and date of birth;
(ii) the dates on which it is mated and the identity of the females;
(iii) the cause of death.

This amount of information is adequate to record the breeding performance and to trace the ancestry of any animal.

Records are often kept in books, but it is becoming increasingly popular to keep a separate record card for each animal. Cards may be sorted and compared (and lost!) more easily than the pages of a book. The information which is needed most frequently—the animal and its cage number—should be written boldly in the top right-hand corner of the card. Each litter is best recorded on a separate card, which is later filed behind the mother's stock card, on which is entered only a précis of the information about each litter. This is desirable for two reasons: (i) litter cards must be kept in the animal room near the relevant cages, and thus are liable to become damaged and soiled, and (ii) they carry

LABORATORY ANIMALS BREEDING DATA FOR FEMALES

SPECIES	Average age generally paired	Average weight at pairing	Mean duration of pregnancy	Average number born	Age of litter at weaning	Average weight of young at weaning	Period between parturition and next possible mating
SEXES WHICH USUALLY LIVE TOGETHER							
MOUSE (*MUS musculus* L.)	6 weeks	18–20 gm	19–21 days	8–11	21 days	10–12 gm	Post-partum oestrus
RAT (*RATTUS norwegicus*)	70–80 days	150 gm	20–22 days	9–11	22 days	35–40 gm	Post-partum oestrus
COTTON RAT (*SIGMODON hispidus*)	6 weeks	80–100 gm	27 days	4–8	22 days	35–40 gm	Post-partum oestrus
GUINEA PIG (*CAVIA porcellus* L.)	12 weeks	500–550 gm	65–72 days	3–4	14 days	180–200 gm	Post-partum oestrus

FEMALES TO MALES AT OESTRUS ONLY							
GOLDEN HAMSTER (*MESOCRICETUS auratus*)	6 weeks	100 gm	16 days	5–7	21 days	40 gm	End of lactation (28 to 32 days post parturition)
CHINESE HAMSTER (*CRICETULUS griseus*)	8–12 weeks	35–40 gm	20–21 days	4–8	25 days	6–8 gm	Post-partum oestrus
RABBIT (large) (*ORYCTOLAGUS cuniculus* L.)	9 months	2,500–3,000 gm	32 days	6–8	8 weeks	1,500 gm	3rd week of lactation
RABBIT (small)	6 months	1,500–2,000 gm	30 days	6–8	6 weeks	1,000 gm	3rd week of lactation
FERRET (*MUSTELA putorius furo* L.)	9–12 months	750–800 gm	42 days	6–10	8 weeks	450 gm	End of lactation or next breeding season
DOG (*CANIS familiaris*)	14 months	Variable	60 days	4–8	8 weeks	Variable	Next breeding season
CAT (*FELIS catus* L.)	7–9 months	2,500 gm	64–66 days	3–6	6 weeks	700–800 gm, but variable	4th week of lactation
MONKEY (*MACACA mulatta*)	F. 3 years ♀	4·5–9 Kg	24 weeks	1 only	3 months	700–800 gm	3 months
MONKEY (*MACACA mulatta*)	M. 4 years ♂	6·75–11 Kg					

See following notes for further details much day-to-day information which need not be entered fully on the mother's permanent record card. The litter card should show:

(i) the mother's identity and cage of origin;
(ii) date of birth of litter;
(iii) numbers and sex born;
(iv) date of weaning;
(v) number, sex, and body weight of weanlings (individually or as a group);
(vi) the fate of the litter, e.g.

to breeding stock (quote identity numbers);
to reserve stock (quote identity and/or cage number);
to experiment (quote name of experimenter and nature of experiment, and identity numbers if known).

Space should be allowed for day-to-day notes on the progress of the litter.

CATS

Cats may be bred under laboratory conditions and litters produced in each month, although peak production can be expected from March to August. The cat is polyoestrus, showing recurrent oestrus at fourteen-day intervals lasting about three to six days, but if the cat is not mated, can last up to ten days. Oestrus behaviour and calling may occur one or two days before the female will accept the male. After accepting him, mating may occur several times a day for 3–4 days.

Oestrus behaviour in a female may continue after she will no longer accept a male. Three or four services should be sufficient for a female at her first mating, and two should suffice at subsequent matings.

The average length of gestation in the cat is sixty-four to sixty-six days, but actual length of individual cats vary from fifty-seven to seventy days, and there is a variation in days from litter to litter. This wide variation is probably because cats can mate on three or four consecutive days.

Pseudo-pregnancy

This is not nearly so common in cats as in bitches. A sterile mating will cause this condition, which usually lasts about 23 days after copulation. Scott *et al.*[1] had the impression that sterile matings resulting in pseudo-pregnancy can occur in a cat colony when a male is running with the females, especially during the winter months, even when the male is known to be fertile.

A close watch should be kept on lactating females for signs of oestrus. Fertile mating can often be effected at this time, inducing three pregnancies in a year. An average litter size of about four kittens per litter can be expected.

RABBITS

Pregnancy in the rabbit usually lasts some thirty-one days. Several factors influence the duration, such as the size of the litter and the weight of the female. Young born between twenty-eight and thirty-four days after mating usually survive. But these extremes are rare. The milk of rabbits is very rich, containing 14 per cent protein, 11 per cent fat, and 2 per cent sugar. The milk contains 1,000 calories per lb. Cows' milk contains about 350 calories per lb, and a bottle of milk 750 calories.

Different breeds will reach sexual maturity at different ages; factors influencing this are size of breed and nutrition. In general, the larger the breed, the longer it takes to reach sexual maturity. Small breeds take about five months, and large breeds about seven months. The average fertile mating to infertile in most rabbitries is about 60–40.

Young can be weaned at six weeks of age or earlier, but whether the young are weaned or not, the mother should be mated at about four weeks after parturition. If the litter is weaned at six weeks the doe will have two further weeks with them and two weeks before the next litter is born.

The doe should always be taken to the buck's cage for mating. Taking the buck to her may result in fighting. Some bucks refuse to mate in strange surroundings. Mating will usually occur in a few moments and should always be observed.

Does may be palpated for pregnancy at about twelve days. Practice is needed to become proficient at this early stage. The following method is recommended: place the rabbit on a table, hold the ears with the right hand, slip the left hand under the tummy of the rabbit, and with the tips of the fingers gently press the abdomen just in front of the pelvis. The embryos if they are present can be felt, and at twelve days will be the size of small marbles. To palpate successfully both the animal and the handler need to be completely relaxed.

Young rabbits from mothers who die or from litters of high numbers can be fostered. Fostering should be carried out within ten days of birth and should be within three days in age of the litter to which they are being fostered. When transferring the young into the nest of the foster doe it is advisable to remove the doe for a short while and keep her occupied with a titbit. It may also be necessary to mask the smell of the new young in her nest as suggested by Sandford[2] (1957). The doe's sense of smell should be changed by rubbing a little 'Vick' ointment or paraffin on her nostrils and forelegs.

Pseudo-pregnancy

It is only when the doe ovulates, but the eggs are not fertilized, that pseudo-pregnancy occurs. The stimulation which causes the doe to shed eggs is usually the behaviour of the buck, but may be caused by does riding each other.

Pseudo-pregnancy continues for fourteen to sixteen days; during this period the doe will behave as though pregnant, i.e. the mammary glands are stimulated to activity and the uterus increases in size. This change is caused by hormones from the corpora lutea. Pseudo-pregnancy may be prevented by letting the doe be mated twice within a period of 5 hours.

GUINEA PIGS

The period of gestation in this animal is determined, to some extent, by the litter size. When the litter conceived at a post-partum mating contains only a single offspring the period of gestation may be as long as seventy-two days.

Rowland[3] (1949) reported gestation periods ranging from sixty-two to seventy-two days; the average, when the litters were conceived at post-partum mating, was sixty-eight days. Bruce and Parkes[4] (1948) found that the percentage of fertile post-partum matings was 74 per cent, but it is suggested by Rowland that the occurrence of post-partum mating is adversely affected if the proportion of females to males exceeds 12–1, but it is considered not unlikely that floor area may be a factor in this connexion. Thirteen animals may be kept in a floor area of about 25 sq ft. If the number of animals housed together is increased, say to thirty-six females and three males, then one male will become dominant and will attempt to mate all the females, to the exclusion of the remaining males. By utilizing the post-partum oestrus, the average number of young weaned from each sow in a year has been reported to be as high as 18·9.

COTTON RAT

Short[5] (1957) recommended the monogamous-pair system of breeding cotton rats, but suggests that it is possible to rotate one male among several females. Sexually mature females of this species can be extremely pugnacious, and may inflict fatal damage to the male. To prevent this occurring early pairing at six weeks of age is recommended.

The provision of a nest box or plenty of nesting material is important when young are due to be born so that the mother may retire from the presence of humans.

HAMSTER (GOLDEN)

The hamster has a four-day oestrus cycle, Orsini[6] (1961). The obvious indication of this cycle is the appearance of the post-oestrus discharge on the morning of *day-2*; i.e. the morning subsequent to the night in which the female is in heat.

This discharge may appear protruding from the vaginal orifice when the animal is picked up, or may surge forth from the apparently sealed vagina in response to slight pressure at the sides of the vaginal orifice. This post-oestrus discharge is extremely regular in its occurrence on each fourth day in the cycling animal. It is a thick, opaque-white, mucous which is very viscous, adhering to the finger and 'stringing out' from the vagina from 2 to 8 in. as the finger is removed. Later in *day-2* the inner portion of this vaginal discharge thickens and becomes less stringy, beginning to resemble a whole waxy plug. On *day-3* many of the animals will show a distinct waxy plug; this is non-mucous and can be expressed. It is not always present, and may be lost prior to examination. On *day-4* there is no special discharge characterizing the day; if the waxy discharge of day-3 has not been extruded it may be found at this time or the vagina may be moist with serous fluid. *Day-1* is the day the animal comes into heat, i.e. the day prior to the recurrence of the post-oestrus discharge, which first manifests itself as a slight, translucent secretion and is not always present on day-1. Oestrus itself extends from the evening of day-1 into the morning of day-2.

HAMSTER (CHINESE)

Post-partum oestrus

Yerganian[7] (1962) reported post-partum oestrus in this animal but added, 'only the gentlest of females permit such absence, no matter how brief, from their newborn. Advantage could be taken of females who allow handling and absence from their newborn at post-partum as a method of selecting future stock with greater docility.'

Management

Sexually mature females of this species are extremely pugnacious, and if the sexes are not separated immediately after mating the females inflict considerable damage to testes and tails of the males. Mating generally takes place during the late evening, so it is necessary for someone to observe the mating and remove the male. To overcome this inconvenience Yerganian[8] (1958) described a reversed-lighting scheme of 11 hours of darkness starting at 7.0 a.m. By 9.0 a.m., 2 hours later, adult females were entering their peak of oestrus and matings are conducted routinely at this time. Females displaying hostility towards their mates could be readily separated before inflicting injury. Sufficient light with which to work during the dark period could be provided by low-voltage bulbs (15–20 watts). A reversed-lighting scheme may be set to any timing cycle that is convenient for individual workers, so long as mating trials are conducted within 2 hours of the lights being turned off.

Mating

When the sexes are put together repeated copulatory attempts by the male and lordosis in the female can be witnessed within seconds. When mating is effective the male retains his hold and forces the female to fall to one side. If the male has been successful the female may suddenly turn and attempt to bite his scrotum. Both sexes will rest for a while before repeating the mating pattern. In time the male fails to respond to the female's desire to continue the mating. The female will then become aggressive and attack the scrotum or tail. This is the time to part the pair.

MONKEYS

Monkeys may be bred in the urban animal house without special feeding, Short and Parkes[9] (1949). One of the secrets is the age of the male. Males mature later than females, and should be at least four years of age; from six to fifteen is their full reproductive age. Van Wagenen[10] (1950) reports that the first menstruation occurs in females around the second birthday, while the onset of the growth and development of the testes is seen about the third birthday or early in the fourth year.

Of primary importance is the day-to-day record of the menstrual history of each female. The same person should make a daily inspection of all females in the colony at the same time each day for the purpose of observing the perineum of each animal (the perch on which she sits may give a clue), and a record of bleeding or not bleeding should be made. With the menstrual records at hand the females may be allotted to the male cages on the eleventh or twelfth day of their cycle. The first day of menstruation is day-1 of the cycle.

Monkeys could be mated at noon each day and removed at noon on the first or second day; this places the animals together at a time when there is no immediate competition for food.

Rectal palpation is necessary to determine early pregnancy, because the Macaca mulatta almost always menstruates once after conception (implantation bleeding). The bleeding is often delayed for a few days; that is, the conception cycle is longer than the menstrual cycle characteristic at the same time for that particular monkey, and the implantation bleeding is also longer.

Together these•two signs enable one to diagnose pregnancy around the twenty-third day, but palpation of the uterus is needed to confirm.

Pregnancy in the Macaca mulatta is around six sexual cycles, about 164 days. Young have been born between 147 and 180 days.

FERRETS

The ferret has a well-defined breeding season of about six months of the year which in Great Britain extends from March to August. Signs of the onset of the season are seen first in the males (hobs) about the middle of January. The testes begin to enlarge and descend, and by the end of February they are eager to mate. The onset of oestrus in the females (gills) is easily recognized by the swollen state of the vulva. The extent of the swelling is variable, and in the absence of mating this swollen condition will persist throughout the breeding season, because ovulation does not occur spontaneously.

As a guide, mating should occur at about the fourteenth day from the onset of the swelling of the vulva. Copulation may vary from 10 minutes to 3 hours, but the average is about 1 hour. The vulva should be examined about seven days after mating and should be shrinking rapidly if ovulation has occurred. If the condition remains unchanged the female should be remated.

The gestation period is forty-two days, and there appears to be little variation whether the litter is large or small. Females may be palpated for pregnancy at three weeks. Pseudo-pregnancy occurs after an infertile mating and will last forty-two days.

The young can be weaned from the mother at six weeks old. Mothers resent interference with their nests during the first fortnight after giving birth, and it is not practicable to count the number in the litter at birth.

Females will come into oestrus about sixteen days after weaning. It is not uncommon in the early part of the season for a female suckling a small litter to come into oestrus during the early part of lactation. According to Grinham[11] (1952), when this condition is observed the female should be mated lest the oestrus condition interferes with the normal course of lactation.

DOG

Bitches come into season for the first time when they are about eight months old, and the season recurs every six to eight months, usually in the spring and autumn. (Basenjis are unique among dogs in having only one season each year, in the autumn.) Bitches are not full grown at eight months old, so it is customary not to mate them until their second season, when they are more nearly mature.

The approach of the season (or oestrus period) is marked by a slight swelling of the vulva. The bitch ovulates spontaneously, and this swelling, which becomes more pronounced during oestrus, subsides at the end of the season whether or not she has been mated. The endometrium is shed at the beginning of oestrus, causing the bloody discharge from the vagina. The duration of the season is twenty-one days.

Most bitches will accept the dog only from about the tenth day until the end of the season, though the dog remains fertile and capable of mating throughout the year. Most successful matings take place from the tenth to the fourteenth day of the season.

The gestation period is sixty-three days, though variations of several days in the length of this period are not uncommon. Pups are usually weaned at the age of six weeks, and should not be left with the bitch longer than eight weeks, when she will have stopped lactating and will be worried by the continued presence of the pups.

Pseudo-pregnancy

This may occur in unmated or unsuccessfully mated bitches. Its duration is the same as that of pregnancy (sixty-three days). During this time the mammary gland develops as in pregnancy, and the bitch may prepare a bed in which to litter. At the end of pseudo-pregnancy this behaviour ceases and the mammary gland regresses to its normal state.

MICE

Most mouse breeders accept the traditional idea of a four to five-day oestrus cycle which is both regular and spontaneous. Bruce[12] (1962) reports this is only true if a male is present. If female mice are housed singly the cycle is longer (five to six days) and more irregular: if they are housed in small groups there is a natural suppression of oestrus, with an increase in the number of spontaneous pseudo-pregnancies: if they are housed in large groups (thirty females per box) they may become anoestrus for weeks on end.

The introduction of a male initiates a new cycle, so that when grouped females are paired with stud males oestrus is synchronized and the majority of females mate on the third night of pairing. Only if females have been maintained singly prior to the introduction of the male are matings spread fairly over the first four or five nights.

Ross[13] (1961) outlines three systems of management to ensure matings on particular days.

(i) Exposure of female mice housed in a stock box to caged males for two days prior to pairing, thus achieving peak matings on first and second night after pairing with stud male.

(ii) Confining the stud male behind a perforated metal division in a small box for two days before release to the females, resulting in a peak mating on the first night after release.

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(iii) The use of intact males caged, or castrated males free, to ensure short regular oestrus cycles, thus increasing the number of females on heat available for pairing on demand.

It is desirable when breeding mice to select for high productivity, that is for the largest number of healthy offspring per female in a given time.

Carter[14] (1951) suggested that the measure of productivity be the number of mice born and weaned divided by the number of prenatal days. This index will be less than 1·0, and occasionally less than 0·1.

Lane-Petter *et al.*[15] (1959) proposed using Carter's index multiplied by 100 as a measure of productivity, and calling this index *Q*. It was suggested that *Q* should be called the index of productivity and to calculate it cumulatively from the date of the first exposure to the chance of mating to the birth of successive litters up to the last.

DEFINITIONS OF TERMS

The lists below have been prepared under the guidance of Dr M. Sabourdy and published by the International Committee on Laboratory Animals (ICLA Bulletin, No. 12 (annex), March 1963). It is recognized that the definitions given here will undoubtedly be modified in the light of public comment, and any such amendments will be published from time to time in the *ICLA Bulletins*. However, these lists are a noteworthy first attempt to provide an internationally acceptable set of definitions, and as such are welcomed by all persons working with laboratory animals.

The *ICLA Bulletin*, No. 12 (annex) says: 'The recommended definitions are the outcome of much investigation, in which full weight has been given to the opinions of a large number of competent experts. The Executive Committee of ICLA urges that very serious consideration be given to their widespread adoption. The Committee recognizes, however, that in spite of all the care that has been taken to formulate satisfactory definitions, there are some that will inevitably prove controversial. The definitions are, consequently, divided into three groups, arbitrarily designated *non-controversial*, *slightly controversial*, and *strongly controversial*. Comments on this list will be welcome, and should be addressed to Dr M. Sabourdy, Centre de Sélection des Animaux de Laboratoire, 5, rue Gustave Vatonne, Gif-sur-Yvette, (S.-et-O.), France.'

LIST I. NON-CONTROVERSIAL

TERM	RECOMMENDED DEFINITION
Closed colony	A colony not recruiting members from outside itself.
Primary type colony	A colony of any laboratory animals defined genetically and of known nutritional and disease status. Its function is to provide breeding stock for subcultivation elsewhere. Also known as Foundation Stock.
Primary type colony centre	A centre where one or more primary type colonies are being maintained. Also known as a Foundation Stocks Centre.
Secondary type colony	Represents a direct derivation of the individuals within the group from an earlier foundation stock.
Foundation stock	Breeding pairs of any inbred strain descended from recent common ancestors, maintained with elimination of sublines.

TERM	RECOMMENDED DEFINITION
Pedigreed expansion stock	Breeding pairs of any inbred strain derived from the foundation stock, propagated for a few generations to increase the number of animals descended from the common ancestors of the foundation stock.
Family	A breeding group generally descended from a single pair of parents.
Substrain	As defined in 'Standardized Nomenclature for Inbred Strains of Mice', *Cancer Research*, Vol. 12, No. 8, 602–13 (1952): Any strain separated after *eight to fifteen* generations of brother × sister inbreeding and maintained thereafter in the same laboratory without intercrossing for a further *fifteen to twenty* generations shall be regarded as substrains. It shall also be considered that substrains have been constituted (*a*) if pairs from the parent strain (or substrain) are transferred to another investigator, or (*b*) if detectable genetic differences become established.' Ibid., Vol. 20, No. 2, 145–69 (1960): '. . . after *eight to nineteen* . . .', '. . . further *twelve or more* . . .'
Inbred strain	As defined in 'Standardized Nomenclature for Inbred Strains of Mice', *Cancer Research*, Vol. 20, No. 2, 145–69 (1960): 'A strain shall be regarded as inbred when it has been mated brother × sister (hereafter called b × s) for twenty or more consecutive generations. Parent × offspring mating may be substituted for b × s matings, provided that in the case of consecutive parent × offspring matings the mating in each case is to the younger of the two parents.'
Subline	A division of a line.
Outbred	Individuals resulting from outbreeding.
Inbred	Resulting from continued matings between closely related animals.
Back cross	The cross of an F_1 hybrid to either of its parents; in mice, to animals of either parental strains.
Homozygous	Having identical alleles at a given locus.
Heterozygous	Having different alleles at a given locus.
Standards	Defined and accepted characteristics, tests, and regulations established by authority as a rule for the measure of value or quality.
Performance tested	Refers to an animal population (usually a strain or one of its subdivisions) shown to possess a continued ability to exhibit certain responses or characteristics for which the population is primarily maintained.
Commercial grade	Refers to animals of undetermined quality that do not necessarily conform to accepted minimum standards.
Accredited supplier	A supplier who raises stocks of laboratory animals which conform to defined and accepted minimum standards of production.
Laboratory animal technician	A person qualified by experience and training to provide specialized care and handling of laboratory animals.

LIST II. SLIGHTLY CONTROVERSIAL

TERM	DEFINITION
Species	(*a*) All the animals of the same kind that can (actually or potentially) mate together and produce fertile offspring. (*b*) A group of organisms with distinguishing characteristics reproductively isolated from other groups of organisms. (*c*) A group of actually (or potentially) interbreeding organisms reproductively isolated from other such groups.
Colony	(*a*) An animal population maintained under some degree of control for the purpose of reproduction. (*b*) All the animals of a species being maintained for reproduction in one laboratory or centre.
Strain	(*a*) A group of animals of known ancestry maintained by a deliberate mating system; generally with some distinguishing characteristics. (*b*) A stock of known ancestry maintained by a deliberate mating system; generally with some distinguishing characteristics. (*c*) A group of animals of known ancestry; generally with some distinguishing characteristics.
Random breeding	(*a*) Matings made entirely at random within a population herd or breeding group without regard to either genotypic or phenotypic resemblance in the mated animals. (*b*) Mating of animals by chance, without regard to relationship. (*c*) Mating system in which the average relationship between mated individuals is the same as the average relationship between contemporary animals, i.e. the matings are random with respect to relationship.
Random bred	(*a*) Random breds are individuals resulting from random breeding. (*b*) Produced by random mating; generally implying also a fairly large number of parents in each generation, i.e. more than about ten pairs. (*c*) Refers to a population resulting from matings which are independent of relationship.
Outbreeding	(*a*) Mating system in which the relationship between mated pairs is less than the average relationship of contemporary individuals, i.e. the deliberate avoidance of inbreeding, even to the extent of introducing animals from outside. (*b*) Mating of animals less closely related than the average for the stock, especially when repeated for several generations.
Hybrid	(*a*) The immediate product of (*a*) an inter-specific cross, or (*b*) a cross between two inbred strains. (*b*) Individual resulting from a cross of parents having different inheritance. The hybrids may have various degrees of heterozygosity. (*c*) Progeny of mating between animals of unlike genetic constitution; in mice between animals from two inbred strains.
Selection	(*a*) Causing or permitting some kinds of individuals to produce more offspring than other kinds do. (*b*) A choice of individuals from which the breeder desires to obtain progeny.

TERM	DEFINITION
Selection	(*c*) Delete reference to 'the breeder' unless 'artificial selection' is to be defined. Selection, in laboratory strains, may be due to unconscious breeding practices, to heterosis, and other causes. (*d*) Matings of animals of designated types to increase or decrease the frequency of specific characteristics.
Isogenic	(*a*) Having identical genotypes. (*b*) Individuals, two or more different lines or families that have identical or, at least, most genes identical.
Enzootic	(*a*) A disease within an animal group which remains over a considerable period of time within the group. It has the same connotation as endemic diseases in man. (*b*) The occurrence in a colony of an illness or a group of illnesses of a similar nature not in excess of normal incidence and derived from a common or propagated source.
Epizootic	(*a*) A disease which affects many animals at one time having the same connotation as epidemic in man. (*b*) The occurrence in a colony of animals of an illness or a group of illnesses of similar nature, clearly in excess of normal incidence and derived from a common or a propagated source.

LIST III. STRONGLY CONTROVERSIAL

TERM	DEFINITION
Population	(*a*) Any group of animals of the same species whose characteristics as a group are being studied or described. (*b*) An interbreeding group of individuals of the same species, living at the same time on the same territory, and at least partially separated from other such populations. (*c*) Any group of individuals under consideration, either real or imaginary.
Stock	(*a*) A collection of animals being grown or maintained for breeding or for experimental use. (*b*) Represents a breeding group having some distinguishing characteristics of interest as geographical origin or genotypic content.
Line	(*a*) Part of a family of animals separated from other parts by one or more generations of independent ancestry. (*b*) Part of a strain, not necessarily an inbred strain, separated from other parts by one or more generations of independent ancestry. If part of an inbred strain, the separation is by an arbitrary fairly small number of generations. (*c*) Part of a strain separated from other parts by one or more generations of independent ancestry.
Mating system	(*a*) Any plan for propagating animals. (*b*) The manner in which pairs are chosen for mating for the production of offspring, the choice being governed by the relationship between the members of the pairs. (*c*) That method of animal breeding designed to produce a desired genetic result.

TERM	DEFINITION
Mating system	(*d*) In my opinion this definition as given is completely erroneous. Mating systems need have nothing to do with planning; a naturally outbreeding or inbreeding group of animals or plants has its own mating system. In this generally accepted sense 'mating system' should probably not be included in the list at all. If you call it something like 'breeding programme', at least it is clearly distinct. But as you have already both 'selection' and items referring to inbreeding, etc., why an additional definition which presumably would have to cover both? (M.S.).

THE FOLLOWING ARE UNCLASSIFIED

TERM	DEFINITION
Production stock	Breeding pairs or trios of any inbred strain derived from a pedigreed expansion stock for further multiplication of animals descended from common ancestors of the foundation stock.
Genetically heterogeneous stock	A stock that has not been specifically bred for genetic uniformity.
Inbred derived	Progeny of mating between animals of a given inbred strain with recent common ancestors, but not necessarily related as brother and sister.
Phenotype	The appearance or properties of an organism.
Genotype	The genetic composition of an organism.
Coisogenic	Having identical genotypes except for a designated difference.
Congenic	Having similar genotypes.
Certified grade	Refers to animals endorsed by competent authority as conforming to certain defined and accepted minimum standards.
Laboratory animal attendant	A person engaged in the care of laboratory animals whose primary duty is the maintenance of environmental sanitation and stability.
Specific pathogen free	Animals proved to be free of the causative agents or agents of one or more specific named diseases, but not necessarily free of the others not named.
Germ free	Animals that are free of all demonstrable organisms, resulting from use of a closed-system sterile technique.
Gnotobiotic	(*a*) An organism whose microbiota, if any, is known. (*b*) Pertaining to a gnotobiote or gnotobiotics.
Gnotobiotics	The science of rearing laboratory animals, the microfauna and microflora of which are specifically known in their entirety.
Gnotobiote	A specially reared laboratory animal, the microfauna and microflora of which are specifically known in their entirety.

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CHAPTER TWENTY-THREE

Mammalian Physiology

Physiology is the study of the functions of the organs and tissues of the body. It is convenient to classify the functions of the body into systems. For instance, the cardiovascular (or circulatory) system comprises the heart and the blood vessels together with the blood. The function of this system is very similar in all mammals, and the information gained from the study of the smaller mammals will help us understand the function of our own circulatory system. In this chapter four aspects of the physiology of mammals are considered:

1. Structure and functions of the blood.
2. The cardiovascular system.
3. Structure and functions of the kidney.
4. The structure and function of the respiratory system.

1. STRUCTURE AND FUNCTIONS OF THE BLOOD

Blood is the material which the circulatory system continually moves about the body in a system of tubes called blood vessels. Most of the functions of blood are related to satisfying the nutritional needs of the tissues through which the blood passes. It carries its own machinery for plugging any holes that might occur in a blood vessel, and in addition is concerned in the protection of the body against infection.

The functions of blood can be briefly summarized as:

1. Respiratory

The blood carries to all the remote cells of the body the oxygen which is absorbed in the lungs. From the outlying tissues it removes the carbon dioxide which the cells accumulate and carries this to the lungs for removal from the blood.

2. Nutrition

All the food that the tissues need is conveyed to them in the blood. In addition, blood removes ingested materials from the intestine and transports these materials as necessary, either to stores, to the liver for further processing, or to tissues that require the material.

3. Hormonal transport

Many of the body's functions are regulated by substances, called hormones, which are released into the blood by one organ to affect the working of another distant organ.

4. Transfer of heat

Heat is generated in different parts of the body at different times. For instance, in running muscles get hot. In travelling through muscles that are working, the blood removes the heat and distributes it more evenly. Blood normally circulates freely in the skin, where it is cooled. By varying the amount of blood going to the skin, and thus the amount of cooling, the body can maintain an even temperature.

5. Protective

Blood contains cells and chemicals which can overcome bacterial and virus invasion. It also has a clotting mechanism to form a protective plug when a blood vessel is damaged.

CONSTITUENTS OF THE BLOOD

Blood consists of fluid and solid components. The fluid is a complex mixture of chemicals called plasma, and suspended in this plasma are vast numbers of blood cells which are the solid component.

The plasma constituents are:

Water	90–92 per cent
Proteins	7 per cent
Organic salts	1 per cent
Inorganic salts	1 per cent

Gases in solution and chemicals such as hormones . . . less than 1 per cent.

The plasma proteins may be classified in three main groups:

Serum albumin	4·0 per cent of plasma
Serum globulin	2·7 per cent of plasma
Fibrinogen	0·3 per cent of plasma

Fibrinogen is the protein actively concerned in the coagulation of blood. The substances which give us immunity to certain diseases are in the globulin fraction.

The blood cells

The blood cells (or corpuscles) are sometimes referred to as 'the formed elements of blood'. There are two main types of blood cells—the red cells and the white cells.

(a) The red corpuscles

A fully developed red corpuscle is called an erythrocyte. They are very small disc-shaped cells, and in mammals have no cell nucleus (Fig. 1). A cubic millimetre of human blood contains between 4·5 and 6 million erythrocytes.

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The characteristic red colour of blood is due to the pigment haemoglobin, which is present in erythrocytes.

Erythrocytes have a life of about 120 days, thus they are continually being formed and destroyed. The cells are made in the bone marrow and the process of erythrocyte formation is called erythropoiesis—old erythrocytes are broken down by the spleen.

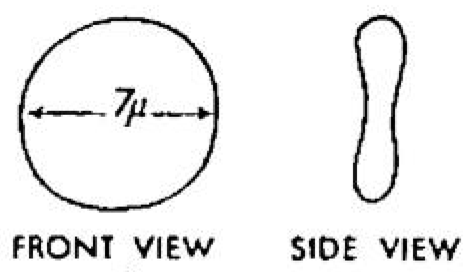

FIG. 1

The pigment haemoglobin which is contained in the erythrocyte is concerned largely in the transport of oxygen in chemical combination. By this means blood is able to absorb more oxygen than if the oxygen was in simple solution.

(b) The white corpuscles (leucocytes)

There are several different types of white cells in the blood. They are usually larger than erythrocytes and possess a nucleus but no haemoglobin. There are two main groups. There are the granulocytes—which are recognized by the presence of easily stained granules in their cytoplasm—and there are the agranulocytes—which as their name suggests—do not have any cytoplasmic granules.

There are far fewer leucocytes than erythrocytes, normally between 5,000 and 10,000 per cu. mm. of human blood.

Agranulocytes

There are two main types of agranulocytes:

(*a*) LYMPHOCYTES. Identified under the microscope by a nucleus that occupies most of the cell (Fig. 2). These cells are non-mobile and originate in lymph

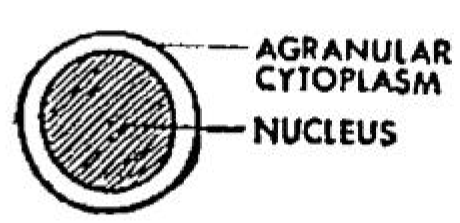

FIG. 2

nodes and in the spleen. Usually subdivided into two classes—large lymphocytes and small lymphocytes. The lymphocytes may be concerned in immunological reactions such as the production of antibodies and γ-globulins.

(*b*) MONOCYTES. Recognized microscopically by the kidney-shaped nucleus (Fig. 3). About three times the size of an erythrocyte, formed in red bone

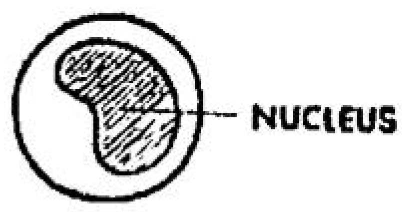

FIG. 3

marrow and have the ability to absorb bacteria and other foreign bodies in tissues (phagocytosis).

Granulocytes (polymorphonuclear cells)

They have peculiar multi-lobed nuclei, and their cytoplasm has granules which can be stained. There are several types of these cells with various staining properties. They are mobile cells—moving like amoeba by the use of pseudopodia, and they can absorb bacteria and other particles of matter. They are also able to leave the blood vessels and move into tissue spaces.

Three main types of granulocytes—which are identified by the staining properties of their cytoplasmic granules:

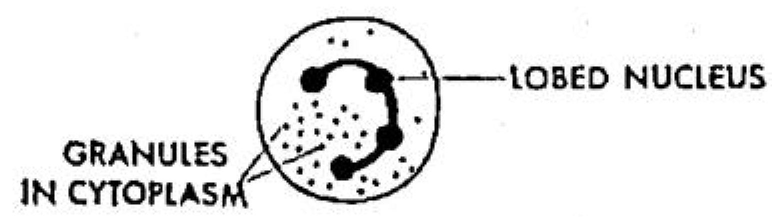

FIG. 4

(*a*) NEUTROPHILS. Granules stain with both acidic and basic stains, purplish with Leishmann's stains. These cells are active phagocytes.

(*b*) EOSINOPHILS. Granules stain with acid dyes (e.g. eosin), and thus appear red with most staining procedures. Function not understood, but the numbers of these cells circulating increase in allergic states or when an animal or man is exposed to stress (such as examinations).

(*c*) BASOPHILS. Granules stain with basic dyes, and hence are usually blue. They appear in chronic inflammatory states. They may also be concerned with the production of a naturally occurring material called heparin which prevents blood from clotting.

COAGULATION OF BLOOD

When a blood vessel is cut bleeding occurs. This bleeding is normally stopped by a combination of two factors—the local constriction of the blood vessel and the formation of a clot at the site of injury. The clot is composed of a

Damaged tissue

↓

Platelet disintegration

↓ Antihaemorrhagic globulin

Thromboplastin + Prothrombin + Calcium
from plasma

↓

Thrombin
+
Fibrinogen

↓

FIBRIN

protein called fibrin which is precipitated from its soluble precursor in the blood called fibrinogen.

In the blood are numbers of minute elements called platelets or thrombocytes. Much smaller than red cells, there are 200–400 per cu mm. It is their disintegration at the site of injury which initiates the clotting process, for they produce thromboplastin (also called thrombokinase) when they disintegrate, and this initiates the process, summarized on p. 317, which ultimately causes the release of fibrin.

Blood clots whenever it comes in contact with a foreign surface. Thus, when it is necessary to prevent this an anti-coagulant is used. An anti-coagulant may be a chemically complex expensive material, such as heparin, or more simple materials, such as potassium citrate or oxalate, may be added to the blood. Citrate and oxalate work by rendering the Calcium in the blood unavailable, and thus interrupt the formation of thrombin.

2. THE CARDIOVASCULAR SYSTEM

The cardiovascular system consists of the heart and the blood vessels. There are three types of blood vessels: arteries, capillaries, and veins. *Arteries* are thick-walled blood vessels which lie deep in the tissues of the body and carry the blood which leaves the heart at high pressure. The large arteries divide into a network of small arteries which ultimately lead to the *capillaries*. Capillaries

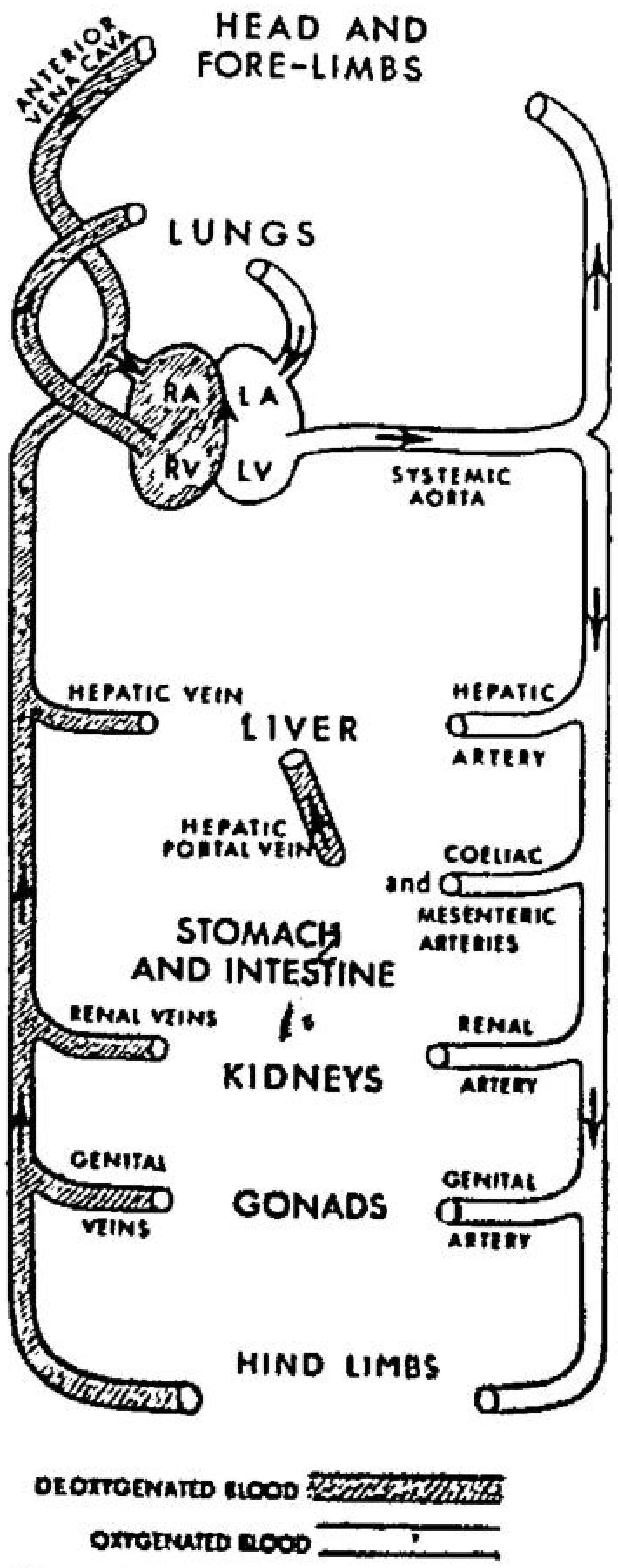

FIG. 5

are vessels with a very thin wall, usually only one cell thick, and here materials such as oxygen, carbon dioxide, etc., can readily enter or leave the blood vessels. Blood capillaries are in close association with the thin alveolar walls, and gaseous exchange takes place between the blood and the alveolar air. Blood returns to the heart in thin-walled blood vessels which lie quite superficially, called *veins*. The blood in the veins is at very low pressure, and veins are readily compressed. There is a system of one-way valves in the veins so that blood cannot flow backwards.

THE HEART

The blood is pumped around the body by the contractions of the heart. In the mammal the heart consists of two pumping systems working together. Each pumping system consists of two chambers, an auricle and a ventricle.

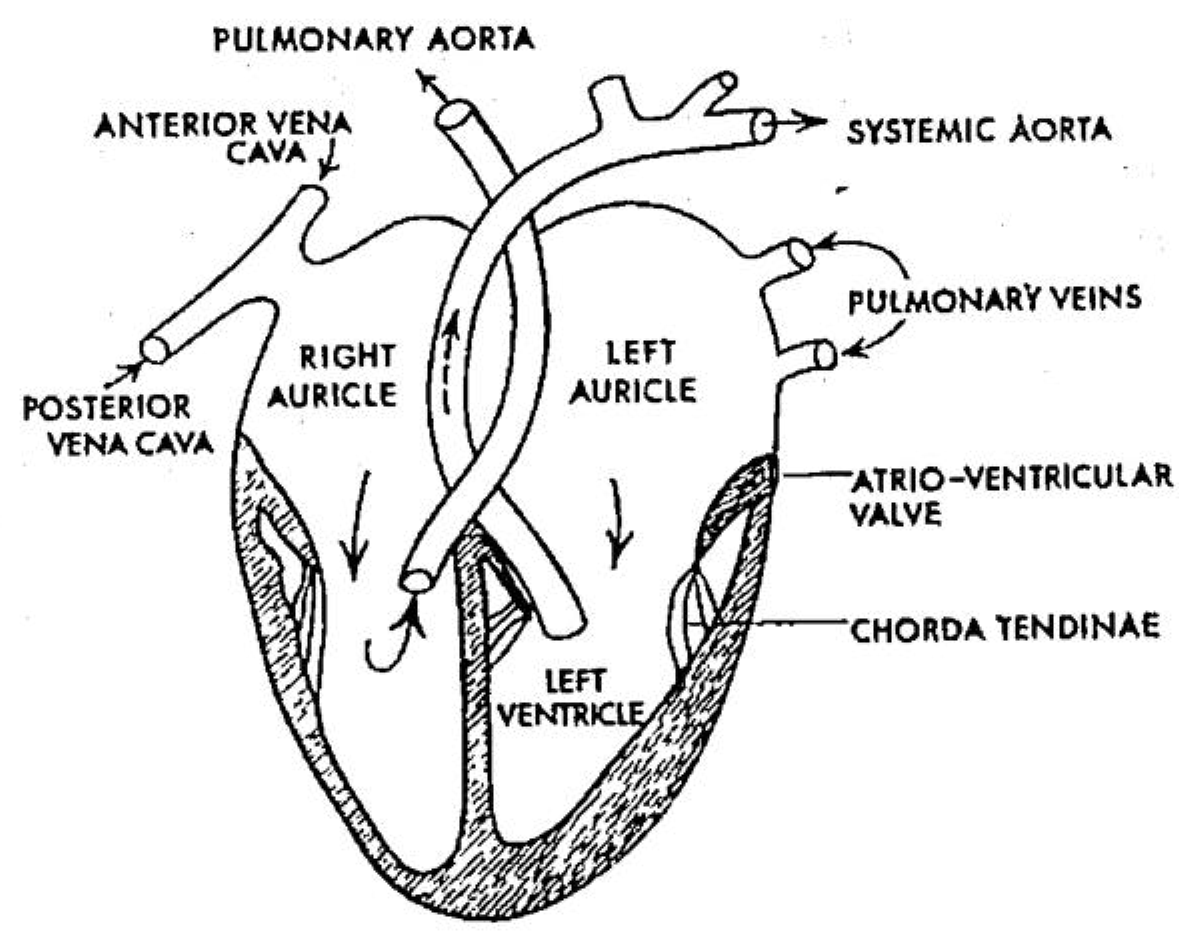

FIG. 6

The auricles (sometimes called atria) are thin-walled sacs which receive blood from the large veins and by their contractions force the blood into the thick-walled chambers called ventricles. The ventricles can develop great force during their contraction. The left ventricle develops enough force to pump blood round all the organs of the body except the lungs. The blood leaving the right ventricle is pumped through the lungs and then enters the left auricle.

It is important that the contractions of auricles and ventricles are synchronized, and this is accomplished by a special system in the heart. There is a conducting system which is arranged so that a contraction of the auricles causes activity in the system (Bundle of His), which is conducted to the ventricles through the Purkinje fibres. In this way the ventricles are caused to contract after the auricles and expel the blood which has been forced into them by the auricular contraction. The presence of valves between the auricles and ventricles and at the entry of the auricles prevents blood from regurgitating.

3. STRUCTURE AND FUNCTION OF THE KIDNEYS

The mammal normally has a pair of kidneys situated high in the abdomen, on the posterior wall and not far from the diaphragm. Each kidney is supplied with a large artery (the renal artery), and the blood is drained by a large vein (the renal vein). It has been calculated that one-sixth of the blood leaving the heart enters the kidneys.

A tube called a ureter leaves each kidney and enters the posterior wall of the bladder.

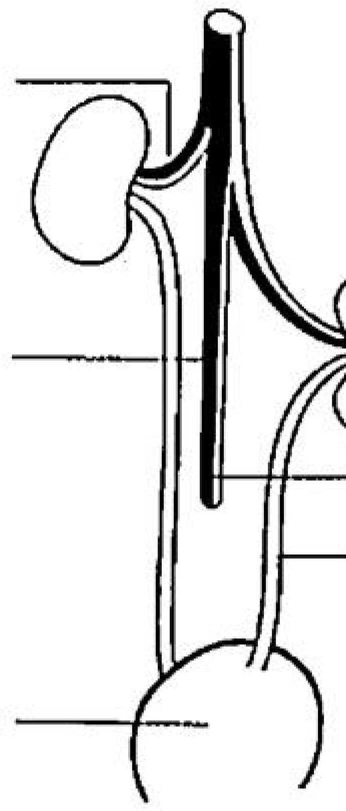

FIG. 7

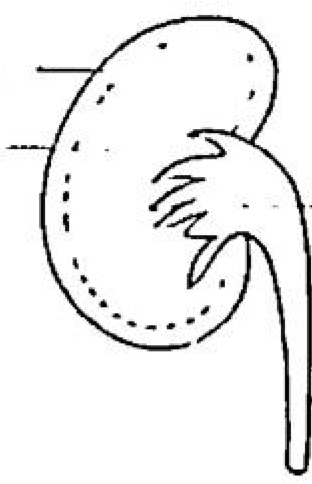

FIG. 8

STRUCTURE OF KIDNEY

If a kidney is cut down its length three regions are clearly recognizable: (*a*) an outer layer—the renal cortex; (*b*) a middle layer—the renal medulla; (*c*) an inner spongy layer—the renal pelvis.

Microscopic examination shows that the renal cortex has many circular bodies in it. A high-power view of one of these bodies (the glomerulus) is shown below. Blood from the renal artery is led to the capillary network of the glomeruli, and here a high proportion of the substances in solution in the plasma are filtered off and enter the tubules. These tubules then dive towards the renal pelvis through the renal medulla. The medulla consists almost entirely of tubules surrounded by blood vessels. The blood vessels surrounding the tubules are formed from the veins leaving the glomeruli, and in the tubule they reclaim a lot of the useful materials from the filtered plasma. The materials that are not reclaimed are carried to the renal pelvis, where all the tubules converge into the ureter. These substances in solution are then carried to the bladder, from which they are periodically voided as urine.

FUNCTIONS OF KIDNEY

By the process of continuous filtration and reabsorption the kidney, together with the lungs, is able to control the composition of the blood. The metabolism of proteins leads to the continual production of materials such as urea, uric acid, and creatinine. If the concentration in blood of these substances is allowed to rise, then they may poison the organism—and the kidney normally removes these materials as they are formed, thus keeping their blood concentration at a safe low level.

The kidneys also regulate salt and water metabolism, getting rid of any excess that has been taken in, and retaining material when insufficient salt or water is taken. About 1½ litres of urine are produced by the adult human each day from about 170 litres of glomerular filtrate formed in Bowman's capsule.

Water excretion is under the control of a hormone released from the posterior pituitary gland. This hormone, called the anti-diuretic hormone, is

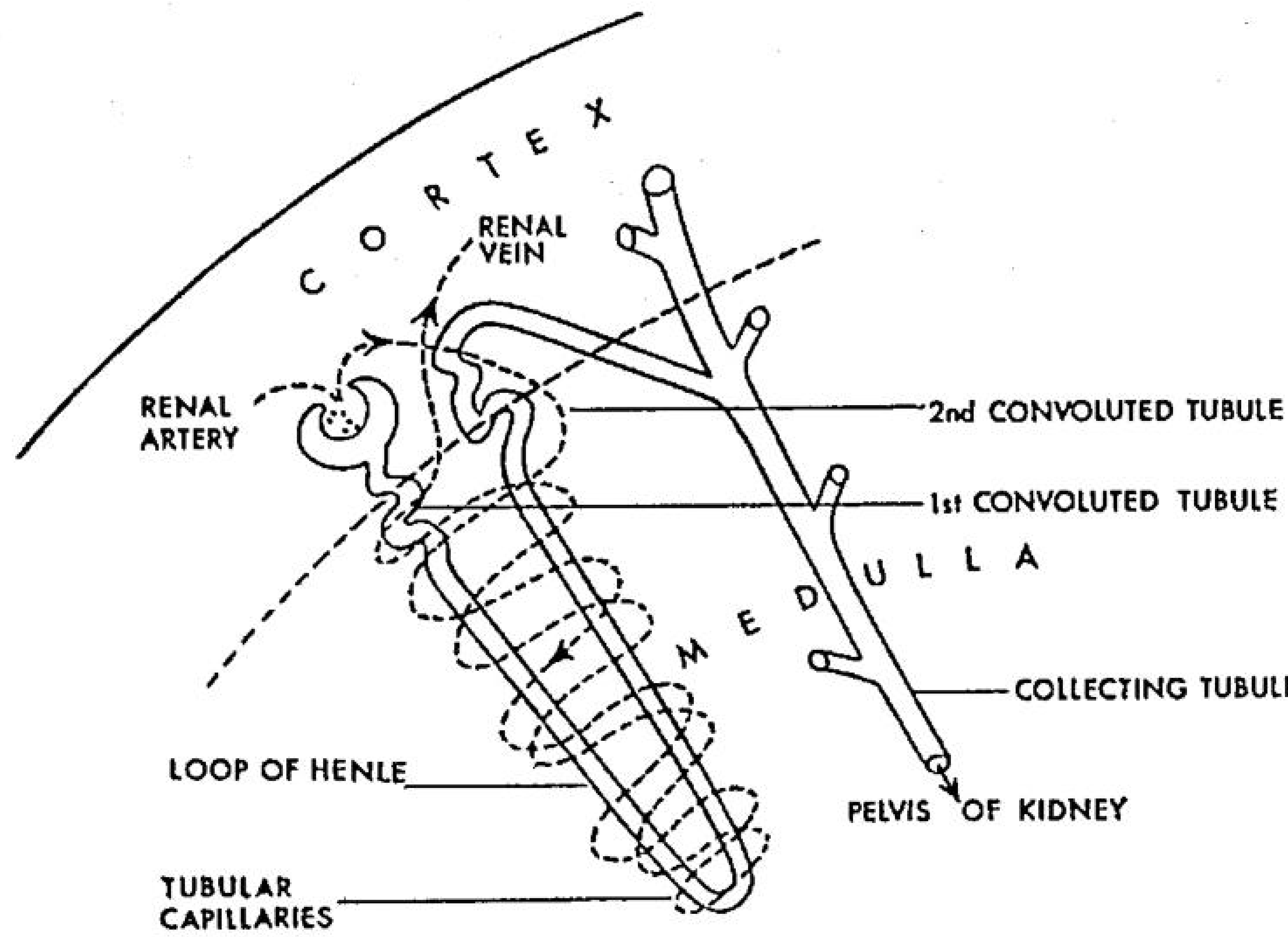

FIG. 9

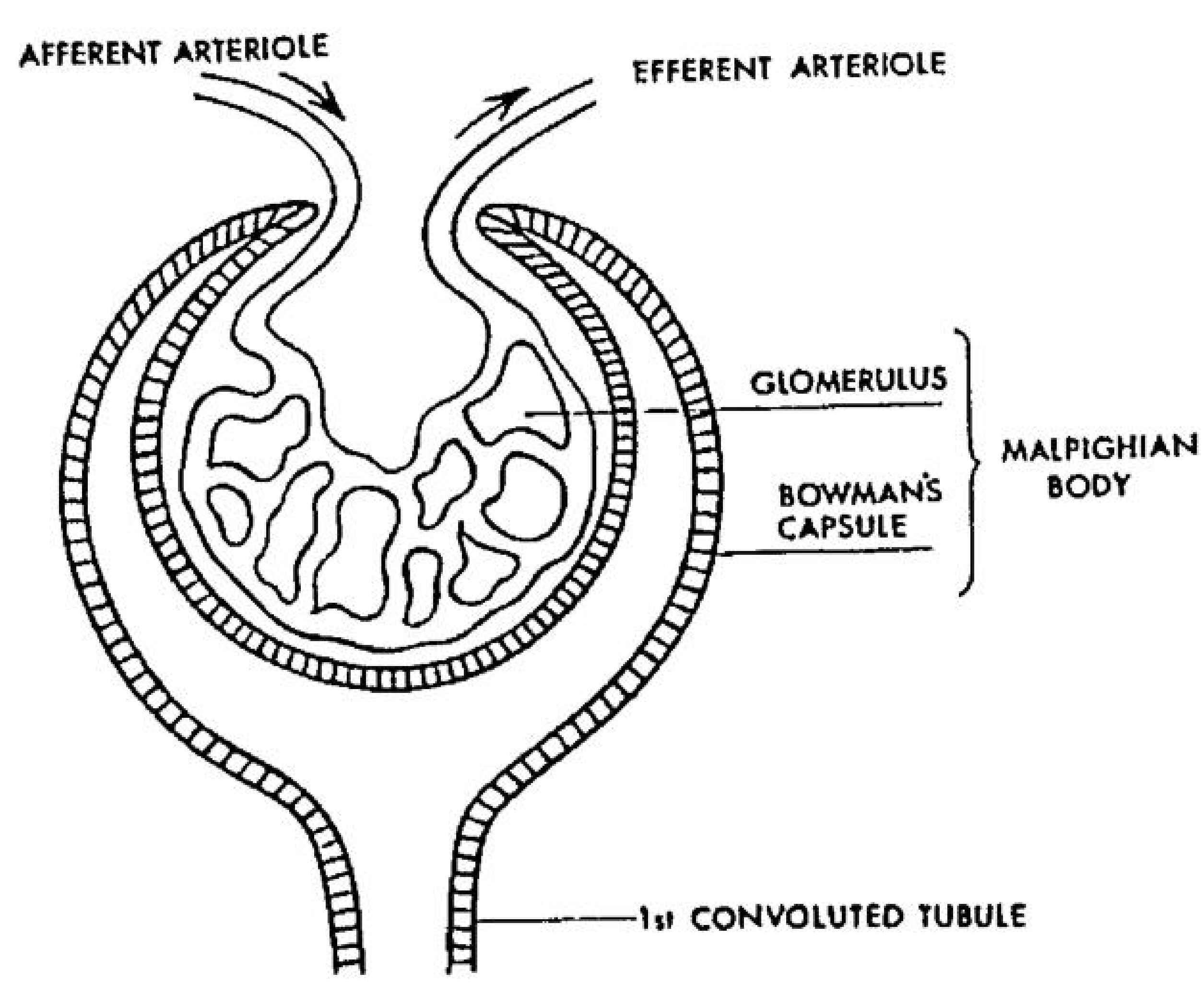

FIG. 10

released into the blood whenever there is a need to conserve water. Thus, a higher proportion of the filtrate in the tubules is reabsorbed. The release of anti-diuretic hormone is governed by cells in the brain which are sensitive to the osmotic pressure of the blood.

4. THE STRUCTURE AND FUNCTION OF THE RESPIRATORY SYSTEM

The term respiration is used to cover two processes: (*a*) the process whereby air is breathed into and out of the lungs (external respiration); (*b*) the chemical processes taking place within cells whereby oxygen combines with foodstuffs to provide energy (internal respiration). Both processes are closely linked, external respiration being necessary for the gaseous requirements of internal respiration. For internal respiration to proceed, a system must exist to supply the cell with oxygen and to remove the carbon dioxide, which is formed during respiration. In unicellular organisms the exchange takes place at the cell surface, but an animal of any size must have a special system to ensure that the circulatory system can send to the cells a fluid rich in oxygen, and can remove the carbon dioxide from the fluid. In the insects organs have been developed called trachae, fish have a gill system, and mammals use lungs. All these organs arrange for a large surface to be available for gaseous exchange.

The possession of internal organs such as lungs means that a mammal has to pump air in and out of its body. This process constitutes the mechanics of respiration.

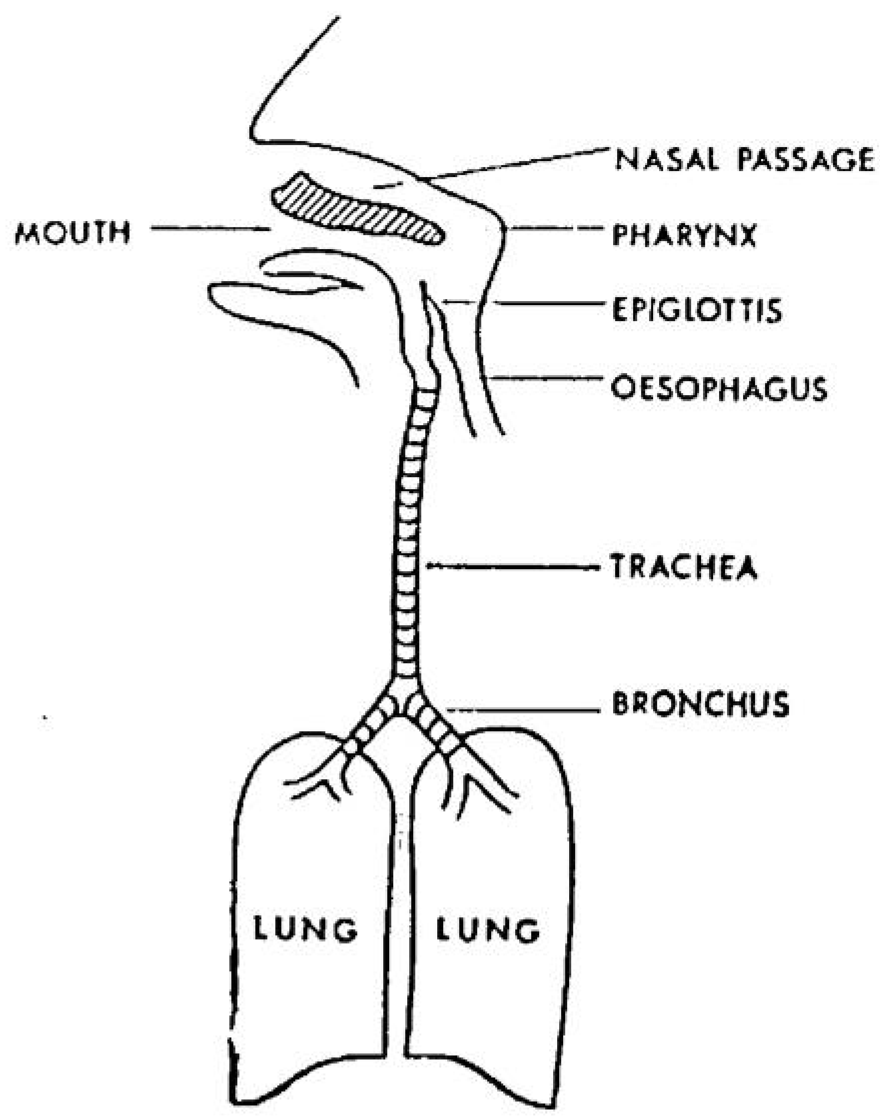

FIG. 11

MECHANICS OF RESPIRATION

The act of drawing air into the lungs is called inspiration, the act of blowing air out of the lungs is called expiration. Certain muscles of the body are responsible for the chest movements which lead to inspiration and expiration. The muscles are: (*a*) the diaphragm—a sheet of muscle which separates the abdomen from the thorax, and (*b*) the muscles of the rib cage, which can move the ribs.

Inspiration is caused by the muscles expanding the chest and thus reducing the pressure in the chest. The opening into the chest leads into the lungs, and thus air entering the body from outside to equalize the pressure enters the lungs. For expiration to occur, the chest cavity is reduced, thus compressing the air in the chest and forcing air out of the lungs.

The tubes leading to the lungs are called the respiratory passages.

The trachea passes from the pharynx into the thorex, where it divides to form two bronchi, which enter the lungs.

The actual tubes in the lungs are the *bronchi*. These are tubes with walls stiffened with cartilage. The bronchi divide into smaller tubes, the *bronchioles*, which lead to blind sacs in which the actual gas exchange takes place. These blind sacs are called *alveoli*.

The amount of air entering and leaving the lungs is closely related to how hard the body is working. Hard work requires the consumption of more oxygen, so the amount of air breathed per minute is substantially increased. A number of terms are commonly used to describe the quantities of air involved in respiration.

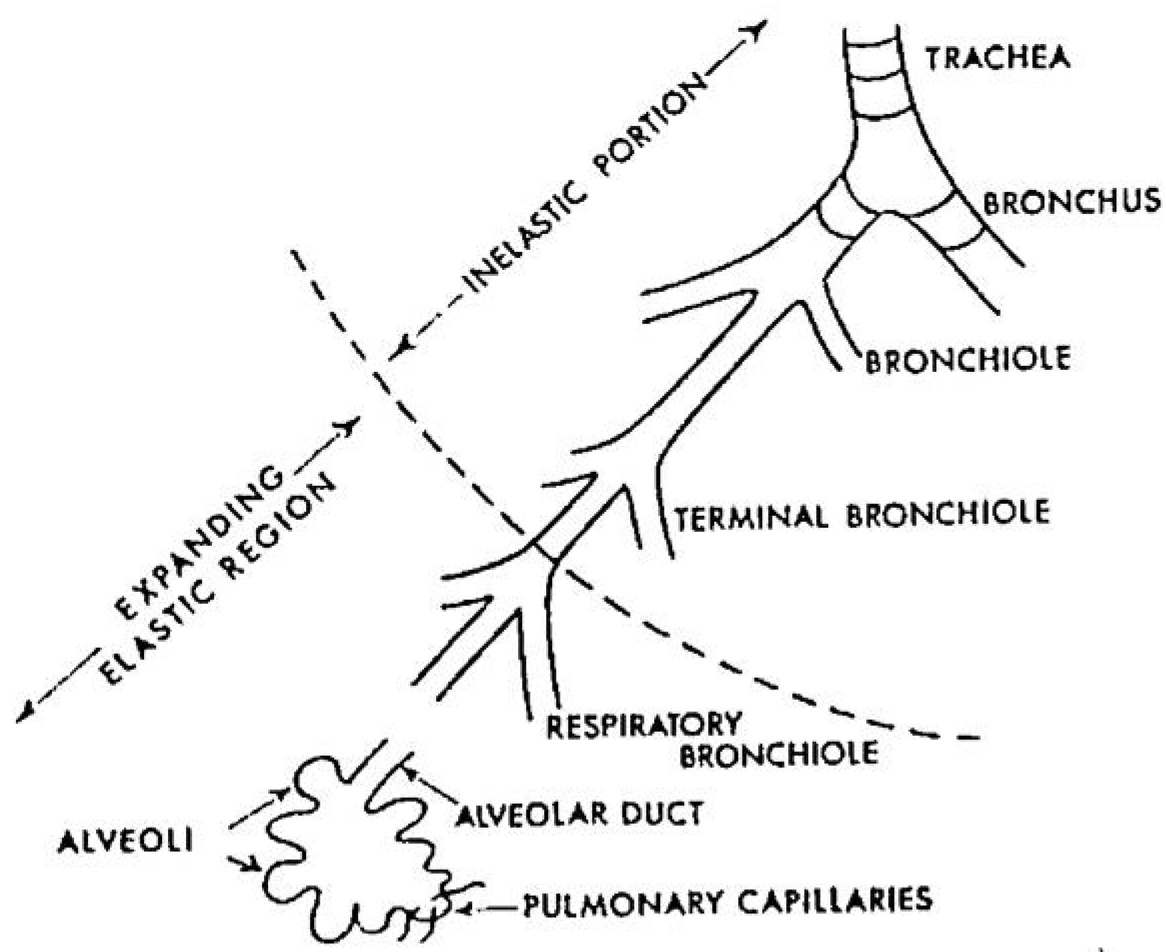

FIG. 12

Total capacity

This is the theoretical amount of air which maximally distended lungs can hold. We divide this into two fractions.

(*a*) RESIDUAL AIR. This is the amount of air which is left even when we breathe out hard—we cannot totally collapse our lungs—so some air is always left in the lungs.

(*b*) VITAL CAPACITY. This is the amount of air that we can expel from our lungs after a maximal inspiration (a really deep breath).

When breathing quietly we only breathe out a much smaller amount of air, and this volume is called '*tidal air*'. The amount over and above the tidal air which we can breathe out in a forced expiration is called '*complemental air*'.

Composition of inspired, expired, and Alveolar air

The composition of the inspired air is naturally the composition of the air around us. Since the lungs absorb oxygen from the inspired air and get rid of carbon dioxide, expired air is poor in oxygen and rich in carbon dioxide. Since the inspired and expired air is not directly exchanged with the air in the alveoli, the concentration of the gases in alveolar air is different again.

	Per cent oxygen	Per cent carbon dioxide	Per cent nitrogen
Inspired air	21·0	0·04	79·0
Expired air	16·5	4·0	79·5
Alveolar air	14·0	5·5	80·0

The depth and frequency of respiration is controlled by the brain. Obviously, say when speaking, movements of air in and out of the lungs are dependent on two factors, the oxygen requirements of the body and the requirements of speech. Thus respiratory control is very complex, involving, in man and higher mammals, the whole of the brain. There is evidence, though, of lower, more primitive centres which can adjust the level of respiration to the concentration of carbon dioxide in the blood. An increase in activity quickly raises the carbon dioxide content of the blood.

Transport of gases in the blood

A proportion of gases in the plasma is in simple solution, but this carrying capacity is very limited. The carrying capacity of the blood is vastly increased by the formation of chemical complexes with the gases, these chemical reactions being readily reversible.

(*a*) TRANSPORT OF OXYGEN. Oxygen is normally carried combined with the red pigment of the erythrocytes, haemoglobin. The reaction may be summarized.

$$O_2 + Hb \rightleftharpoons HbO_2$$

$$\text{Oxygen} + \text{haemoglobin} \rightleftharpoons \text{Oxyhaemoglobin}$$

This is a reversible reaction.

The oxyhaemoglobin is bright red, and haemoglobin is a much darker colour. This accounts for the differences in colour between arterial and venous blood.

(*b*) TRANSPORT OF CARBON DIOXIDE. Carbon dioxide is more soluble than oxygen, and hence a larger proportion of the gas is carried in solution. In addition, it forms a complex with haemoglobin after the haemoglobin has given up its oxygen. This complex is called carbamino-haemoglobin. Most of the carbon dioxide is carried in the plasma as sodium bicarbonate.

The actual formation of bicarbonate takes place in the erythrocytes. The formation of bicarbonate in the erythrocytes is accompanied by a loss of chloride from the erythrocytes—the 'chloride shift'.

CHAPTER TWENTY-FOUR

Nutrition

The aim of this chapter is to give in simple terms, first, the chemical components of foodstuffs and the purpose for which the animal body needs these components, and second, to discuss how the nutritional requirements of animals may be met. Most breeding stocks of laboratory animals are maintained to provide a continuous supply of normal, healthy animals for use in experiments. Such animals cannot be reared on diets which are deficient in either quality or quantity. Since most stock diets are fed *ad libitum*, a deficiency in quantity is rare and occurs only through accident or carelessness, e.g. food placed out of reach of animals, or providing wet mashes which are allowed to sour and become unpalatable. Deficiencies in quality are more common and may be difficult to detect. The state of chronic, sub-optimal nutrition may reveal itself only by an all-round lowering of the standard of performance, e.g. slower growth rates, or fewer litters which are small both in number and weight, or numbers of pups killed by dams who are unable to suckle their litters. If the deficiency is acute it may reveal itself quickly, e.g. an outbreak of scurvy among guinea pigs deprived of a dietary source of vitamin C, but such a deficiency is unusual in an age which is attuned to the publicity the vitamins have enjoyed in this century. Today the complex relationships between the metabolism of the protein and that of the energy-rich (fat and carbohydrate) components of the dietary are being revealed, but before these can be discussed the components themselves must be considered.

All the material needed for the growth and maintenance of the animal body must be taken in by mouth. The nutrients required are proteins, carbohydrates, fats, salts, vitamins, and water. These are the digestible and absorbable constituents of foodstuffs. The fibre (roughage) of foodstuffs is non-digestible, but some animals (especially the ruminants) are able to utilize it to various degrees owing to the action of the micro-organisms present in their digestive tracts.

Food contains chemical energy which is converted, in the body, to work and heat. Protein and carbohydrate yield energy equivalent to 4 Calories per gram; fat yields 9 Calories per gram. (A Calorie is the amount of heat required to raise the temperature of one litre of pure water from 16–17°C.)

Foodstuffs have to be broken down into simple substances before they can be utilized by the body. First, the digestive enzymes act upon foodstuffs, splitting them into less-complex substances which can be absorbed through the gut wall. The breaking-down process is continued by other enzymes in the body tissues. This breakdown is called *catabolism*; the reverse process (e.g. the building up of body proteins) is called *anabolism*; the term *metabolism* includes both anabolism and catabolism. Some enzymes can only function properly when in combination with co-enzymes. Many vitamins act as co-enzymes.

PROTEINS

These are composed of some twenty different *amino acids* (nitrogen-containing organic compounds). Protein is broken down to its constituent amino acids in the body. Some of the mixture of amino acids is used to form new body proteins, and the rest is broken down with the liberation of energy. The waste product from protein metabolism is *urea*, which is excreted through the kidney.

Amino acids may be divided into two groups—the *essential amino acids*, which must be provided from the diet, and the *non-essential amino acids*, which can be made in the animal body. The amino acids essential for most animals are lysine, methionine, leucine, isoleucine, valine, tryptophan, histidine, phenylalanine, and threonine.

Plants can synthesize amino acids using simple nitrogen-containing salts, such as ammonium sulphate, but animals have only a limited ability to synthesize amino acids from nitrogenous organic compounds.

Animal sources of protein (meat, fish, eggs, milk) contain all the essential amino acids in approximately the right proportions for optimal growth. Plant proteins (e.g. protein from cereals, beans, nuts) may contain all the essential amino acids, but not in the best proportions for animal growth, or may contain only some of the essential amino acids. A mixture of plant protein may, however, contain all the essential amino acids in the right proportions for animal growth and be equivalent to animal sources of protein.

Proteins constitute about one-sixth of the body mass. Animals therefore require a liberal supply of protein throughout life for the growth and replacement (maintenance) of body tissues.

CARBOHYDRATES

As their name implies, these are composed of the elements carbon, hydrogen, and oxygen. They are the chief source of energy for the animal body. Carbohydrates are catabolized (broken down) to simple sugars and then to carbon dioxide and water with the liberation of energy. Sugars and starches (which are complex forms of sugars) are carbohydrates.

Plants, but not animals, can synthesize carbohydrates from carbon dioxide and water. Glucose is the commonest simple carbohydrate which animals can utilize.

Cellulose (fibre or roughage), a complex carbohydrate, is not itself digestible, but some animals (e.g. rabbits and ruminants) are able to utilize cellulose, which is first broken down into digestible components by micro-organisms normally present in the gut.

Animals store carbohydrate as *glycogen* ('animal starch') in the liver and muscle. Glycogen is the most readily available energy store of the animal body. Carbohydrate can also be converted to fat, and stored as such, within the animal body.

FATS

Plants and animals contain substances, insoluble in water but soluble in chloroform, ether, and benzene, which are properly called LIPIDS. This group includes neutral FATS and *other similar compounds*. Quantitatively and economically the fats are the most important members of the group, but some of the other compounds have important physiological functions.

Fats are composed of carbon, hydrogen, and oxygen and are metabolized in the body to carbon dioxide and water. Like carbohydrates, they are a

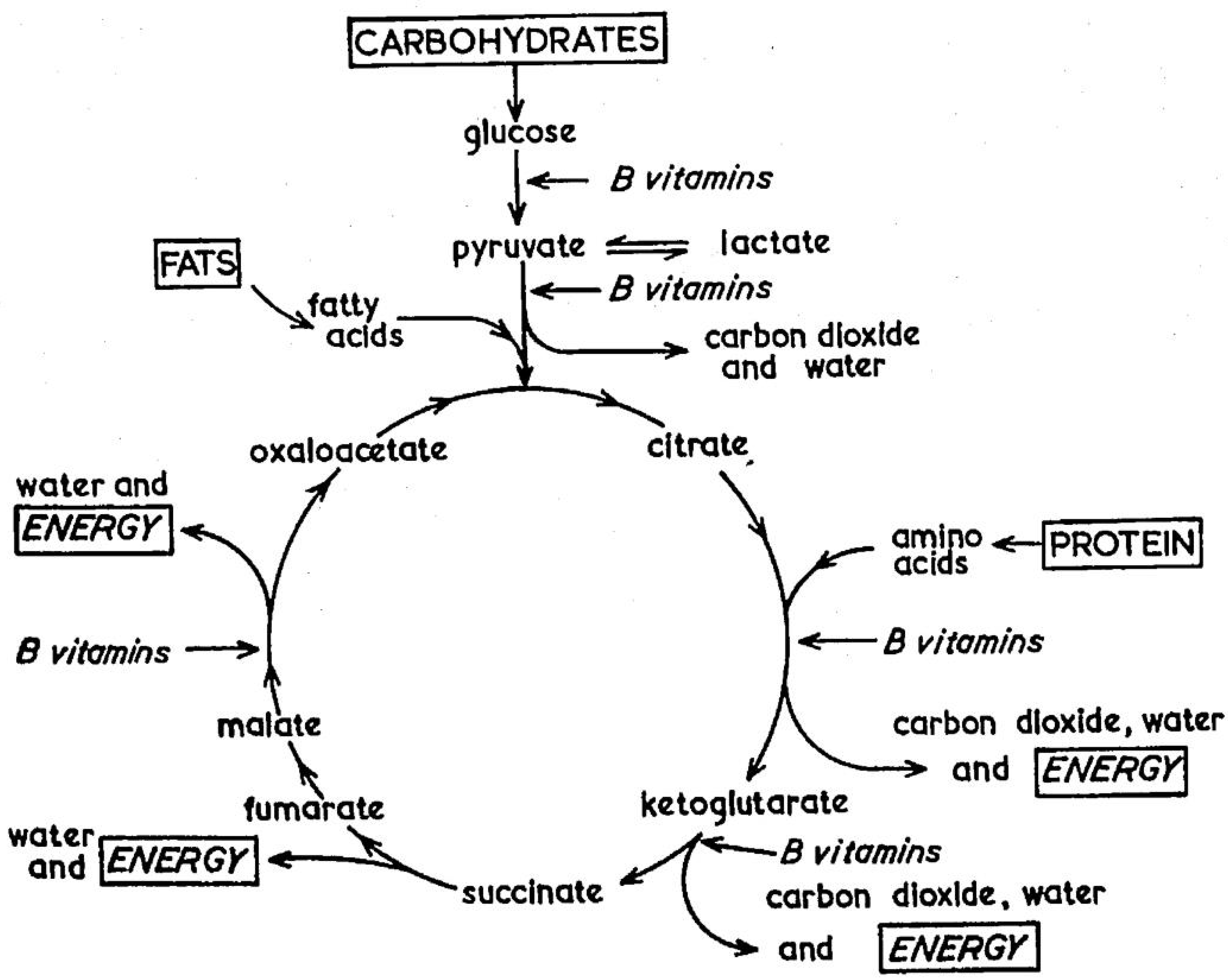

Fig. 1

Diagram to indicate how protein, carbohydrate, and fat may be oxidized to yield energy through a common metabolic path known as the citric acid cycle.

source of energy, and weight for weight, fats yield more than twice as much energy as carbohydrates. Although carbohydrate may replace fat as a source of energy, it is desirable that diets should contain some fat to promote the absorption of fat-soluble vitamins. Furthermore, *choline* and the *essential fatty acids*, which are known to be essential food factors, occur in foodstuffs as components of lipids. Oils obtained from plants are much better sources of the essential fatty acids than are animal fats. In practice, it is impossible to devise a diet composed of natural foodstuffs which would also be devoid of fat.

MINERAL SALTS

Mineral salts are widely distributed in nature and occur in all foodstuffs. They are required by animals for the growth, replacement, and proper functioning of all body tissues.

Calcium, *phosphorus*, and a small amount of *magnesium* are the chief constituents of bones and teeth. Calcium and phosphorus cannot be efficiently utilized except in the presence of vitamin D. During the period of rapid growth considerable amounts of calcium and phosphorus (and vitamin D) are needed, but the requirement falls off as the adult state is reached. During pregnancy and lactation there is a renewed demand for calcium and phosphorus for the formation of bone in the foetus and because these salts are secreted in milk. Calcium also occurs in blood plasma, and plays an essential role in the clotting of blood.

Sodium, potassium, and chlorine are present in the soft tissues and fluids, where their function is to maintain the osmotic pressure and the acid–base equilibrium. These salts also play an important part in water metabolism. Sodium, potassium, and chlorine are not stored in the body, any excess being excreted in the urine. These salts must be available from the diet throughout life.

Iron is present in the body in a small, but important, amount. It is a constituent of the respiratory pigment haemoglobin, present in red blood cells. Once iron has been absorbed by the body it is held tenaciously and is not readily excreted, even in milk. The requirement for iron in adult life is therefore low, except during reproduction. Most young are born with considerable reserves of iron. The notable exception are piglets, which frequently suffer from anaemia (perhaps because sows have been bred to throw numerically large litters), and it is good practice to administer iron to young pigs. Anaemia due to iron deficiency can occur in any animal at any time of life should the supply of iron fall below the requirement for haemoglobin formation, e.g. following haemorrhage.

It should be remembered that there are other types of anaemia due to specific diseases of the blood-forming organs and to protein and vitamin deficiencies.

Iodine is present in the body in very small amounts and is found chiefly in the thyroid gland. The thyroid secretes an iodine-containing hormone called thyroxine, which controls the metabolic rate of the body. A deficiency of iodine leads to enlargement of the thyroid gland—a condition known as goitre. Iodine must be supplied from the diet throughout life. Goitre is uncommon among laboratory animals.

Other elements are known to be essential, in small amounts, to the animal body (e.g. copper for blood formation). These are known as the '*trace elements*'. Many other elements are normally present in the body, perhaps performing some useful function or perhaps there by accident, having been ingested with the food.

Although mineral salts occur in all foodstuffs, it is customary to ensure that adequate amounts are present in a diet by adding up to 1 per cent of chalk and common salt (to supply calcium, sodium, and chlorine) and a mixture of trace elements. All cereals are rich sources of phosphorus. Other good sources of elements in a natural diet are milk (calcium), fish (iodine), liver and muscle (iron), and vegetables.

VITAMINS

Vitamins act like catalysts in many metabolic reactions; that is, the vitamins take part in these reactions without themselves being used up. Because vitamins behave like catalysts they are required only in minute amounts. Some vitamins are quickly excreted from the body and therefore need to be replaced frequently from the food. Other vitamins (notably the fat-soluble vitamins) can be stored within the body for several months.

FAT-SOLUBLE VITAMINS

VITAMIN A

Sources

Vitamin A does not occur, as such, in plants, but its precursor, *carotene*, does occur in plants. Carotene can be converted to vitamin A within the alimentary tract. Carotene is found in green, leafy materials and, generally speaking, the greener the leaf, the more carotene it contains. Grains, roots, and tubers, with the exception of carrots, are poor sources. Halibut- and cod-liver oils are rich sources of vitamin A.

Storage and stability

Vitamin A is quickly absorbed by the animal body and is stored in the liver. Animals can store sufficient of this vitamin to meet their needs for two or three months.

Outside the body, both vitamin A and carotene are easily destroyed by oxidation when exposed to air and light.

Function and signs of deficiency

Vitamin A is necessary for the physiological process of seeing, and a deficiency results in slow dark adaptation and night blindness.

Further, a deficiency of vitamin A leads to a general degenerative change in the epithelial tissues of the body, thus lowering the resistance of these tissues to attack by infective organisms. The tissues of the eye, skin, and reproductive organs are the most obviously affected, and blindness and sterility may ensue.

The malformation of growing bone in vitamin-A deficiency may result in pressure on nerves by bone. Blindness and deafness have been reported from this cause.

VITAMIN D

Sources

Of all the vitamins, vitamin D has the most limited distribution in natural foodstuffs.

The only rich source is cod-liver oil. Unprocessed milk fat contains a variable amount. Seeds and growing forage crops are poor sources.

Function and signs of deficiency

Vitamin C is essential for the formation of the intercellular material in soft tissues and bone. If the intercellular material breaks down the bones become weak and may fracture; the gums swell and bleed and the teeth loosen; the walls of small blood vessels rupture, allowing the escape of blood into the surrounding tissues, causing haemorrhages throughout the body. Early signs of a deficiency in guinea pigs are loss of weight, staring coat, and a mincing gait, as if the feet were too painful to walk upon.

Scurvy is the name given to the deficiency disease associated with a lack of vitamin C.

THE VITAMIN-B COMPLEX

The known vitamins of the B-complex are aneurin (vitamin B_1, thiamine), riboflavin (vitamin B_2, vitamin G) nicotinic acid, pantothenic acid, pyridoxine (Vitamin B_6), *p*-aminobenzoic acid, biotin, folic acid, cyanocobalamin (vitamin B_{12}), and inositol. All are concerned with the metabolism (the breakdown and rebuilding) of the digested and absorbed components of foodstuffs. If food components cannot be metabolized energy cannot be released for use by the animal, and new tissue cannot be laid down nor existing tissues maintained. It is rare to encounter a deficiency of only one B-vitamin. The clinical picture is usually of a multiple deficiency—loss of weight; loss of appetite; apathy; skin lesions; anaemia; loss and/or greying of hair.

All species require B-vitamins for the proper functioning of their metabolic processes. All species are able to satisfy, to a greater or lesser extent, their requirement for B-vitamins from the synthesis of these vitamins by their intestinal micro-organisms. The ruminants are able to supply practically all the B-vitamins they require from this source. Other species, however, require a supplement of some B-vitamins from a dietary source.

Sources of B-vitamins

Liver; yeast; the germ and outer seed coats of cereals; green, leafy materials; milk.

None of the B-vitamins is stored by the body to any appreciable extent; like vitamin C, they have to be supplied continuously from the diet to all species standing in need of them. *Specific diseases* associated with deficiencies of particular B-vitamins are polyneuritis in birds (beri-beri in man) due to a deficiency of vitamin B_1, and black tongue in dogs (pellagra in man) due to a deficiency of nicotinic acid.

MEETING THE NUTRITIONAL REQUIREMENTS OF ANIMALS

In designing a diet to be composed of natural foodstuffs the first consideration is the amount and quality of protein the diet is to contain. Protein is an expensive item; therefore the amount of protein in a diet should not be greatly in excess of the consumer's requirements, and plant proteins, which are cheaper than animal proteins, should be used to the best advantage.

Enough is now known about the amino-acid content of foodstuffs and the amino-acid requirements of animals for it to be possible to equate, with some accuracy, the supply of these nutrients with the demand for them.

The quality of cereal proteins is usually limited by their lysine content. Lysine is one of the essential amino acids in which animal proteins are rich. It is common practice to use a mixture of cereals to supply the bulk of the protein portion of a diet, and to add a small amount of animal protein to guard against a possible deficiency of lysine. Animal protein is also a source of vitamin B_{12}, which does not occur in plant foodstuffs.

The quantity of protein in any one cereal depends on the variety of the cereal (e.g. soft, English wheat contains about 10 per cent of protein; hard, Manitoba wheat about 15 per cent). Recent work indicates that the soil on which a crop grows and the weather conditions during growth exert a great influence on the yield per acre, but little or no influence on the chemical composition of the crop.

It will be found necessary to have 80–90 per cent of cereals in a diet to obtain sufficient protein from this source. This amount of cereal provides not only protein but also carbohydrate in quantity, fat, fibre, minerals, and some vitamins. Fat of plant origin is a good source of essential fatty acids. In effect, the cereal provides all the required carbohydrate and fat, and most of the protein and vitamins. Thus, the remaining considerations are: (i) to enhance the quality of the protein by the addition of some animal protein, and (ii) to study the vitamin and mineral content of the diet and to make any necessary additions, e.g. calcium would need to be added to balance the high phosphorus content of cereals.

Since the major problem in designing a diet is to supply protein of the right value, i.e. of adequate quality in sufficient quantity, it is worth while considering this problem in a little more detail.

The need for protein is greatest during the period of rapid growth and reproduction, when new tissues are being laid down, and during lactation, when protein is secreted in the milk for the sustenance of the suckling. The qualitative requirements of different species for amino acids show a remarkable similarity, though the amounts required differ both from species to species and within any one species for different purposes, e.g. tissue maintenance and reproduction. The biochemical reactions involved in the metabolism of amino acids are only beginning to be understood.

Two diets having the same protein content as determined *chemically* do not necessarily have the same nutritive value when fed to animals, because the protein *quality* may be different. Diets having a high protein quality are those which supply all the necessary amino acids in approximately the right proportions and amounts for maintenance, or growth, or reproduction. Such a diet would give maximum results for minimum protein intake. Another diet may have its amino acids so ill balanced as to be inadequate to support growth (or maintenance, or reproduction) no matter how much of it is eaten by the animal. Amino acids which are not utilized for their specific function of protein nutrition are not entirely wasted, but are catabolized and provide heat and energy. The provision of energy is the function of the carbohydrates and fats; to use protein (the most expensive component of the diet) for this purpose is wasteful and uneconomic.

Ever since it has been known that the quality of proteins can differ widely much effort has been devoted to evaluating these differences. Methods of measuring protein quality have been developed which take into account *digestibility* (defined as the percentage of the ingested protein which is absorbed) and the *biological value* (the percentage of the absorbed protein which is retained in the body). These measurements are made by biological assay in which diets under tests are fed to young, growing rats. The product of the figures obtained for digestibility and biological value, divided by 100, is termed *Net Protein Utilization* $\left(\frac{D \times BV}{100} = \text{NPU}\right)$, and is a measure of protein *quality*.

However, the efficiency of protein utilization also depends on the total energy value of the diet, that is, the amount of energy which could be obtained from the diet if *all* the protein, as well as the carbohydrate and fat, were burned for this purpose.

The animal body does not distinguish between protein, carbohydrate, and fat as possible sources of energy in order to reserve all the available amino acids for tissue growth and replacement, and some protein is always burned to supply heat and energy.

The value for NPU falls as the protein concentration of a diet is increased above a certain level. When the protein concentration is high the concentration of carbohydrate and fat is correspondingly low, and therefore the body must burn protein in addition to carbohydrate and fat to satisfy its energy requirements.

If food intake is *restricted* so that an animal cannot completely satisfy its hunger, then some of the protein has to be burned to provide energy, and the efficiency of protein utilization (NPU) is again impaired. In this case the body has been forced to burn protein to satisfy its immediate need for energy at the expense of its need to renew or build body tissues. The feeding of additional carbohydrate and fat to supply energy would *spare the protein* for its proper function, and protein utilization (NPU) would be improved.

Obviously, then, when assessing the protein value of the diet as a whole account must be taken not only of the quality and quantity of protein present but also of the total calorie (energy) content of the diet, for this must always be sufficient to meet the energy requirement of the animal being fed.

Classically, the *quantity* of protein in a diet is measured by determining the nitrogen present in the diet and converting the nitrogen figure obtained to protein, using the assumption that proteins contain 16 per cent of nitrogen (i.e. protein = nitrogen × 6·25). But the quantity of protein in a diet can also be expressed in terms of energy, i.e. as the percentage of the total calories of the diet which could be supplied by the protein portion if it were used for energy purposes only. Energy is measured in Calories (Cals), and each gram of protein could provide 4 Cals. Then,

$$\text{Protein Cals } \% = \frac{\text{Nitrogen of diet} \times 6{\cdot}25 \times 4}{\text{Total Cals per 100 gm of diet}} \times \frac{100}{1} = \frac{2{,}500\ \text{N}}{\text{Total Cals}}$$

The product of the values obtained for protein quality (NPU) and protein quantity (protein Cals per cent), divided by 100, is termed *Net Dietary-*

protein Calories per cent (NDp Cals per cent) and is a measure of both quality and quantity of protein. Where direct biological assay methods are not available for the determination of NPU, protein values can be 'scored' from data to be found in food composition tables. The method of scoring is illustrated in Appendix I to this chapter, where Diet 41B is used as an example.

It is known that for reasonable growth in rats a diet in which the NDp Cals per cent = 8 is necessary. Such a value cannot be reached by diets having less than 9 per cent protein, however good its quality, nor with a protein having a quality of less than NPU = 50, whatever its concentration in the diet. It is of interest to note that it would be difficult to devise a diet consisting mainly of wheat which had an NDp Cals per cent of less than 8. Stock diets commonly contain about 15 per cent of protein from mixed sources; these well-tried diets are known to support growth and reproduction.

CUBED DIETS

Ready-made diets for laboratory animals have been introduced and widely used during the last twenty years. Such diets should be bought in small quantities (not more than about one month's supply) and used up completely before the next batch is opened. Storage of a diet increases the opportunity for infestation by parasites and moulds, and for rancidity to develop. Furthermore, some considerable time may elapse between the date of manufacture of a diet and the date of its delivery to a purchaser.

In using cubed diets great reliance is placed, initially, on the persons who formulated the diets, and thereafter on the compounders of the diets. It should be remembered that these recipes were designed to provide moderately priced diets which are adequate, rather than optimal, for growth and reproduction. Undoubtedly the growth rate of rats may be increased by supplementing the standard cubed diets. Whether or not it is desirable to do so depends upon personal opinion and preference, economic necessity, and experimental conditions.

The manufacture of cubed diets for laboratory animals represents an insignificantly small part of the milling industry of Great Britain and is consequently unlikely to command the undivided attention of millers. None the less, millers are conscientious persons who manufacture the diets according to the recipes, using ingredients of specified standards. No variations are made to the recipes without consultation with the buyer. It is, on rare occasions, a question of going without diet or accepting diet made up to a modified recipe. In this event it is preferable to have the diet made with a better (and more expensive), rather than a poorer-quality ingredient, since to use a poorer-quality ingredient might so reduce the overall quality of the diet as to render it inadequate for reproduction and/or growth. It is not easy to standardize processed foodstuffs. Fishmeal, which is widely used as a source of protein in animal diets, is one of the most difficult of foodstuffs to process without damage to its protein. It says much for the manufacturers of animal foodstuffs that it is rare for a laboratory to be faced with severe or enduring trouble arising from a dietary deficiency.

APPENDIX I

CALCULATION OF THE PROTEIN VALUE OF A DIET

The quality of a protein may be calculated by 'scoring' the amino-acid pattern of the protein against a known, adequate amino-acid reference pattern (see *FAO Nutritional Studies*, Nos. 15 and 16, 1957). The amino acids of the unknown protein are scored as a percentage of the amino acids of the reference protein pattern, and thus a score of 100 or more indicates that the protein is equivalent to the reference protein pattern. In theory each amino acid should be scored in turn to find which of the essential amino acids is 'limiting' the value of the protein as a whole. In practice, it has been found sufficient to consider only three amino acids: (i) the 'sulphur-containing amino acids' (SAA), *methionine and cystine* (the latter can partly replace methionine) for diets containing animal protein; (ii) *lysine* for diets in which cereals are the chief source of protein; and (iii) *tryptophan* for diets in which maize is the chief source of protein. The Food and Agriculture Organization of the United Nations gives recommendations for the amounts of the essential amino acids which should be present in each gram of protein nitrogen. These factors are:

Sulphur amino acids (SAA)	0·27 gm/gm N
Lysine	0·27 gm/gm N
Tryptophan	0·09 gm/gm N

The following table gives the amounts of amino acids, in grams of amino acids per gram of nitrogen, in some common foodstuffs. These figures were determined by biological assay in the Medical Research Council's Human Nutrition Research Unit.

FOOD	SULPHUR AMINO ACIDS (SAA) (gm/gm N)	LYSINE (gm/gm N)
Rice	0·197	0·155
Maize	0·156	0·127
Potato	0·160	0·130
Oats	0·202	0·168
Wheat	0·195	0·132
Meat	0·216	0·540
Fish	0·243	0·540
Milk	0·230	0·500
Legumes (peas and beans)	0·122	0·450
Groundnut	0·122	0·225
Soya	0·190	0·400

The amino-acid composition of proteins can be found by reference to published food tables, and then the amount of any chosen amino acid per gram of protein nitrogen may be calculated. The appropriate FAO factor is applied to this figure to obtain a 'score' for the protein value of the diet. The quality and quantity of protein in the diet may then be expressed as NDp Cals per cent.

The two following worked examples show: (i) the step-by-step calculation, and (ii) the data set out in tabular form—an arrangement which facilitates the calculation of a diet having several ingredients.

Examples

Diet: Whole wheat 90 per cent.
Skim milk powder (SMP) 10 per cent.

Chosen basis for scoring: sulphur amino acids (SAA).
Relevant data from food tables:

Wheat—8·9 per cent protein: 333 Cals per 100 gm; 0·20 gm SAA/gm N
Skim milk powder—34·5 per cent protein: 326 Cals per 100 gm; 0·23 gm SAA/gm N
Protein is assumed to contain 16 per cent of nitrogen.

Calculation of total nitrogen of diet

90 gm of wheat contains $\frac{8{\cdot}9 \times 90}{100}$ gm of protein

or $\frac{8{\cdot}9 \times 90}{100} \times \frac{16}{100}$ gm of nitrogen

$$= \frac{8{\cdot}9 \times 90}{100 \times 6{\cdot}25} = 1{\cdot}28 \text{ gm N}$$

Similarly, 10 gm SMP contains

$$\frac{34{\cdot}5 \times 10}{625} = 0{\cdot}55 \text{ gm N}$$

Then, total nitrogen in 100 gm diet is

$$1{\cdot}28 + 0{\cdot}55 = 1{\cdot}83 \text{ gm}$$

Calculation of sulphur amino acids in diet

90 gm of wheat contains 1·3 gm N, of which 0·20 gm/gm is SAA (sulphur amino acids).

Then 90 gm of wheat contains

$$1{\cdot}3 \times 0{\cdot}20 = 0{\cdot}26 \text{ gm SAA}$$

and 10 gm of SMP contains

$$0{\cdot}55 \times 0{\cdot}23 = 0{\cdot}13 \text{ gm SAA}$$

Therefore total SAA in 100 gm diet is 0·39 gm OR $\frac{0{\cdot}39}{1{\cdot}83} = 0{\cdot}21$ gm SAA/gm N.

Scoring

But FAO recommend 0·27 gm SAA/gm N
therefore score is

$$\frac{0{\cdot}21 \times 100}{0{\cdot}27} = 79 \text{ per cent}$$

This scoring gives numerical expression for the quality of the protein in the diet. The quantity of protein in the diet must now be expressed in terms which can be related to this expression of the protein quality.

Calculation of protein calories per cent of the diet

90 gm of wheat contains

$$\frac{90 \times 333}{100} = 299{\cdot}7 \text{ Cals}$$

10 gm of SMP contains

$$\frac{10 \times 326}{100} = 32{\cdot}6 \text{ Cals}$$

Therefore 100 gm of diet contains 332·3 Cals.

The total nitrogen in 100 gm of diet, calculated above, is 1·83 gm, which is equivalent to 1·83 × 6·25 gm of protein.

Proteins yield 4 Cals per gram.

Then the protein from 100 gm of diet would yield 1·83 × 6·25 × 4 Cals.

This amount as a percentage of the total Calories is

$$\frac{1{\cdot}83 \times 6{\cdot}25 \times 4 \times 100}{332{\cdot}3} = 13{\cdot}8 \text{ per cent}$$

It is now necessary to read off the value for NDp Cals per cent of the above diet from a nomograph which has been constructed to allow for the effect on NPU of the concentration of protein in the diet. On this nomograph it will be seen that the horizontal line for a protein score of 79 per cent intersects the vertical line for a

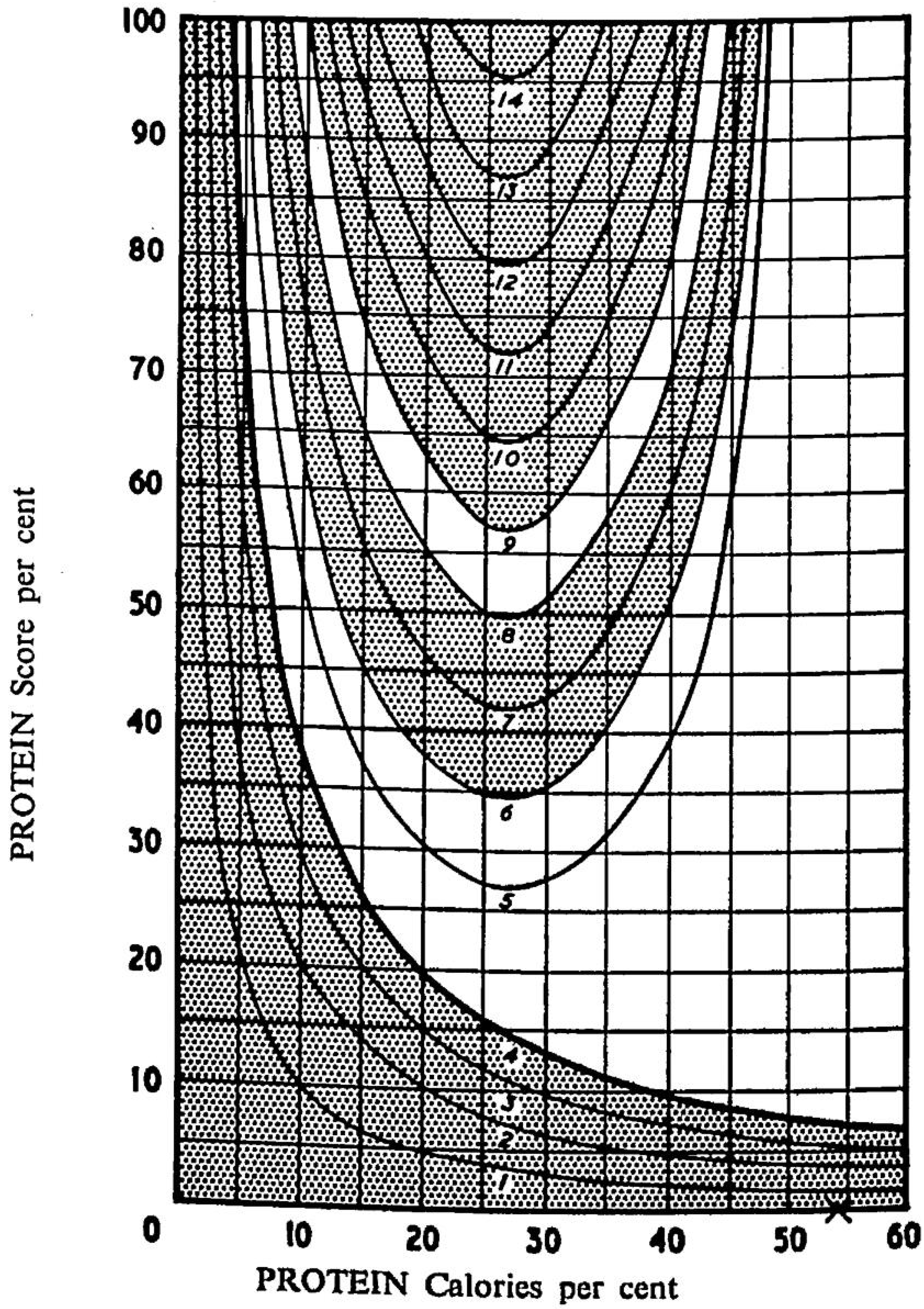

Fig. 2

Nomograph for the prediction of protein values of diets. The curves are lines of equal NDp Cals per cent.

protein Cals per cent of 13·8 close to the parabola marked 9. The above diet therefore has a calculated NDp Cals per cent of 9·2.

The foregoing calculations may be simplified by charting the data for any given diet as shown below, where diet 41B is used as an example.

Inspection of the nomograph reveals that: (i) when the protein Cals per cent is increased beyond 27 for a protein of any score, then the NDp Cals per cent begins to fall, because the protein is being metabolized for heat and energy; (ii) if the protein Cals per cent is less than 9 it is never possible to achieve an NDp Cals per cent of 8—the value thought to be required for a reasonable growth rate in rats; and, similarly, (iii) if the protein score is less than 50 it is never possible to achieve an NDp Cals per cent of 8.

DIET 41B

CHOSEN BASIS

SULPHUR AMINO ACIDS (METHIONINE AND CYSTINE)

Weight	Food	% P	% Cals	AA/gN[3]	Ng	Cals	AAg
a		*b*	*c*	*d*	$\frac{ab}{625}$	$\frac{ac}{100}$	$d\frac{ab}{625}$
47	Wheat flour[1] English, 100% extr.	8·9	333	0·20	0·67	156·0	0·134
40	Oatmeal[1]	12·1	404	0·20	0·77	162·0	0·154
8	White fishmeal[2]	61·0	365	0·22	0·78	29·2	0·172
1	Dried yeast[2]	41·5	360	0·10	0·07	3·6	0·007
3	Skim milk powder[1]	34·5	326	0·23	0·17	9·8	0·039
1	Salt (NaCl)	Nil	Nil	Nil			
100	Mixture	—	—	—	2·46	360·6	0·506
					e	*f*	*g*

Protein Cals % $= \frac{e \times 2500}{f} = 17{\cdot}1$

Score $= \frac{g}{e} \times \frac{100}{\text{FAO}} = 76\%$

NDp Cals % $= 9{\cdot}7$

SOURCES

1. *The Composition of Foods*, by McCANCE, R. A., and WIDDOWSON, E. M.
2. *Bulletin No. 48 of the Ministry of Agriculture, Fish, and Food.*
3 See table in the text.

APPENDIX II

NOTES ON CHEMICAL COMPOSITION OF FOODSTUFFS USED IN ANIMAL DIETS

Cereals

Wheat has a protein content of 8–15 per cent, depending on the variety. Soft, English wheats contain less protein than the hard wheats grown in Canada. The average figure for mixed wheats is 11·5 per cent of protein, 62 per cent of carbohydrate, 2 per cent of fat, 13 per cent of water, and fibre and mineral salts. The fibre content is low, about 2 per cent.

Oats and oatmeal contain about 12 per cent of protein. The fibre content of Sussex ground oats is about 11 per cent, but oatmeal (from which the hull has been removed) contains less fibre. Oats contain more fat than other cereals; ground whole oats 5–6 per cent of fat, and oatmeal 7–8 per cent.

Maize contains about 10 per cent of protein, but is notoriously deficient in tryptophan and lysine, two of the essential amino acids. *Yellow* maize, alone among the cereals, contains carotene (the precursor of vitamin A) equivalent to about one International Unit of vitamin A per gram. Maize contains about 4 per cent of fat as corn oil and is a good source of essential fatty acids.

Barley and barley meal contain about 10 per cent of protein and 5 per cent of fibre.

All cereals yield about 3·5 Cals per gram, and the whole grains are good sources of B-vitamins. Vitamin E is present in cereals, notably in wheat, but vitamins A, D, and C do not occur in cereals. Cereals contain considerable amounts of phosphorus and other minerals.

Cereal by-products. Bran, middlings, and offals are by-products of the milling industry obtained during the manufacture of flours and meals when the outer layers of the grains are removed. *Fine middlings* from wheat has a composition similar to that of whole wheat, but with a slightly higher fibre content. *Fine bran* (wheat) has a fibre content of 9–10 per cent. *Wheat germ* is rich in protein (30 per cent) and oil (9 per cent), and also contains considerable amounts of B-vitamins and Vitamin E. Wheat germ is liable to become rancid during storage, because of its high fat content. Vitamin E is destroyed in the presence of rancid fat.

Hays and grass meals

The protein content of hays varies with the ripeness of the crop. Hays cut before flowering and seeding have higher protein and lower fibre content than late-cut hays. The ranges are 7–15 per cent of protein and 20–30 per cent of fibre. Timothy, sainfoin, and seed hays have a higher fibre content than good-quality meadow hay. *Sun-cured* hays contain small and variable amounts of vitamin D.

Lucerne and grass meals also vary in composition with the ripeness of the crop. Lucerne in bud contains 22 per cent of protein and 18 per cent of fibre, but in early flower the values are 16 per cent and 25 per cent, respectively. The figures for grass meal are similar. These meals are good sources of carotene.

Greenfoods

Cabbage and similar vegetables are fed chiefly to supply vitamin C, though they are also good sources of carotene. Greenfoods contain about 90 per cent of water and about 2 per cent of protein. Good, fresh cabbage contains about 1 mg/gm of vitamin C. The carotene content is associated with the colour of the leaf, and the greener the leaf, the higher its carotene content. Cabbage leaves may contain carotene equivalent to between 50 and 2,500 IU of vitamin A per 100 gm. Kale contains more vitamin C and carotene than cabbage.

Food supplements in common use

Raw ox liver is a good source of vitamins. It contains about 75 per cent of water, 16 per cent of protein, and 14 mg/100 gm of iron.

Cod-liver oil is an excellent source of vitamin A (750 IU/gm) and vitamin D (9 IU/gm). It also contains iodine.

Whole milk has about 87 per cent of water, but the solids consist of all the nutrients, in approximately the right proportions, known to be required for animal growth. The vitamin A and D content of summer milk is higher than that of winter milk. The vitamin C content falls rapidly during storage, from 1·5 to 0·5 mg/100 gm in one day. Riboflavin is destroyed rapidly if the milk is exposed to sunlight.

Dried yeast is a good source of B-vitamins.

Protein-rich foodstuffs

Skim milk powder contains about 35 per cent of protein of good quality, 1 per cent of fat, 50 per cent of carbohydrate (as lactose or 'sugar of milk'), 5 per cent of water, and minerals. *Whole milk powder* (full-cream milk powder) contains about 27 per cent of protein of good quality, 30 per cent of fat, 40 per cent of carbohydrate, 1 per cent of water and mineral salts. Milk powders also contain B-vitamins and some vitamin C. Vitamins A and D are present in whole milk powder, but not in skim milk powder. Whole milk powder is sometimes *fortified* by the addition of vitamins A and D.

White fishmeal is a very variable product, its protein *quality* depending on the amount of heat-damage which had occurred during manufacture. Though, when analysed chemically, a fishmeal may be shown to have a high protein content, some of the protein may have been so badly damaged during processing that it can no longer be fully utilized by the animal body. The biological value of fishmeal can range from 20 to 80 (the maximum biological value attainable is 100). White fishmeal is manufactured from non-oily fish. In addition to protein it contains mineral salts, and is an important source of iodine and calcium (from the fish bones).

Oil-seed cakes and meals. These may be from linseed, groundnut, cottonseed, palm kernel, rapeseed, soya-bean, and many other oil seeds. They are by-products of the margarine, cooking fat, soap, and cosmetic industries. The removal of the oil leaves a product relatively rich in protein (30–50 per cent). The amount of residual oil depends on the extraction process, the lowest oil content being found in meals which have been solvent extracted. Meals which have been extracted with chlorinated hydrocarbons are toxic to animals, but other solvents are harmless. Oil-seed cakes and meals are specified as 'decorticated' or 'undecorticated' (indicating whether or not the seed coat or shell has been removed before processing) and as 'solvent extracted' if they have been subjected to such treatment.

Before cottonseed is used as animal feed it should be ascertained that the sample is free from gossypol—a toxic substance which sometimes occurs in cottonseed. Groundnut meal has recently suffered unfavourable criticism because some samples were found to be toxic, though the toxic effect does not manifest itself until the groundnut is fed at the 20 per cent level. Manufacturers of oil-seed cakes and meals are aware of the possibility of the presence of these toxins, and batches of oil seeds are tested and treated, if necessary, before they are put on the market.

The *quality* of the protein from oil-seed cakes and meals is not good, and these substances cannot be relied on as the sole source of protein in a diet. Nevertheless, oil-seed meals obtained from suitable processing remain a valuable source of protein for animals.

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TABLE 1

SUGGESTED DIETS AND SUPPLEMENTS FOR LABORATORY ANIMALS

All amounts are in gms or mls per animal/day unless otherwise stated

SPECIES	BASIC DIET	AVERAGE AMOUNT EATEN DAILY	SUPPLEMENTS	AVERAGE DAILY INTAKE OF WATER
Mice	Any of the recognized cubed diets, e.g. 41B	5	Usually none necessary, though some inbred strains may have to be supplemented	6
Rats	" " "	15	None necessary	24
Cotton Rats	" " "	15	5 cabbage	24
Hamsters	" " "	10	5 cabbage	8
Guinea pigs	SG1 pellets + a source of vitamin C + hay	30	VITAMIN C ESSENTIAL, usually supplied by 50 cabbage	85
Rabbits	SG1 pellets	100	None necessary except hay for lactating does	330
Ferrets	50 raw meat + up to ¼ pt. whole milk	—	—	Give water in addition to whole milk
Monkeys	As for mice + a source of Vitamin C	4% of body weight	VITAMIN C ESSENTIAL, usually supplied by 70 cabbage	Varies with size
Dogs	40 proprietary tinned dog meat (NOT meat and cereal) + 30 skim milk powder + 41B powder to satisfy appetite (up to 300) + 10 mls cod-liver oil. Mix and damp slightly with water		Double meat and milk ration for pregnant and lactating bitches. Warm bread and milk for pups from 3 to 10 weeks of age (in addition to basic died)	Varies with size
Cats	No complete proprietary diet yet available Suggested diet: Cooked horsemeat 50% Cooked peeled potatoes 43·25% Biscuit meal 6·5% Mineral and vitamin mixture 0·25%	Adults, 4% of body weight Growing, 6–8% of body weight	1·0 gm. special. feeding bone meal per cat per day ...	100–150
Fowls	Chicks: chick pellets (25% protein fortified) Growing: pellets 16% protein Adults: complete pellet	110–225	None	Varies with age

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