CHAPTER ONE

The Law and Laboratory Animals

Act. 39 & 40. Vict. Ch. 77, 1876 is popularly known as the Cruelty to Animals Act, and it regulates the use of living vertebrate animals (other than man) for experiments calculated to cause pain. It is administered by the Home Secretary in England, Scotland, and Wales. Licences granted by the Home Secretary are not valid in Northern Ireland, Isle of Man, Eire, and certain other places which are administered separately.

Experiments performed under the Act of 1876 are expressly excluded from the operation of the Protection of Animals Acts of 1911 and 1954.

I DEFINITIONS

The terms listed below are not defined in the Act of 1876; the following is only a guide to their interpretation.

(i) EXPERIMENT—a procedure the outcome of which is not known in advance (e.g. the inoculation of horses with known toxins to produce antisera is not an experiment and is therefore outside the Act).

(ii) LIVING—an animal is regarded as living so long as it is breathing and its heart is beating and any part of its cerebrum and basal ganglia is intact.

Experiments on pithed frogs or on cats in which the cerebral hemispheres and basal ganglia are destroyed are outside the scope of the Act. However, the pithing of warm-blooded animals (but not frogs) is regarded as an experiment under the Act; this is by recommendation of the Second Royal Commission of 1906.

(iii) VERTEBRATES—by convention the following are excluded from the provisions of the Act: larval forms of fish and amphibia (tadpoles before metamorphosis); avian and reptilian embryos before hatching; mammalian foetuses *in utero*, providing the mother is counted as an experiment under the Act.

Ambiguous cases should be referred to the Chief Inspector at the Home Office.

(iv) CALCULATED TO CAUSE PAIN—a procedure is calculated to cause pain if it is liable to interfere in a material degree with an animal's health, comfort, or integrity, e.g. pregnancy tests in which the injection of urine into an animal may induce ovulation or spermatogenesis (normal, physiological processes

which do not cause pain) are outside the scope of the Act. The inoculation of calves with material which may be tuberculous—and which may therefore interfere with the animal's health—comes within the scope of the Act.

II REGISTRATION OF PREMISES

Registration of premises where experiments are to be performed is a statutory requirement. There is no application form for this purpose. Written application, requesting premises to be registered under the Cruelty to Animals Act, 1876, should be made to the Under Secretary of State, Home Office, Whitehall, London, S.W.1, by the person or body in authority in the establishment, e.g. vice-chancellor of a university, senior officer of a government department, chairman or secretary of a management committee. A memorandum entitled *Experiments on Living Animals—Registration of Premises* is obtainable from the Home Office and gives information about the conditions which must be fulfilled before premises may be registered.

III LICENCES

Licences permitting the holder to perform experiments are granted by the Home Secretary. Every experiment under a licence alone is subject to restrictions, among which are the following:

(i). Throughout the experiment the animal must be under the influence of an anaesthetic of sufficient power to prevent the animal feeling pain. A local anaesthetic *may* satisfy this requirement.

(ii). The animal must be killed while still under the influence of the anaesthetic if any serious injury has been inflicted upon it or if pain is likely to continue after the effect of the anaesthetic has ceased.

In practice, recovery is rarely permissible in experiments under licence alone.

(iii). The experiment must be for the advancement by new discovery of physiological knowledge or knowledge which will be useful for saving or prolonging life or alleviating suffering in man or animals.

IV CERTIFICATES

Certificates are given by the statutory signatories, and the Home Secretary has the power to allow, disallow, or suspend (in whole or in part) certificates, but the Home secretary has no power to extend the scope of certificates. Possession of Certificate A releases a licensee from restriction (i) above attached to the licence, Certificate B from restriction (ii), and Certificate C from (iii).

CERTIFICATE A allows the simple procedures of inoculation and superficial venesection where an anaesthetic is neither necessary nor appropriate.

CERTIFICATE B allows the recovery of an animal from an anaesthetic where this is necessary to achieve the object of the experiment, providing the animal is killed at the end of the experiment.

CERTIFICATE C allows animals under general anaesthetic, without recovery, to be used in demonstrations before students and learned societies. However, there is no objection to *suitable* persons witnessing experiments performed in accordance with the provisions of the Act, whether under licence alone or under licence and any certificate.

CERTIFICATE E in conjunction with Certificate A and CERTIFICATE EE in conjunction with Certificate B are required for experiments with cats and dogs.

CERTIFICATE F, with or without A and B, is required for experiments with horses, asses, or mules.

V CONDITIONS ATTACHED TO LICENCES

Ten conditions are attached to and printed upon all licences. Additional conditions may be attached at the discretion of the Home Secretary.

CONDITION 1 lists the registered place(s) where the licensee may perform experiments. Animals may not be moved from one premises to another without the permission of the Home Office.

CONDITION 2 states that the licensee may not perform any experiment until he has been notified that the certificate covering the experiment has not been disallowed.

CONDITION 3, known as the 'pain condition', epitomizes the purpose of the Act, and the administration of the Act depends upon its strict observance. It applies to all experiments (except those under Certificate C) and states that:

(*a*) If, at any time, an animal is suffering pain which is *either* severe *or* likely to endure, and if the main object of the experiment has been attained the animal shall be painlessly killed forthwith.

(*b*) If, at any time, an animal is suffering *severe pain which is likely to endure* the animal shall be painlessly killed forthwith (whether or not the main object of the experiment has been attained).

(*c*) If an animal appears to an Inspector to be suffering considerable pain and the Inspector directs such an animal to be destroyed it shall be painlessly killed forthwith.

CONDITION 4, the 'limitation condition', states that under Certificate A (and E or F) no procedures more severe than simple inoculation and superficial venesection may be adopted.

CONDITION 5 applies to all experiments under Certificate B (and EE or F) and requires that all operative procedures shall be conducted under strict antiseptic or aseptic conditions and with adequate anaesthesia. If these precautions fail and pain results the animal shall be painlessly killed forthwith.

CONDITION 6 applies to all experiments under Certificate C, and requires that on the completion of the experiment the animal shall be killed forthwith by, or in the presence of, the licensee.

CONDITION 7 states that no experiments using curare (or substances exerting a similar action*) shall be performed without the special permission of the Secretary of State, and that forty-eight hours notice of the performance of any such permitted experiment, or series of experiments, shall be given to the District Inspector.

This condition arises from the fact that curare is not for the purposes of the Act deemed an anaesthetic.

CONDITION 8 states that the licensee must keep a written record of all experiments which shall always be available for examination by an Inspector, and he (the licensee) shall, annually, report the number and nature of his experiments to the Secretary of State.

CONDITION 9 states that cinematograph films of experiments on animals may not be made and/or exhibited without the consent of the Secretary of State. Films of apparatus used in such experiments are exempt from this condition. Consent under this condition may be sought in general terms and not with reference to a particular film.

The object of this condition is to prevent the exhibition of such films to non-scientific audiences.

VI OTHER PROVISIONS OF THE ACT

i. SECTION 6

Any exhibition to the general public of experiments on living animals calculated to give pain is illegal. This prohibition does not apply to a licensee's colleagues or assistants, but otherwise only the Home Office Inspector has a legal right to see animals under experiment.

Penalties for infringement—£50 for first offence.
—£100 or imprisonment for a period not exceeding 3 months for second and subsequent offences.

ii. SECTION 10

The Secretary of State appoints Inspectors who must visit, periodically, all registered premises to ensure compliance with the Act. These inspections are unannounced.

The Royal Commission (1906–12) recommend, endorsed by the Howitt Committee (1951), that all Inspectors should hold medical qualifications.

iii. SECTION 21

Protects licensed persons from prosecution under the Act except after the written assent of the Secretary of State. It is doubtful if the Home Secretary has ever given this permission, since his powers of revocation and cancellation are such as to render the need to prosecute unlikely. The effect of this section is to protect licensees from irresponsible or malicious prosecutions.

* 'Curare-form' substances are those which, in the dose used, produce motor paralysis without anaesthesia.

iv. SECTION 8

The Secretary may license any person whom he considers qualified to perform experiments upon living animals. Graduate scientists are normally licensed to do any experiment which their duties demand and their skill permits. Senior students may be licensed, usually with a supervision condition attached. Technicians may be licensed for procedures of a repetitive nature, or to cover emergencies, and may have a supervision condition attached.

VII ON FILLING IN FORMS

The following notes are complimentary to, and should be used in conjunction with, those printed on the various forms of application.

Application forms for licence (price 4*d.* each) and certificates (3*d.* each) are obtainable through any bookseller or direct from H.M. Stationery Office at:

York House, Kingsway, London, W.C.2.
13a Castle Street, Edinburgh 2.
39 King Street, Manchester 2.
2 Edmund Street, Birmingham 3.
1 St Andrews Crescent, Cardiff.
Tower Lane, Bristol 1.

i. APPLICATION FOR LICENCE

(*a*) PREMISES—these should be registered.

Where it is necessary to conduct experiments in the field the licence may be made available provided the Inspector is given sufficient notice of the performance of such experiments to enable him to be present if he so desires. As and when additional or alternative places are needed the licence must be endorsed by the Home Office before it is valid at the new place(s).

(*b*) NATURE OF PROPOSED EXPERIMENT. A broad description only is needed here, since the licence covers all vertebrates, except equidae, for experiments conducted wholely under anaesthesia and from which there is no recovery.

ii. CERTIFICATE A

For experiments in which an anaesthetic is impracticable. The procedure involved should be described in terms such as: injection; inoculation, withdrawal of body fluids; administration of substances by enteral or parenteral routes; inhalation; external applications; feeding experiments, the animal being allowed to satisfy its hunger and thirst; exposure to infections, to rays,* to variations of temperature* and/or atmospheric pressure.*

ANIMALS TO BE USED—vertebrates other than cats, dogs, and equidae.

* The circumstances necessitating these procedures should be explained.

iii. CERTIFICATE B

For experiments under anaesthetic from which the animal is allowed t recover. The certificate is appropriate to minor procedures, such as biopsy or if an anaesthetic is administered to immobilize an animal for a proce dure specified under Certificate A. However, its main purpose is to cove surgical operations which must be accurately indicated under 'descriptio of experiments to be performed', and the words used should not b capable of a broad interpretation—if biopsy only is intended, stat 'biopsy'. The species or class of animals to be used must be specified.

iv. CERTIFICATE C

Covers experiments to illustrate lectures in medical schools, colleges, etc. No experiment or demonstration may be performed on a conscious animal. A description in broad terms suffices, e.g. 'experiment to demonstrate the fundamental facts of physiology and pharmacology'. As Certificate C applies also to demonstrations before learned societies, it is convenient to apply for permission to conduct experiments at: (i) the normal place where teaching is done, and (ii) 'Meetings of learned societies held in premises registered under the above Act'.

Further (under description of proposed experiments) to specify 'Demonstrations (i) to students of science/medicine at the place first named above, of the fundamental facts of physiology/pharmacology, and (ii) to members of learned societies, of newly discovered physiological facts or facts which will be useful to them in alleviating suffering or saving or prolonging life'. The Home Office will always require notice of the licensee's intention to perform experiments before learned societies, and this will be written into Condition I of the licence.

v. CERTIFICATE D

Has been obsolete for many years.

vi. CERTIFICATES E, EE, AND F

The purpose of these certificates is well explained in the notes printed upon them.

It is *essential* that the description of proposed experiments be the *same* as that on the accompanying A or B certificate.

Certificate A accompanies Certificate E and/or F; Certificate B accompanies Certificate EE and/or F. Though one Certificate F can accompany both Certificates A and B, in practice it is better to submit two F's, one to cover A and one B.

vii. AN UNDERTAKING

Is generally required on behalf of all applicants from overseas. The undertaking is in set form (obtainable from the Home Office) and should be signed by some senior member of the department, who thus makes himself responsible for the proper observance by the applicant of the provisions of the Act.

TABLE SUMMARIZING THE REQUIREMENTS FOR LICENCE AND CERTIFICATES IN DIFFERENT CIRCUMSTANCES

Procedure	Vertebrates other than cats and dogs	Vertebrates other than equidae	Vertebrates other than cats, dogs, and equidae
Under anaesthesia without recovery	Licence + Cert. F	Licence	Licence
Under anaesthesia with recovery	Licence + Certs. B & F	Licence + Certs. B & EE	Licence + Cert. B
No anaesthesia employed	Licence + Certs. A & F	Licence + Certs. A & E	Licence + Cert. A
Lectures and demonstrations, under anaesthesia without recovery	Licence + Certs. C & F	Licence + Cert. C	Licence + Cert. C

VIII ANNUAL RETURN OF EXPERIMENTS

In mid-December all licensees receive a printed form for the return of experiments. This form must be completed and returned to the Home Office *by* January 14th.

(*a*) Normally, *one animal counts as one experiment*. Some trivial procedures under Certificate A ONLY may leave the animal entirely normal; if such an animal is returned to stock and subsequently used for another experiment, then it is counted as another experiment.

(*b*) An experiment involving procedures under more than one certificate is returned as *one* experiment under the more severe certificate.

(*c*) An experiment begins with the first interference with an animal's health, comfort, or integrity, and ends at its death or complete recovery and (under Certificate A *only*) its return to stock.

(*d*) An experiment conducted by more than one licensee is returned as a conjoint experiment by all the licensees concerned unless one licensee performed the main part of the experiment (the others acting only as assistants), in which case the experiment is attributed to the principal licensee only.

Returns from licensees reporting conjoint experiments should tally.

Note. A useful memorandum entitled *Notes on Plurality of Experiments* may be obtained from the Home Office.

VIII DELEGATION

Licences and Certificates are legal documents, personal to the holder, and delegation of authority under them is expressly forbidden. It is stressed that there is no relaxation of this prohibition under Certificate C.

The Home Office gives the following guide to the interpretation of the term 'delegation':

(i) There is no delegation where two or more persons, each holding authority under the Act to perform a particular experiment, carry out conjointly the operative or other procedures involved.

(ii) Where necessary a licensee may permit anyone to administer anaesthetics to an animal subject to his experiment.

(iii) He may allow another person to carry out mechanical duties. Thus, a licensee may, for instance, employ an assistant to hold an animal while he gives an injection or to administer a diet he has prescribed, or, while he carries out operative procedures, to control haemorrhage, hold retractors, or to undertake equivalent subaltern duties.

(iv) Subject to the above, the prohibition on delegation is absolute, and a licensee may not allow another person, licensed or unlicensed, to take part in his experiment, even under his supervision or when he himself is present.

IX OBSERVANCE TO THE ACT

The Home Office looks to licensees to give strict observance to the Act, the conditions attached to licences, and to the wording of certificates. Infringement can lead to revocation of a licence or even the cancellation of registration of premises. From neither of these decisions is there any appeal.

X ON PURCHASING CATS AND DOGS FROM DEALERS

Dogs seized by the police under the authority of the Dogs Act of 1906 may not 'be given or sold for the purpose of vivisection'. They could conceivably be handed over for laboratory procedures outside the Act of 1876 (e.g. the preparation of distemper vaccine), but in practice this has never been done. This ban does not apply legally to cats, but in effect stray cats are equally inaccessible. There is consequently an ever-present danger that cats and dogs offered by dealers may be stolen animals, and laboratory workers are advised to take every precaution against being incriminated in this way. The practice in many laboratories is to require the dealer to sign a statement to the effect that the animal which he is selling is his own property; the following is a suggested form of undertaking for such a guarantee:

'I certify that these.....are my own property and have been obtained by legal means.

Signed.....

If a further safeguard is thought necessary the dealer may be asked to state the source of each animal.

PUBLICATIONS RELEVANT TO THE FOREGOING SECTION

Pamphlets entitled *Experiments on Living Animals—Registration of Premises* and *Notes on the Plurality of Experiments* are both obtainable from the Home Office.

The final Report of the Royal Commission on Vivisection (1912) and the Report of the Howitt Committee (1951) are obtainable from H.M.S.O.

Notes on the Law Relating to Experiments on Animals in Great Britain is issued by the Research Defence Society, 11 Chandos St., London, W.1.

CHAPTER TWO

Animal House Design

Let us begin by stating the obvious, lest it be forgotten. The animal house is where laboratory animals live and animal technicians work: it must therefore be built to accommodate both these functions as efficiently and conveniently as possible.

It is a matter of general policy, outside the scope of this chapter, whether the animal house be attached to the laboratory or in a separate building; on the top storey, in the basement, or somewhere in between: a single floor or several; serving all departments of a university or research institute collectively, or only one. Other considerations will also determine whether the animals used will be bred on the premises or imported from elsewhere. However, all these factors affect the layout, and they must therefore be known before work begins on the design of the animal house.

In Chapter 2 of the *UFAW Handbook*, 2nd edition, it is explained that there are four basic subdivisions of all animal houses. These are:

(1) Accommodation for normal animals, whether bred or held between importation and use.
(2) Accommodation for animals under experiment.
(3) Stores for clean materials—food, bedding, clean cages, and utensils.
(4) Dirty area—for cage cleaning, disposal of soiled bedding, etc.

These four subdivisions must be physically separate, and will in nearly all cases be themselves subdivided. Their siting, in relation to each other, must take into account the pattern of traffic between them, so that, for example, routes for the conveyance of clean material are not crossed, at least at the same time of day, by dirty material—an elementary precaution of hygiene all too easy to overlook.

In the *clean animal area* will be kept breeding colonies and all stock animals awaiting use, whether they come from an indigenous breeding unit or from elsewhere. In the latter case provision will be made for suitable quarantine. The rule here is that animals only go out, to the experimental side; no animal will enter, except imported animals, and then only after adequate quarantine. No experimental animals, not even long-term ones, must ever be given house room in the clean side.

The *experimental animal area* normally draws all its animals from the clean area, although exceptionally, imported animals may go directly into the experimental area after suitable quarantine.

The *clean store area* has to accommodate supplies of food and bedding, and to store clean cages, water bottles, and other utensils. Here may also be found storage for special clothing, stationery, and, most important, a small office for the head technician, where records are kept and the general housekeeping is controlled.

The *dirty area* is devoted to cleaning cages and utensils, sterilizing, and disposal of soiled bedding and carcases, by incineration or otherwise. Cages after treatment are then returned to the clean area.

Thus we have a key to the general layout of any animal house.

Because the movement of all material will be on wheeled vehicles, steps are ruled out. If difficulties of level are inevitable gentle ramps must be provided, bearing in mind that a heavily loaded trolley can run away with a technician on a steep or awkward slope. If the animal house is on more than one storey a lift is essential; preferably two lifts or hoists, one for clean traffic, and the other for dirty.

Corridors should be wide enough for the traffic expected. In most cases it should be easy for two trolleys to pass in the corridor without obstruction. The animal technician has the duty of working out the volume of this traffic, so that he can advise the architect on the requisite width of corridors.

ANIMAL ROOMS

Wherever possible, rooms should be designed for use by any species, rather than specifically for mice, guinea pigs, rabbits, and so on. However, certain animals, such as dogs, cats, farm animals, and monkeys, are likely to require special accommodation, such as soundproof rooms, fixed stalls, exercise runs, and internal wire subdivisions. This must be accepted, but rather as a penalty: it limits the flexibility of the animal house. For the common species, animal rooms should be adaptable and interchangeable.

In considering the size and shape of animal rooms it is useful to visualize something that has for most purposes many advantages. Departures from this notional animal room can then be worked out for specific reasons. The notional module may be a room 8–10 ft wide and 12–20 ft long. It can be economically racked along the two long sides, with a door at one or both ends, giving a free working area down the middle. But breeding rooms may well be much larger, and rooms to accommodate island racking, rather than wall- or ceiling-mounted racking, may be square and probably two or three times the size of the notional module. Cats, rabbits, and larger animals may require much larger rooms. On the other hand, isolation animal rooms have usually to be small and numerous.

All animal rooms have to be washed or hosed down from time to time. This governs the structure and finish. Floors must be impervious to water, to disinfectants, urine. and, ideally, to acids, alkalis, and organic solvents. There is no flooring that meets all these requirements. and the choice lies between asphalt, granolithic terrazzo quarry tiles, smooth cement, and possibly rubber or some of the new plastics. If they are level they have to be mopped and squeegeed; if they slope the gradient must be enough to ensure a good run away—not less than $\frac{1}{8}$ in. per foot. A gulley or drain is necessary in either case,

either a glazed half channel covered with a removable metal grill or a corner or centre drain; in every case well trapped.

Corners should be coved, with a skirting integral with the floor and, at its upper edge, flush with the wall, rather than standing proud of it.

Walls may be of fair-faced brick or concrete block, hard plaster or cement, covered with a suitable paint; or of granolithic terrazzo or tiles, either all the way up or as a dado up to about 6 ft, with plaster, brick, or other surface above. The corners of all walls must be rounded.

Ceilings do not have to be capable of being hosed, although this is an advantage, but it must be possible to mop them down. Hard gloss paint is a suitable finish.

Every effort must be made to keep plumbing, conduits, and other services buried, either chased into the walls or carried in an impervious service duct. Where this is not possible, pipes should be held an inch or two out from the walls, so that it is easy to clean behind them. Pipes coming through the walls must be closely sleeved as a precaution against insect pests. All electric fittings must be waterproof.

Doors should be close fitting and proof against even a young mouse. This means that not more than a ¼-in. gap can be tolerated. Steel doors have certain advantages, but they are heavy and noisy. Wood is lighter, but requires to be covered over the lower 4 ft or so with metal. The door frames can be of metal or metal clad like the doors. All doors should have observation panels at eye level. Lever-operated door handles are a great advantage; also rising butt hinges.

The provision of windows is open to argument. Bearing in mind that artificial lighting is no disadvantage, at least to rodents, and that ventilation must in all modern houses be ducted in, or out, or preferably both in and out; windows merely complicate the problem of adequate heating and ventilation, as well as letting in direct sunlight, which is usually undesirable. But technicians may prefer to work in daylight and to open the window on occasion, and many people, including the Home Office Inspector, regard natural daylight as highly desirable if not obligatory for dogs, cats, and large animals. If animal-room windows face north-east direct sunlight will not be a serious problem, but if they face south sun-blinds will be necessary. Venetian blinds are the most efficient, but are serious dust traps; they should therefore be fitted in the space between double windows.

The provision of windows, then, is a debatable point. Artificial lighting is no disadvantage to most species of laboratory animal, and in breeding rooms a controlled light cycle, rather than the seasonal variation in length of daylight, may be necessary to regulate breeding programmes, and therefore windows, if present, will need to be screened. But whatever decision is come to, it should be based on functional considerations, and the architect instructed accordingly.

SERVICES

Animal rooms, corridors, and most other parts of the animal house need to be frequently washed down. Drains and gulleys must be generously provided, and of a pattern that makes cleaning out easy. Drains should be trapped to collect sludge, and it must be impossible for wild rats or mice to gain access to

the animal rooms through the drains. (This would seem an elementary precaution, but it has not seldom been overlooked, with unfortunate consequences.)

Each animal room normally needs a small sink, for washing hands, filling water bottles, but *not* for washing up cages and utensils, which should be done elsewhere. Hot and cold water should be provided and, if necessary, a separate cold drinking-water supply from the mains and another point for a hose.

The lighting intensity, both natural and artificial, should be a good working light, but it is often useful to have a movable source of more intense light, such as can be provided by an Anglepoise lamp. It is wise to fix a time switch to the lighting circuit. It may never be used; on the other hand, it is an inexpensive device, and may prove to be invaluable.

There should be at least one 5-amp power source in each room, for aerosol generators, electrical balances, and other pieces of equipment.

High-pressure water for hosing down may sometimes be required, especially for rooms containing monkeys, dogs, and large animals, where cages or stalls may be inconveniently large or fixed. In a new building consideration should be given to piping high-pressure water to each room.

HEATING AND VENTILATING

Modern animal accommodation is expensive, all the more so if extravagant use is made of the available space. To rely for ventilation solely on opening the window will reduce the usefulness of an animal room to the point of extravagance. For this reason, mechanical ventilation of some kind is obligatory in the modern animal house.

Full air-conditioning, which means making provision for heating and cooling, humidifying and dehumidifying, and controlling the quantity of incoming air, is the best of all methods, and also the most expensive. In the equable climate of the British Isles there will be a great economy at small sacrifice in working and living conditions if the cooling and the humidity control are omitted.

It is a sound principle to provide background heating for the whole animal house up to the normal comfort zone, which is usually taken (in England) to be about 65–70°F (18·3–21·1°C). Animal rooms can then be equipped with supplementary heating devices, controlled by individual thermostats, which can raise the temperature to 75° or 80°F (21·1° or 26·7°C). The background heating may be by incoming air, by radiators, by heated panels, heated floors, walls, or ceilings, or by convectors, and the supplementary boosting by extra heating batteries—steam, hot water, or electric—in the air input duct, by electric water or steam tubular heaters, or by convectors. There is a wide choice of method, and this is not the place to discuss it in detail, but it should not be forgotten that the heat produced by the animals and the technicians working in the animal rooms may raise the temperature as much as 8°F (4·5°C).

Heating and ventilation is a highly technical subject, but still much of an art, depending as much on intelligent guesswork as on hard facts. The following points, therefore, are merely a guide for discussion with the heating and ventilating engineer or consultant.

Every part of the animal room must be equally ventilated. Draughts are to be avoided, and also stratification of the air. Air should come in at low level and be removed at high level, on the opposite side of the room, but for the sake of added human comfort, some air may be brought in at head level.

In breeding and other clean animal rooms the pressure of air in the room should be higher than outside, to reduce the possibility of infection being sucked into the room. Conversely, infected animal rooms should have a negative pressure.

The size of ducts should be such as to reduce the air noise to a negligible level, and the openings should be so fitted that they can be hermetically sealed, in the event of it being necessary to close a room for fumigation.

Incoming air should be filtered to remove dust particles. Outgoing air from infected rooms may need to be sterilized, by ultra-violet irradiation or other means. Short-circuiting of air currents between input and output, whether in the rooms or in the main duct system, should be virtually impossible. Re-circulation is not advised. There may be a penalty in the heating bill, but the risk of spread of infection is a more important consideration.

The test of a good ventilation system is the absence of unpleasant smell in the animal rooms; provided, of course, that a high standard of hygiene obtains.

SANITATION

Sanitation includes the washing and sterilizing of cages and utensils, and the measures that have to be taken to keep the animal house generally clean.

The provision of mechanical means of cage and bottle washing should be the normal practice. Only very small animal houses can afford to do without them, for they represent a great saving of labour, and of an unpleasant kind. Most such machines require a supply of steam, water, and electricity, which must be foreseen in the planning of the animal house.

Autoclaves, which are, for practical purposes, the only sure means of bacteriological sterilization, are expensive. They are necessary in an animal house where dangerous pathogens are being used, but in many animal houses the only pathogens are likely to be those associated with accidental intercurrent infections, and free steaming or treatment in an efficient mechanical washer will destroy all of these. And it should be remembered that the cost of an autoclave is about twice that of a cage washer of comparable capacity, and the autoclave does not wash the cages.

Similarly, bottle-washing machines are cheap and labour saving. Their omission from the animal house should be strongly challenged.

HYGIENE

The animal house is a dense population of susceptible creatures, an ideal subject for epidemic disease. The standard of hygiene therefore must be high, as high as in a children's hospital.

There is a tendency today to provide all animal houses with showers for the staff, and this is to be vigorously recommended. Indeed, in the case of specific pathogen free (SPF) animal colonies such a provision is obligatory, and

since such colonies are likely to become more and more common, new animal houses must have showers, with adequate changing rooms.

The incoming technician therefore takes a shower on entering, and puts on special clothing, or at least outer clothing, for working in the animal house. The same rule applies to visitors. Lest it be thought that such a rule is hopelessly impractical, it should be added that it applies mainly to breeding and normal animal divisions where the exclusion of extraneous infection is vital; that it is practised in many animal houses already; and that today's exception has a habit of becoming tomorrow's rule. The animal house that has no provision for this eventuality may therefore soon come to look embarrassingly archaic.

Ample provision needs to be made throughout the animal house for washing hands, when going from room to room, or from animal room to clean store, for example. Foot trays containing disinfectant outside the door of each room are an added hygienic precaution.

AMENITIES

Work in the animal house can be arduous, and the hours, especially at week-ends, may be irregular. A technician's room is seldom an extravagance, and may be a great asset. This is especially so in SPF units, where the internal environment may have a prison-like quality, and a resting place with tea-making facilities will be more than ordinarily welcome.

Toilet accommodation is also a necessity. Showers, which are not absolutely necessary except in strictly isolated units, are nevertheless a much appreciated amenity in almost all animal houses.

CONCLUSION

There is no such thing as the ideal animal house. Each one must be designed to serve the particular purposes for which it is going to be used. In all cases, however, certain general principles must be observed, which have been referred to in this chapter.

No reasonable man would plan a kitchen without seeking the advice of his wife, who will spend much of her time working there. In planning an animal house it would be equally unwise to fail to seek the advice of the animal technician who has to work there. For his part the animal technician must be prepared to give the sort of advice that only he can give, namely on the practical considerations that will affect the efficiency of his daily work.

Certain aspects of animal-house design have been omitted or only lightly touched on in this chapter, because they are more the concern of others than of the animal technician. They are, however, dealt with elsewhere, more particularly in the publications listed in the bibliography.

BIBLIOGRAPHY

WORDEN, A. N. and LANE-PETTER, W., (Editors), *The UFAW Handbook on the Care and Management of Laboratory Animals*, Universities Federation for Animal Welfare, London, 2nd Edition (1957).

Comfortable Quarters for Laboratory Animals, Animal Welfare Institute, New York (1958).

LANE-PETTER, W., *Provision of Laboratory Animals for Research: A Practical Guide*. Chapter 8, 'Physical Environment'. Elsevier Publishing Coy., Amsterdam (1961).

Various articles in the *Journal of the Animal Technicians Association* and *Proceedings of the Animal Care Panel*.

Various references in *Federation Proceedings*, 1960, **19**, No. 4, Part III, Supplement No. 6.

CHAPTER THREE

Animal House Equipment

The term 'equipment' covers a multitude of articles from spring clips, used to hold food containers or water bottles, to batteries of cages or large pens to house the animals. It can confidently be said that the success and efficiency of an animal house depends upon the working conditions and happiness of its technicians. It follows therefore that the suitability of the equipment they have to use is of paramount importance. While the majority of the work is done by hand, there are automatic and labour-saving pieces of equipment on the market which justify examination, and in some cases, installation; thereby relieving the technician for more productive work.

The cage, or pen, is perhaps the most important single item of equipment in the animal house, because its function is to contain the animal. There are many different sizes and types of cage in existence to accommodate the wide variety of animals used in the laboratory today. Almost every worker has his own ideas as to the most suitable design of cage for his particular purpose; an attitude which does nothing towards reducing the multiplicity of cages.

No attempt will be made to lay down hard-and-fast rules about cage design; rather an attempt will be made to offer constructive suggestions of a general nature and to draw attention to some of the pitfalls which may be encountered. Before discussing cage design it would be convenient to consider some of the materials available for their manufacture.

MATERIALS USED IN THE MANUFACTURE OF CAGES

(i) Galvanized iron

This is iron covered with a protective layer of zinc, and is probably the most popular material in use for cage making in Britain. Galvanized iron (or steel) is resistant to attack by alkalis but not by acids, including those found in urine.

Iron is quickly destroyed by oxidation (the formation of rust or iron oxide) when it is exposed to air and water. The protective zinc is also destroyed by oxidation, but the decay of zinc to zinc oxide on exposure to air and water is a much slower process than the rusting of iron. Furthermore, the formation of zinc oxide protects the underlying zinc from further oxidation. As the zinc oxide layer is worn away, fresh oxide is formed. This continues until all the zinc has been oxidized, after which oxidation of the underlying iron begins

and proceeds rapidly until holes appear in trays and the mesh of cages breaks. Items made from galvanized iron can be easily and cheaply regalvanized, providing this is done before the iron work has been allowed to rust. Regalvanized items can be expected to wear as well as new, galvanized items. All equipment made from galvanized iron should be inspected regularly for signs of wear and should be set aside for regalvanizing immediately rusting appears.

Iron may be galvanized either by dipping in molten zinc or by an electrolytic process. The latter method is not suitable for items intended for the animal house, as the layer of zinc deposited is too thin to offer adequate protection to the metal. Although galvanized-iron sheet and wire are readily available, it is customary to construct animal cages and trays from iron and to galvanize the articles after manufacture. Such a procedure ensures that all joins, seams, and bends are properly protected by the zinc coating.

Mild steel (which is iron mixed with a small amount of carbon) is the metal commonly used for cages and trays. Twenty-two-gauge mild-steel sheet is suitable for trays, and 16-gauge mild-steel wire on 10-gauge wire frames is suitable for grids of moderate area. Orders for cages and trays should specify 'galvanized after manufacture'.

(ii) Sheet steel

This is plated with tin (usually by a dipping and rolling process) and is known as *tinplate*. It is an easily worked material, very useful for 'mocking up' some new design of cage, but unsuitable for permanent apparatus, as the surface is soft and easily damaged, so that rusting of the exposed steel soon occurs, and spreads rapidly.

(iii) Sheet zinc

This is used for some equipment. It has all the desirable corrosion-resistant characteristics of galvanized iron, but it is soft and can be chewed through by rodents.

(iv) Aluminium

This is used widely for making trays, mouse boxes, and racking. It is important that 99 per cent pure aluminium is used, as some of its alloys corrode quickly in the presence of urine. Aluminium is a soft metal which is easily formed, but it cannot be joined by the common methods of welding. The special equipment needed for working this metal may not be available in cage-making plants which do not specialize in aluminium work. Aluminium has excellent resistance to corrosion by water and the acids of urine, but it is attacked by alkalis, e.g. common washing soda. An inhibited cleanser should therefore be used for washing aluminium equipment. The resistance of aluminium to corrosion is derived from the protective layer of aluminium oxide (about 0·0000005 in. thick) which forms on aluminium immediately it is exposed to air. This protective film may be increased to more than 600 times this thickness by an electrolytic process called anodizing.

The softness of this metal, which permits easy forming, also means that aluminium articles can be damaged easily. Thus, aluminium has only a limited application to equipment in an animal house.

Ninety-nine per cent aluminium of 16 gauge is recommended for mouse boxes.

(v) Stainless steel

This is a material which is likely to become more popular for cage manufacture. Its present high price arises from its being a difficult material to work and an expensive one to manufacture. It is highly resistant to atmospheric and chemical corrosion, and is extremely hard-wearing. If a number of cage designs became generally accepted and were available to buy 'off the shelf', then stainless steel would be the material of choice for these cages. Production costs would be much reduced by the methods of manufacture applicable to a steady market, and the durability of stainless steel would make the cost/life of such cages comparable with that of cages made from conventional materials.

Not all types of stainless steel are highly resistant to corrosion; the chromium–nickel types are most suitable for cages (Hoeltge, 1961).

(vi) Wood

This material still finds some favour, particularly for the construction of mouse boxes and rabbit hutches. It has a few advantages over the other materials in common use—it is warm, and the animals prefer it to metal. Its disadvantages are many. Wood must be treated with preservative if it is not to be rotted by urine. Wet sterilizing warps the wood, loosens glued joints, and rusts nails and screws. Animals chew at projecting or irregular parts of wooden cages, so much time has to be spent on the repair of such cages.

Mouse boxes made from resin-bonded plywood have proved fairly successful in use. They are the only type of wooden box which can reasonably be recommended.

(vii) Plastic materials

There are five basic plastic materials, three of which, fibre glass, polycarbonate, and polypropylene, can be autoclaved. Styrene–acrylonitrate copolymer and linear (high-density) polyethylene should not be exposed to high temperatures.

The low thermal conductivity of all plastic materials is suitable for most laboratory animals. They are light in weight, and boxes made from fibre glass, linear polyethylene, and polycarbonate will stand repeated impacts from rough handling, and they will not dent.

Most plastic cages can be stacked, and they are not attacked by cleaning agents, most disinfectants (except the cresol group), or animal waste.

Fibreglass reinforced polyester

This material has exceptional thermal stability and will not distort or deteriorate after repeated exposures to heat in the 250–290°F range (120–140°C). Impact resistance is good. The material can be machined.

Polycarbonate

This is a clear material with a very high impact strength and the only transparent plastic which can withstand temperatures of 280–290°F (138–145°C). It contains the optical and thermal properties of glass with the strength

properties of the new thermoplastics. Unlike most plastics, it does not shatter under high-energy impact by sharp objects. Even thin wall sections will not break when subjected to repeated hammer blows. (See Fig. 1 for specimen boxes.)

Polypropylene

This translucent material has good impact strength and a glass-smooth surface. The heat distortion point is in the range 215–230°F (101–109°C). It has excellent chemical properties and is resistant to most chemicals and solvents.

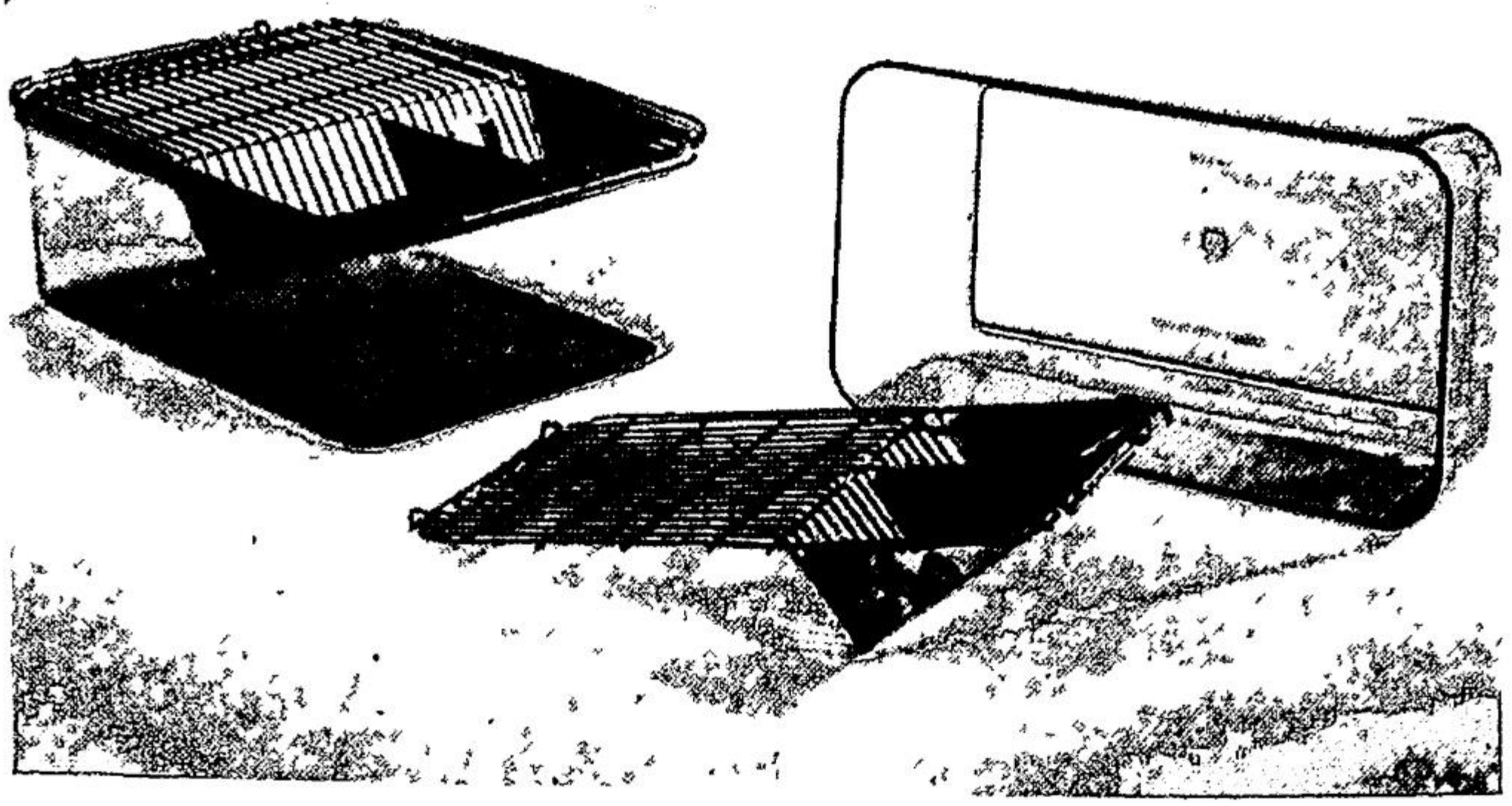

FIG. 1

Linear (high-density) polyethylene

This material is similar to polypropylene in looks and has excellent impact strength, but the heat distortion point is only 180°F (82°C), and if repeatedly exposed to high temperatures tends to warp. It cannot be autoclaved.

Styrene (acrylonitrile copolymer)

A clear material with a medium–low impact strength. The heat distortion point is about 180°F (82·2°C). It cannot be autoclaved.

Polystyrene

This is a clear material with low impact strength. The heat distortion point is about 150–170°F (65·5–76·7°C). Useful for rigid disposable cages.

If agreement could be reached on the standardization of basic cage designs better use could be made of modern materials such as stainless steel and plastics, for the cost of making but a few cages of any one design in these materials is prohibitive.

(viii) Glass

Tanks or jars of glass are sometimes used to house small animals such as mice, reptiles, and insects, but glass has a limited use as an animal container. It is

reasonably cheap, can be cleaned easily, and it allows direct observation of the creature it surrounds. The breakage hazard is considerable, and should always be borne in mind.

The foregoing remarks about the suitability of various materials for cage manufacture apply equally to other items of equipment, such as food baskets and hoppers.

CAGES

All cages have some features in common, whether they are used to house animals for experiments, or for breeding, or for stock. They must be strong enough to stand the day-to-day wear and tear and repeated temperatures of 250°F (123°C) in steam sterilizing and/or the rigours of chemical sterilization. They must also be practical in construction and easy to assemble and clean. A cage must be escape-proof; the animal should not be able to gnaw or break through the fabric of the cage, or be able to undo the door fastening. Doors and other openings should be well fitting, yet easy to open and close by using only one hand. Some animals—dogs, rabbits, and especially monkeys—have the ability and cunning to open cage doors unless these are firmly secured by fastenings placed out of reach of the animals or by locks.

The dimensions of ventilation holes and the mesh of floor grids and the distances between the upright bars forming the top and sides of a cage should be carefully chosen. The sizes selected may be suitable for adults and the weaned young of a particular species, but this could be large enough to permit the escape of sucklings. A day-old rat is a very active creature which can, and will, crawl through a $\frac{1}{2}$-in. square; and, having escaped from the nest, such a creature would die from starvation and exposure.

Wire-mesh grid cage bottoms are best made from welded wire and then galvanized. The size of mesh and the gauge of the wire are important, and should be appropriate to the animal which is to be supported by the grid. 12 s.w.g. wire and $\frac{3}{4}$-in. mesh is suitable for rabbits, and 20 s.w.g. wire and a $\frac{3}{8}$-in. mesh for rats and other small rodents. It should be noted that mesh size is measured from centre to centre of the holes in the mesh; the mesh size therefore *includes* the width of one strand of wire. Floor grids made from square mesh of a size larger than this is unsuitable for guinea pigs. The hind-legs of these animals easily slip through square mesh, and are often broken as the frightened animals struggle to free themselves. Weldmesh grids of 3 in. $\times$ $\frac{1}{2}$ in. mesh are recommended for guinea pigs or a $\frac{1}{4}$-in. mesh with the corners of the floor grid cut away (Fig. 2).

It is impossible to give definite advice about the sizes of cages. Lane-Petter (1957) quoted the following formula as a guide to the minimum dimensions for a cage in which an animal may be confined for a matter of weeks.

$$A = n(3W + 5/W) \text{ or } A' = n(0{\cdot}7W' + 6/W)$$

where A is the floor area in sq in. (A' in sq cm); W the weight of the animal in ounces (W' in grams), and n the number of animals in the cage. Lane-Petter recommends that neither the height nor the width of the cage should be less than the length of the animal, excluding its tail. This formula is said to hold good for the usual laboratory species from the mouse to the rabbit, but it is

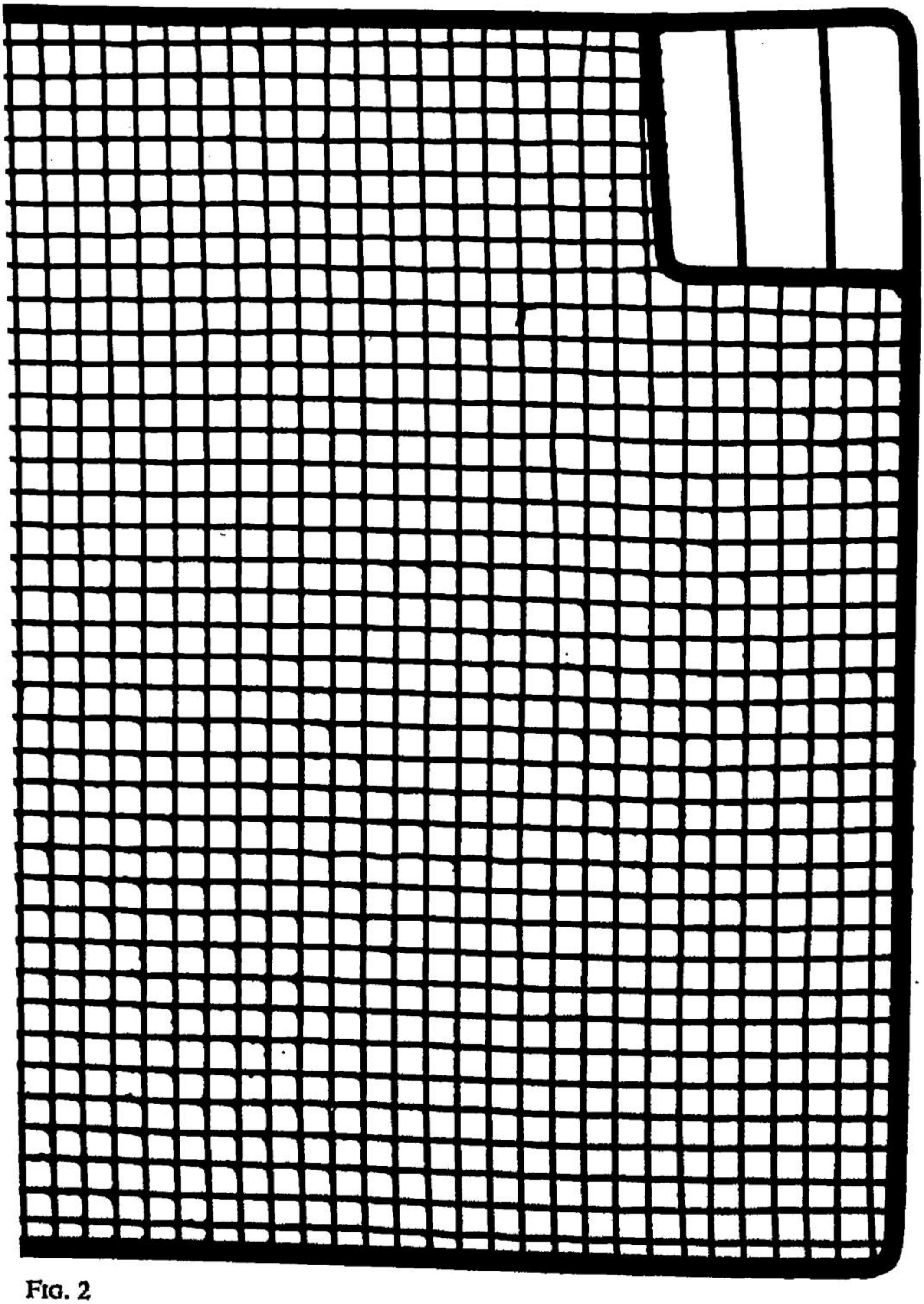

FIG. 2

not applicable to the dog, cat, or monkey. Further, while it may be a useful guide to cage size for animal houses separately, *n* animals would not necessarily need *n* times the floor area that one animal requires. The following table gives the sizes of cages in common use today:

CAGE SIZES

SPECIES	NO. OF ANIMALS	FLOOR AREA	CAGE SIZES
Cat	Doe and litter	6 sq ft	3 ft × 2 ft × 18 in.
Ferret	Doe and litter	4–6 sq ft	3 ft × 2 ft × 12 in.
Guinea pig	Doe and litter	150 sq in.	Various
Hamster	Doe and litter	48–60 sq in.	12 in. × 5 in. × 6 in.
Mouse	Breeding	48–60 sq in.	12 in. × 5 in. × 6 in.
Rabbit	Doe and litter	4½–6 sq ft	4 ft × 18 in. × 18 in.
Rat (Norvegicus)	Doe and litter	140 sq in.	14 in. × 10 in. × 10 in.
Rat (Norvegicus)	Breeding pair	168 sq in.	14 in. × 12 in. × 10 in.
Rat (cotton)	Doe and litter	140 sq in.	14 in. × 10 in. × 10 in.
Other small rodents	Doe and litter	140 sq in.	14 in. × 10 in. × 10 in.

For breeding purposes it is customary to use cages having solid floors and one or more solid, or partly solid, sides. Such a cage offers maximum comfort and protection to the young. Nesting material is trampled through grid floors, leaving the young exposed on the bare grid. Young animals can be badly injured if their limbs are caught in a grid. It is not uncommon to use a box, only the lid of which is of perforated material, for breeding small animals such as rats and mice.

These boxes are often designed to slide in and out of a rack, much as the drawers of a chest slide open and close. Such an arrangement, known as a 'battery' of cages (Fig. 3) uses the available space in an animal room to best advantage. The outstanding disadvantage of a battery is that the whole unit may have to be replaced in order to house a different species. Batteries are now available for housing dogs, cats, rabbits, guinea pigs, and small rodents. Some batteries are designed for suspended cages with grid floors (Fig. 4); this arrangement permits the trays to be removed from beneath the cages without handling the cages. Several cages can be hung over one tray, thus further reducing the amount of work and time needed to clean the tray.

There are some special-purposes cages in use in laboratories, such as the metabolism cage. This cage is used when it is necessary to make quantitative collections of urine and faeces from an animal. The cage is usually suspended over some device which separates urine and faeces and permits the separate collection of both. Most cage manufacturers have models of this type of cage in their catalogues, but few completely fulfil their proper purpose of collecting urine and faeces without mixing with food and water. Fig. 5 shows a cheap but effective home-made metabolism cage for small rodents which is made up from a retort stand, a glass funnel, a test-tube basket, and the necessary clamp with a glass device for separating the urine and faeces.

Cages for housing radioactive animals can be standard cage (Fig. 6) tops which fit on to special funnel bottoms. This allows excreta to be collected into a glass vessel.

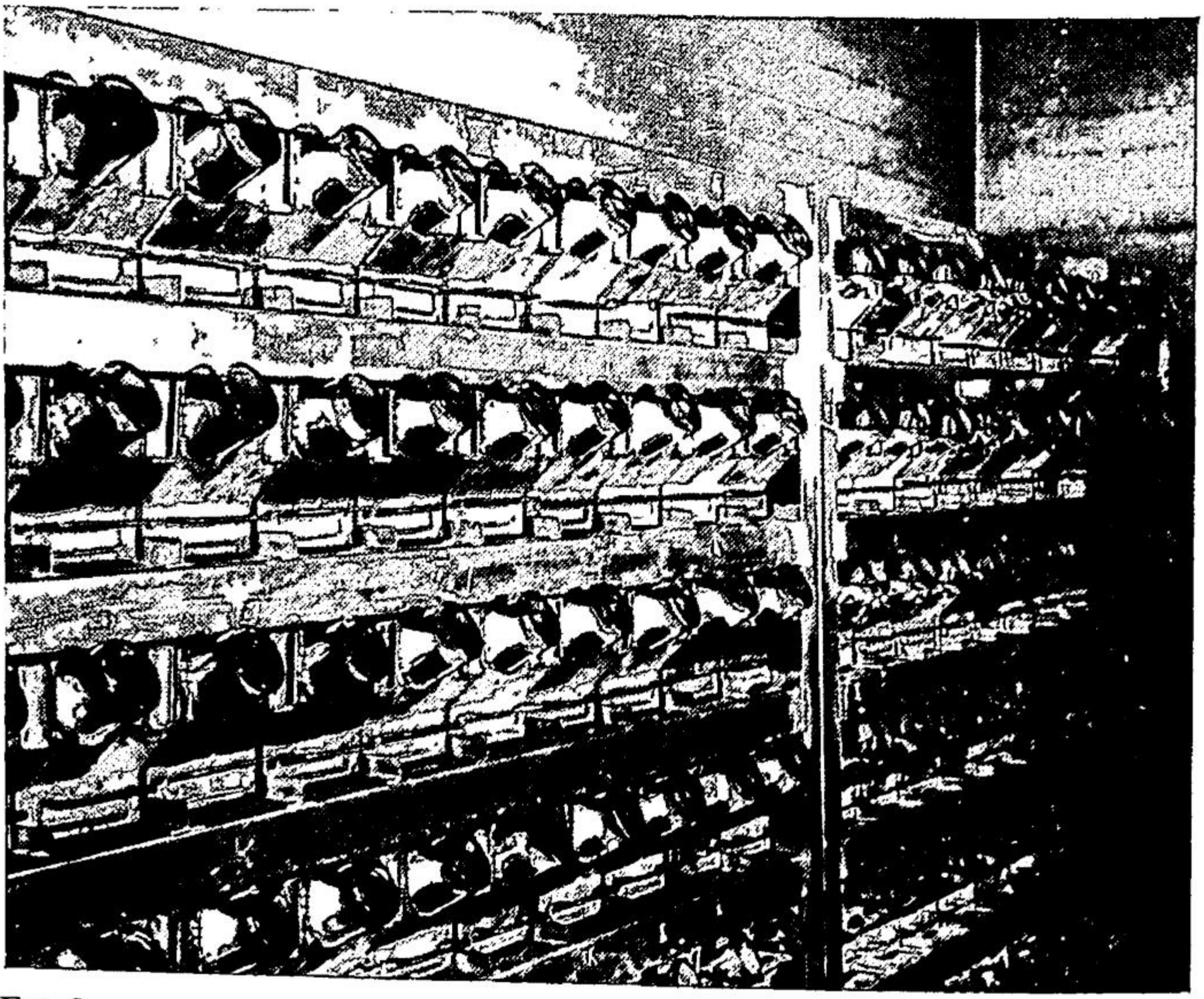

FIG. 3

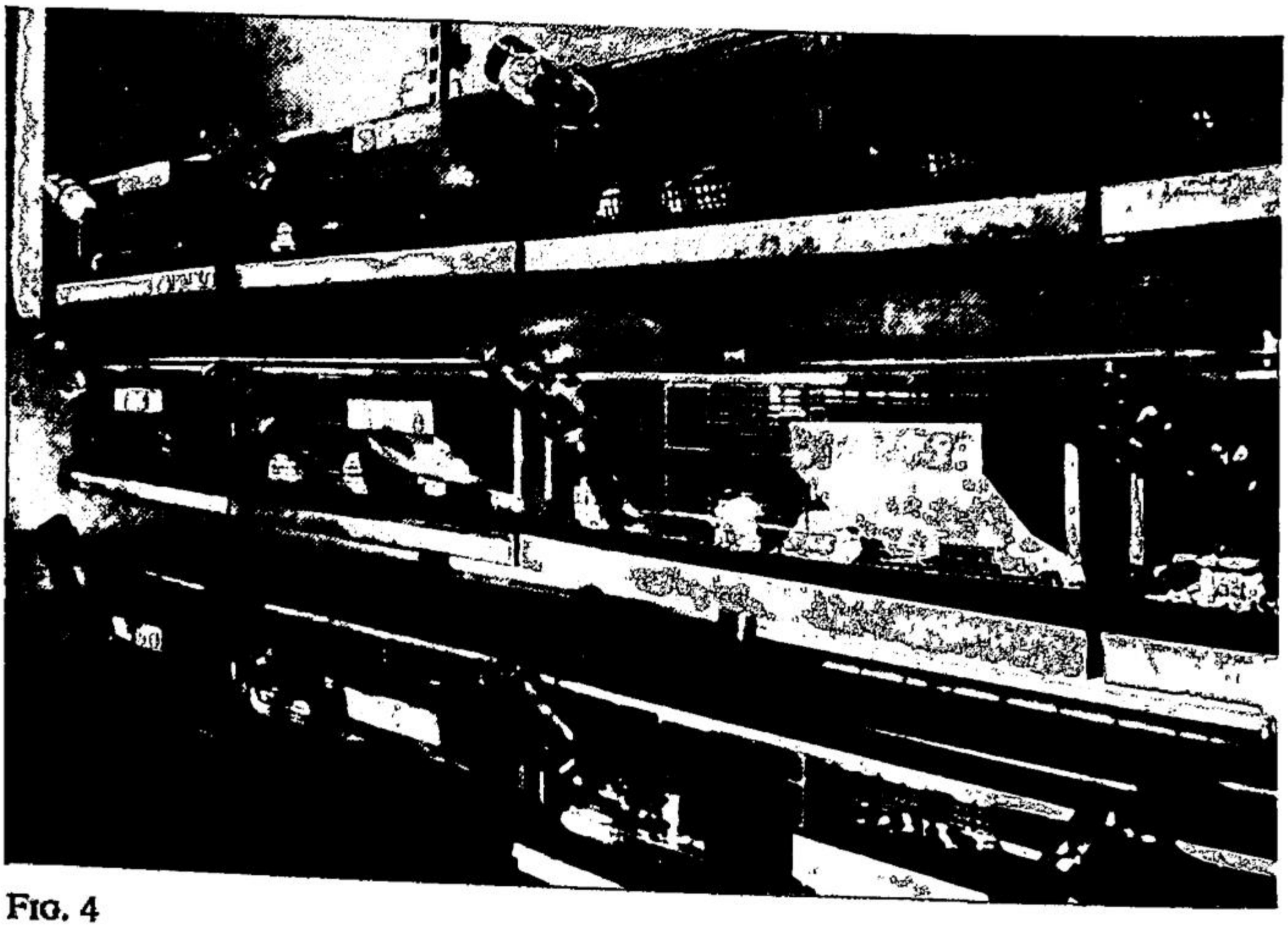

FIG. 4

Fig. 7 shows a three-tier cat breeding cage designed for the cat colony at Mill Hill. This cage, which is of light construction, has a separate diet tray which can easily be removed for cleaning; this avoids the necessity of opening

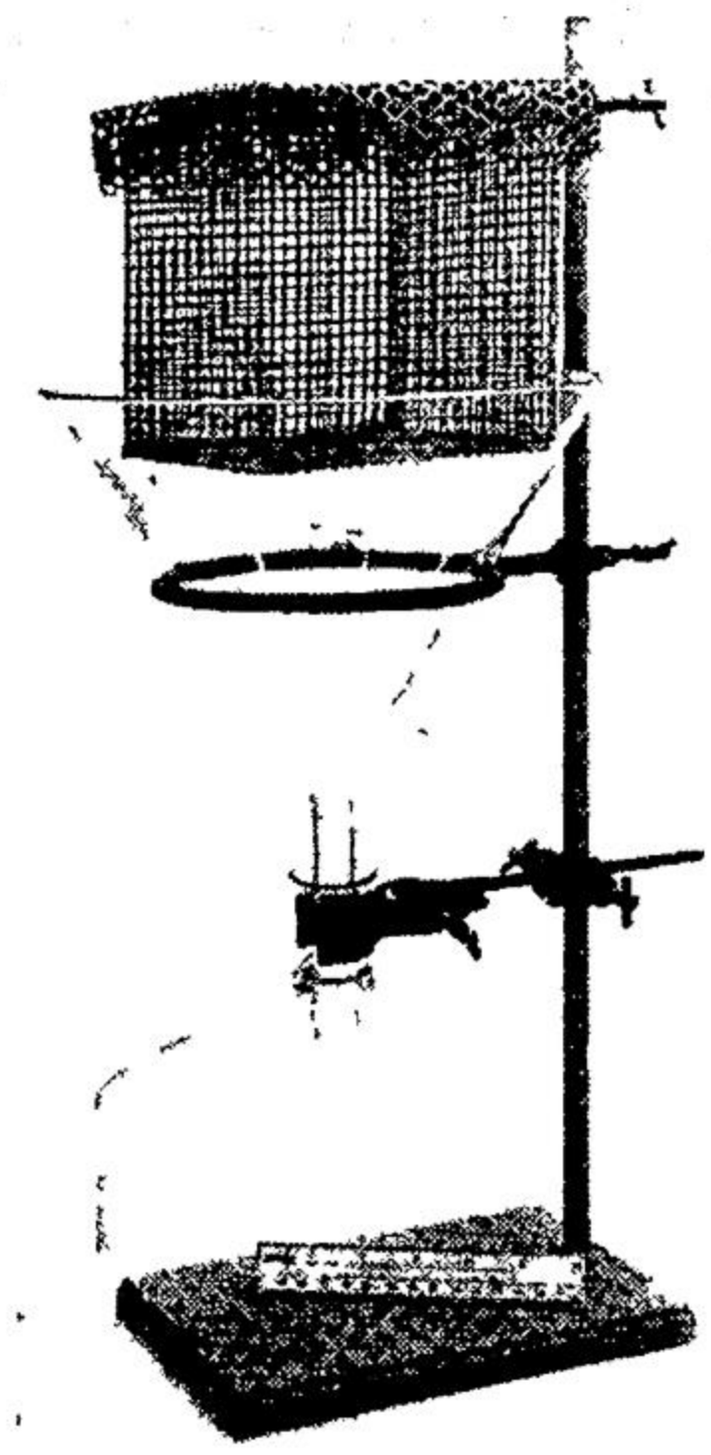

FIG. 5

the cage door and saves time when a litter of kittens are romping around. This battery of cages, being made from light material, is easily moved from place to place.

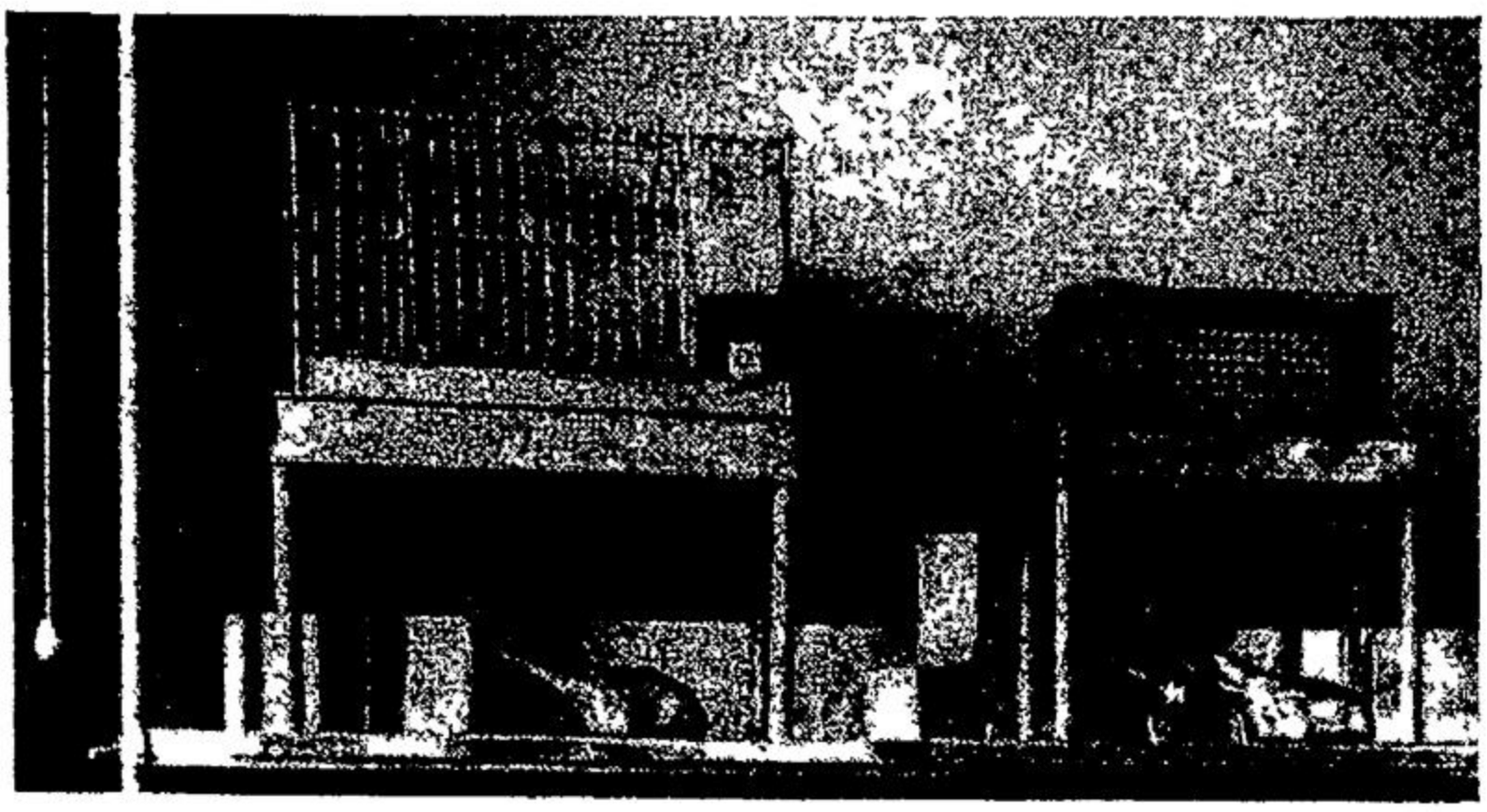

FIG. 6

Cages for a particular use, such as the exercise wheel shown in Fig. 8, with electric recording, is an example of 'do-it-yourself'. This piece of useful apparatus was made out of bits and pieces at an eighth of the cost of bought equipment, but will do the same job. Another special cage is used to retain an

animal in one position for a period of time, as, for example, during the continuous recording of temperature. Animals, particularly rabbits, can be trained to stand still for quite long periods without any restriction being applied.

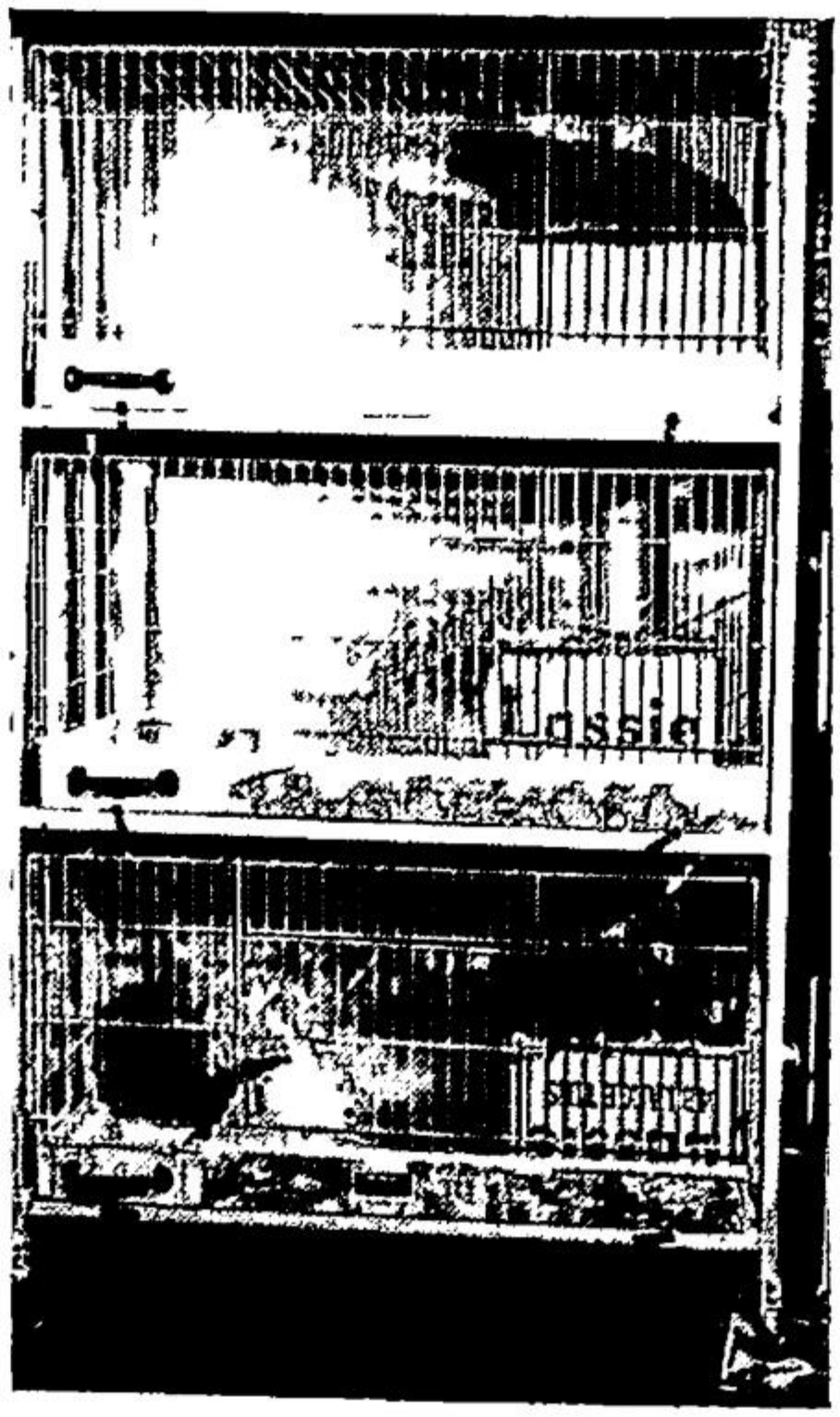

FIG. 7

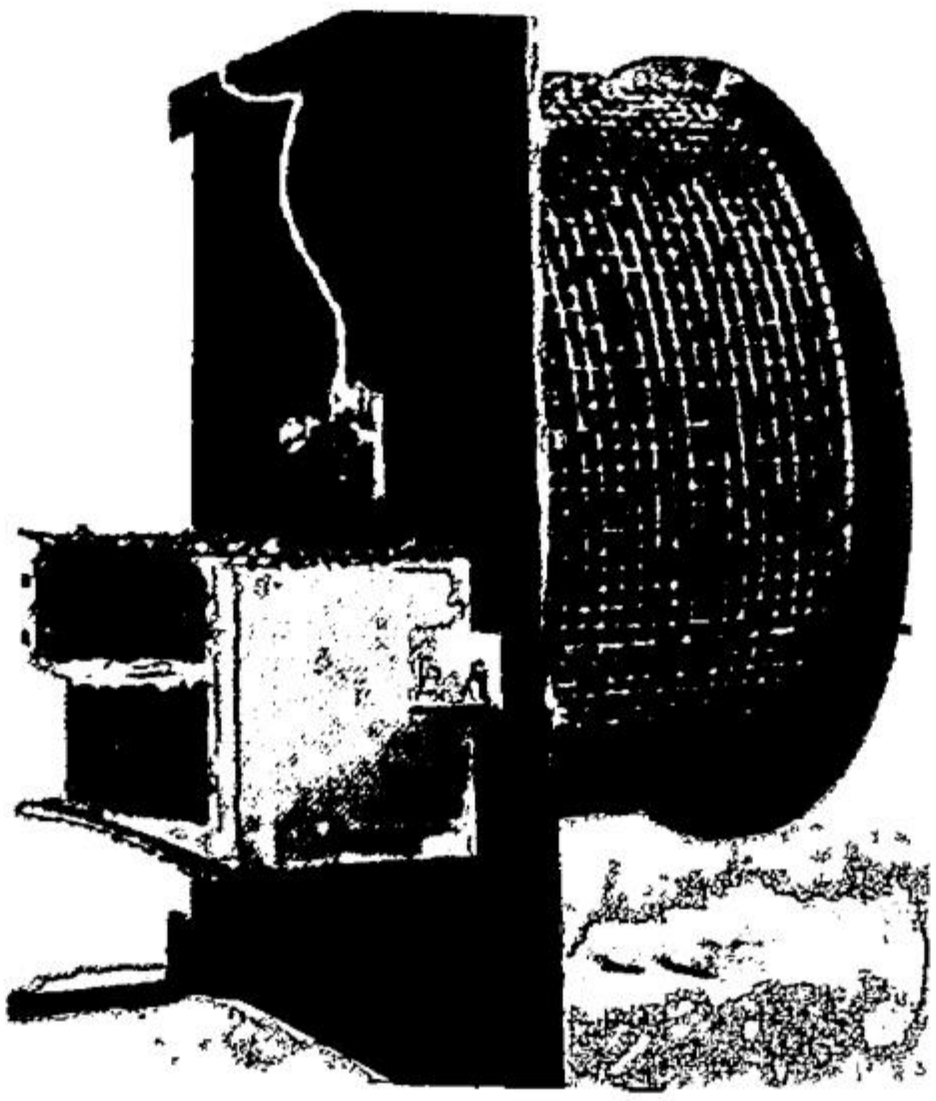

FIG. 8

Short (1960) reported an automatic battery for housing rabbits. Cleaning, feeding, and watering were done mechanically with the minimum of human attention. The initial cost for housing 672 rabbits was soon recovered by the

saving in labour costs. It is possible that large numbers of guinea pigs could be housed using a similar system (Fig. 9).

Braby's (November 1961) described the primate quarters attached to the Medical School, University of Birmingham, Department of Anatomy. The primate quarters were designed for a capacity of 250 monkeys. They consist

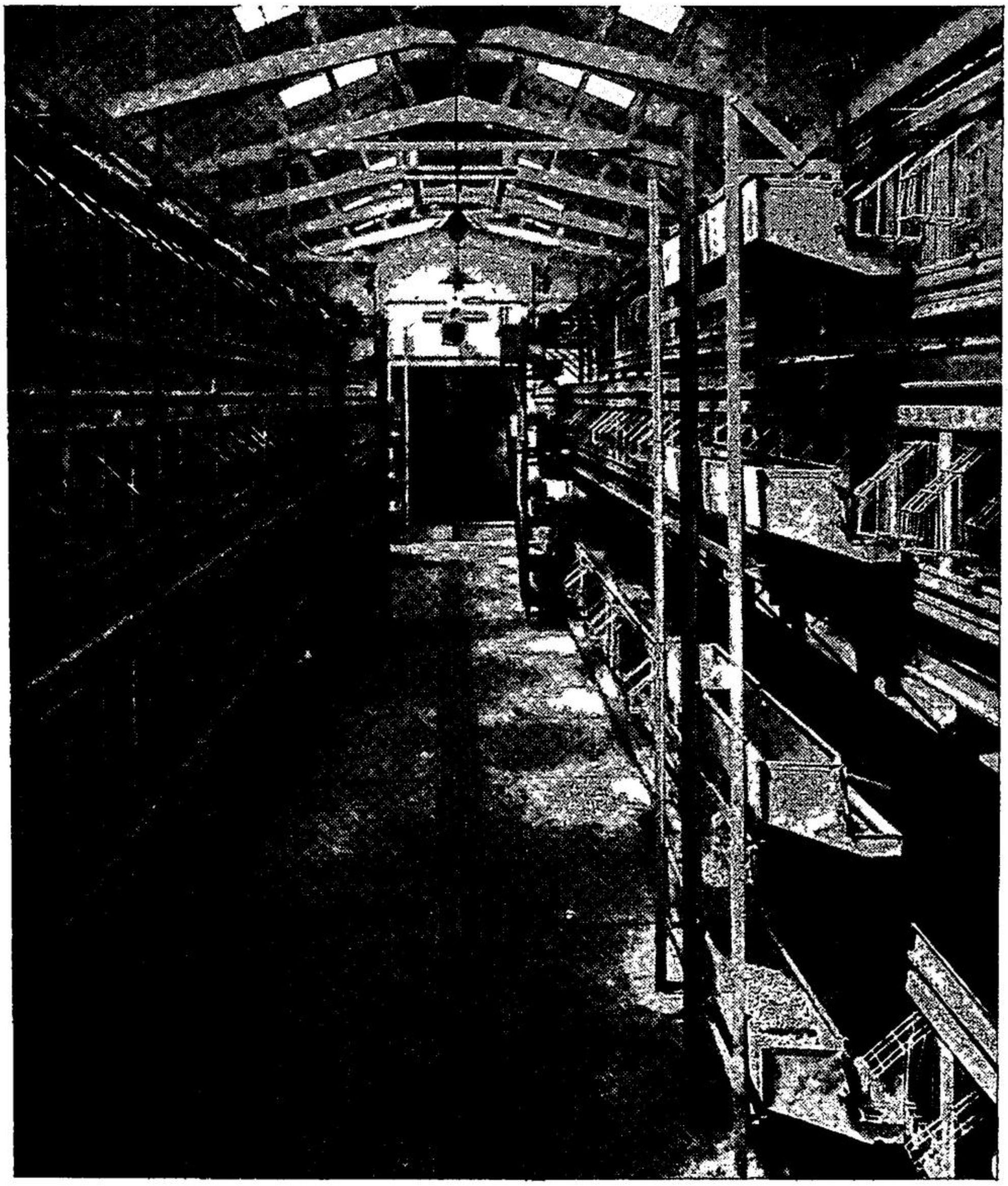

FIG. 9

of a very large fully air-conditioned room (Fig. 10) housing some 125 cages, and two additional smaller rooms for the quarantine and treatment of suspected or known sick animals (Fig. 11). It will be seen from the illustrations of the interior of the large monkey room that full advantage of space has been taken by building a tubular structure supporting a grill-floor to provide a staging for many of the cages (Figs. 12 and 13).

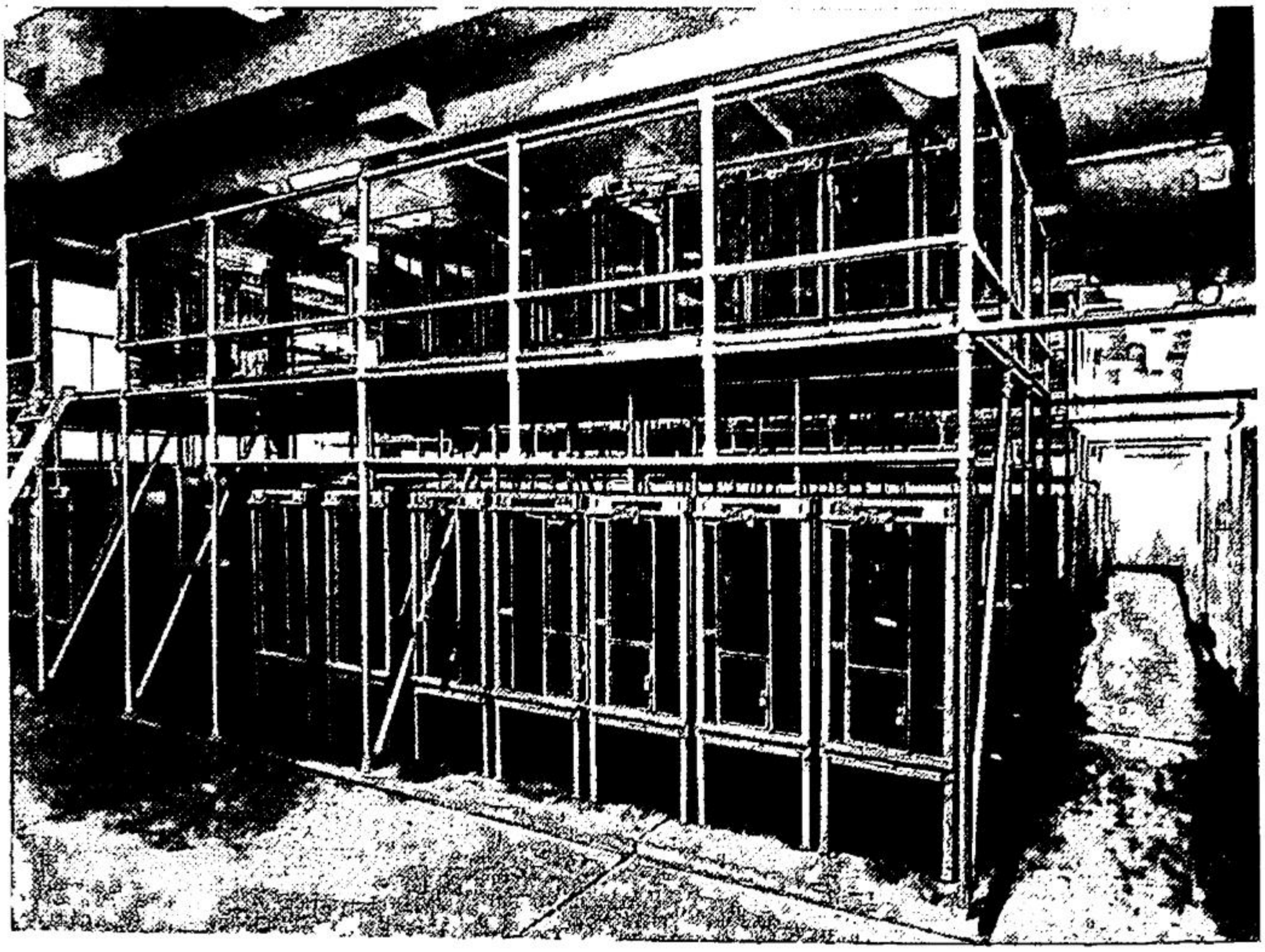

FIG. 10

FIG. 11

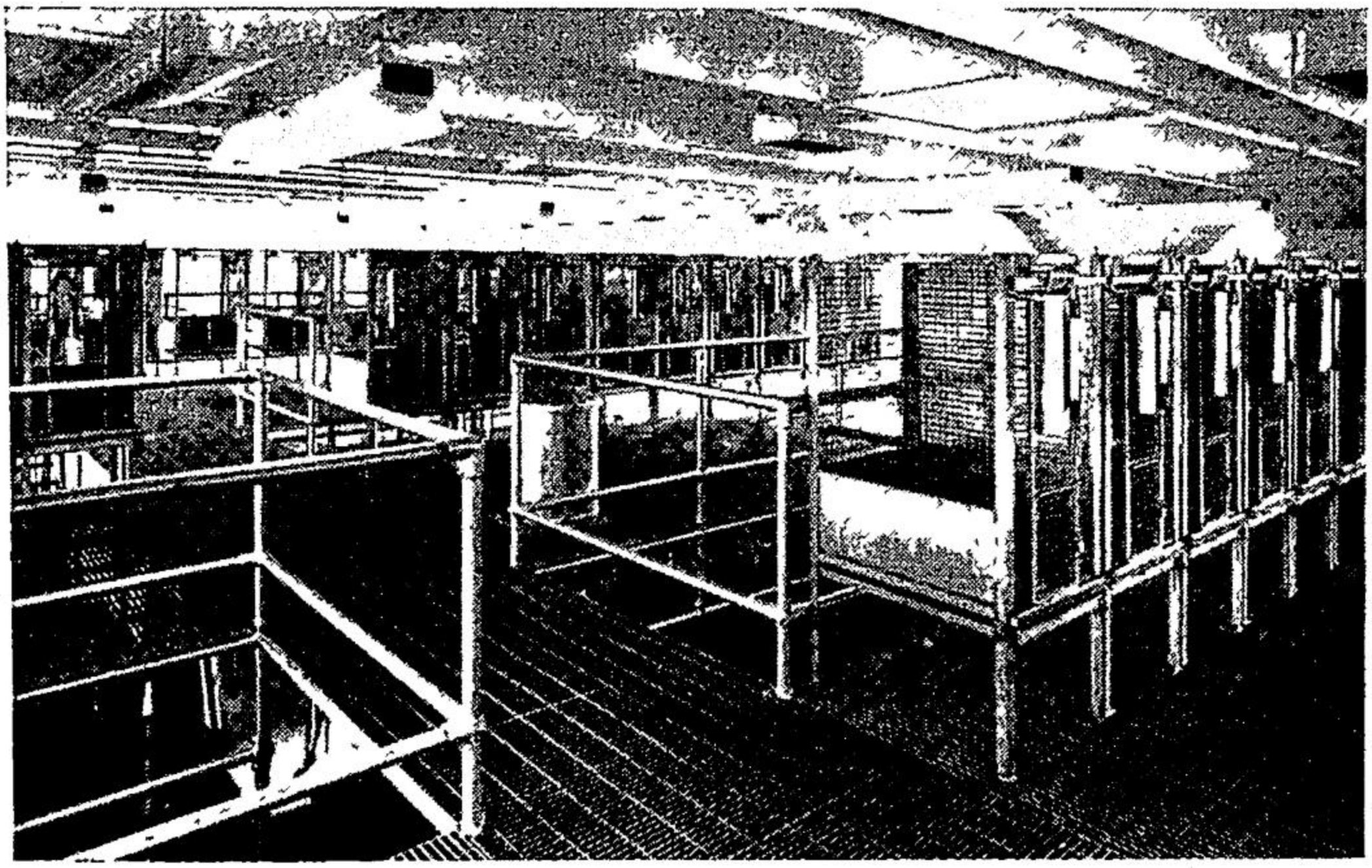

FIG. 12

Both single and double cages (provided with a removable partition) are used and shown in the photographs (Figs. 11 and 14). All are provided with a sliding door moving on a guarded runner, a metal perch, water dispenser, and a pan. They are bolted to a solid backrail to prevent them being 'walked about' by the monkey; doors are secured by spring padlocks.

FIG. 13

Some type of rack or shelf is essential for the economic accommodation of the cages, and, like the cages, these racks may be seen in a variety of forms. They can be built on to walls, suspended from the ceiling, or be free-standing and fitted with castors so that they may be moved about the room.

The materials that are suitable for the manufacture of cages are also generally suitable for the construction of racks; galvanized iron and aluminium are popular. Wood is an undesirable material for several reasons, particularly that of hygiene. Racks should be fully adjustable and easy to dismantle. The ability to adjust the distance between the shelves enables both large and small cages to be accommodated on the same rack. To be able to dismantle a rack completely allows thorough cleaning, and the rack can be stored in the minimum of space.

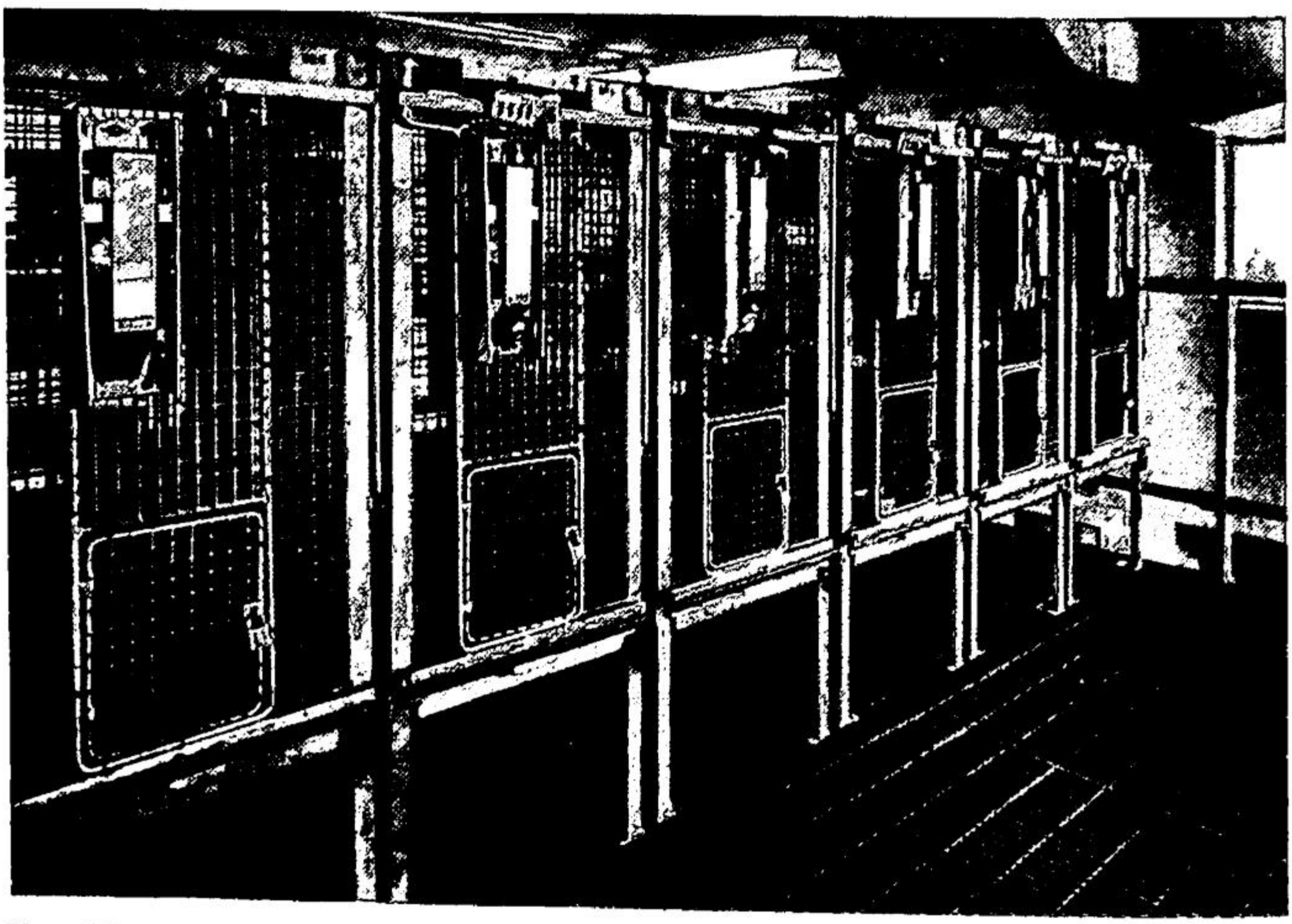

FIG. 14

Some establishments have used racking made from ready-drilled and slotted steel or aluminium members (e.g. 'Dexion' and 'Handy Angle'). These materials are supplied in standard lengths with holes and slots stamped out at regular intervals. A special cutter is available for use with these materials and the angle members are marked at appropriate distances to ensure that the holes and slots match up when cut lengths are bolted together.

The shelves used on the racks can be either solid (sheet metal) or open (rods or bars). While the open type of shelf has the advantage of presenting a small surface area on which dust and dirt can collect, the solid shelf has the advantage that faeces, food, and bedding cannot drop down to contaminate the cages below.

Whatever type of rack is used, good clearance should be allowed between the lowest shelf and the floor so that it is easy to clean beneath the rack.

Suspended racks are ideal from this point of view, as there is no floor obstruction whatsoever (Figs. 15, 16). The mobile type, running on castors, is probably the most useful general rack. While ground clearance is important, such racks can be moved to clean the floor beneath them. Castors for these racks should be of a reasonable diameter and be fairly wide. Castors make the rack easier to move (a loaded rack can be very heavy) and also minimize pitting in soft floors if the loaded rack is kept in one position for any length of time. The castors must be of good quality and be protected from water and dirt; they should be serviced regularly.

Wall racks are best fixed by means of slotted brackets, so that the projecting members can be lifted down and the wall cleaned or painted. Animal rooms should have the minimum of permanent fixtures so that cleaning and redecoration may be easily accomplished.

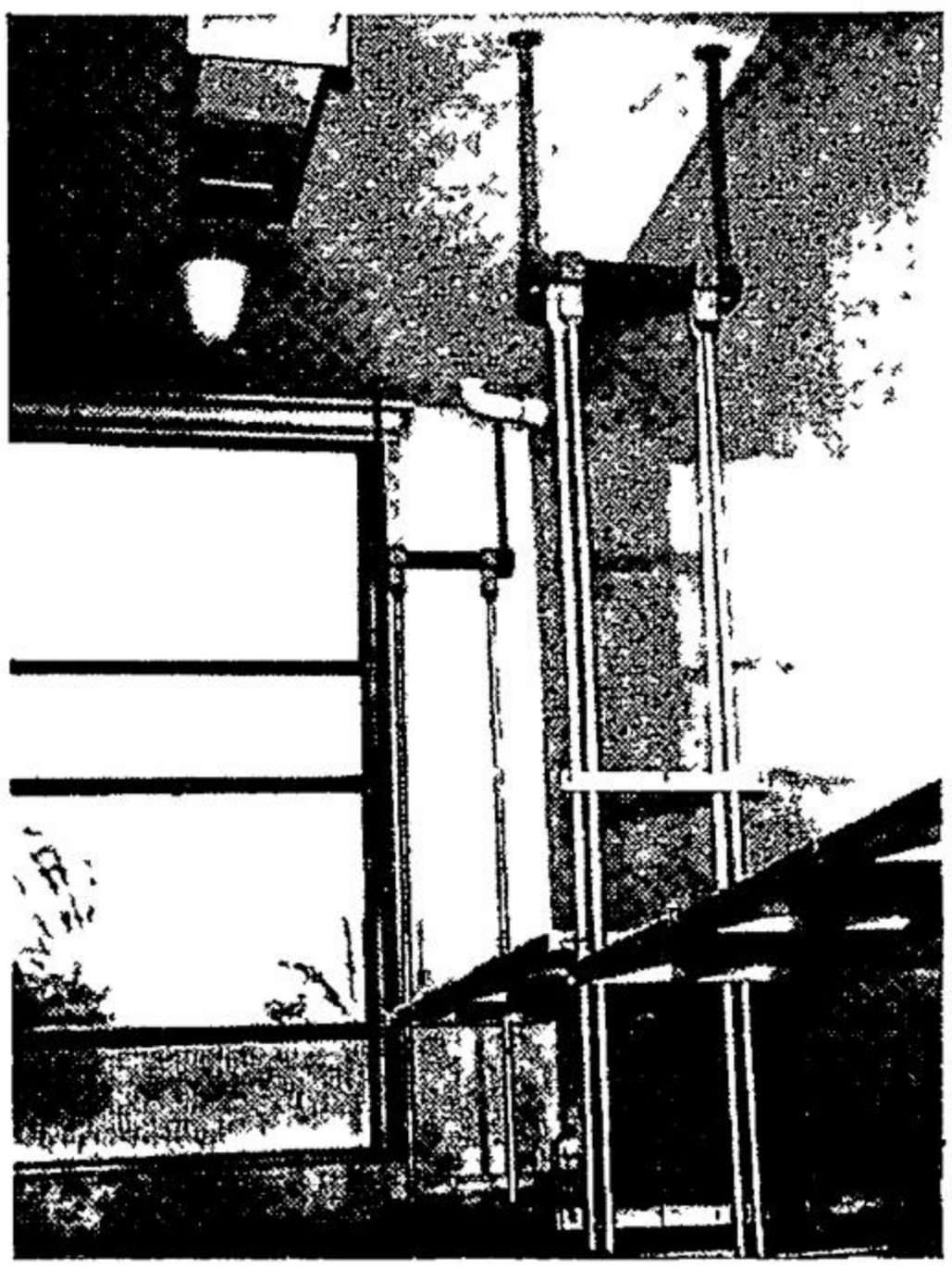

FIG. 15

WATERING DEVICES

Bruce (1950), comparing the relative water requirements of human beings and laboratory animals, says, 'Even allowing for a very large margin or error in the estimate, it is evident that laboratory animals fed on a dry diet only, drink much larger amounts of water than human beings.'

The watering of small animals by means of inverted bottles and glass drinking-spouts is attended by two small problems. The first is that of attaching the drinking-spout to the bottle so that there is no leak and dismantling it for filling and cleaning is easy. The second problem is that although glass drinking-tubes and bulbs are easy to clean and airlocks are visible, the glass is fragile and the breakage rate is high, with the possibility of serious

injury to the technician. Short and Parkes (1949) described a metal drinking-spout which could be screwed on to the standard blood transfusion bottle, and a similar spout to fit the 'medicine flat'. Lane-Petter (1951) described a soft plastic cap which fitted over the neck of the bottle, with a Pyrex glass tube for the spout. Short (1952) described a cheap, hard plastic combined screw cap and drinking-spout designed to fit a 4-oz screw-top glass bottle.

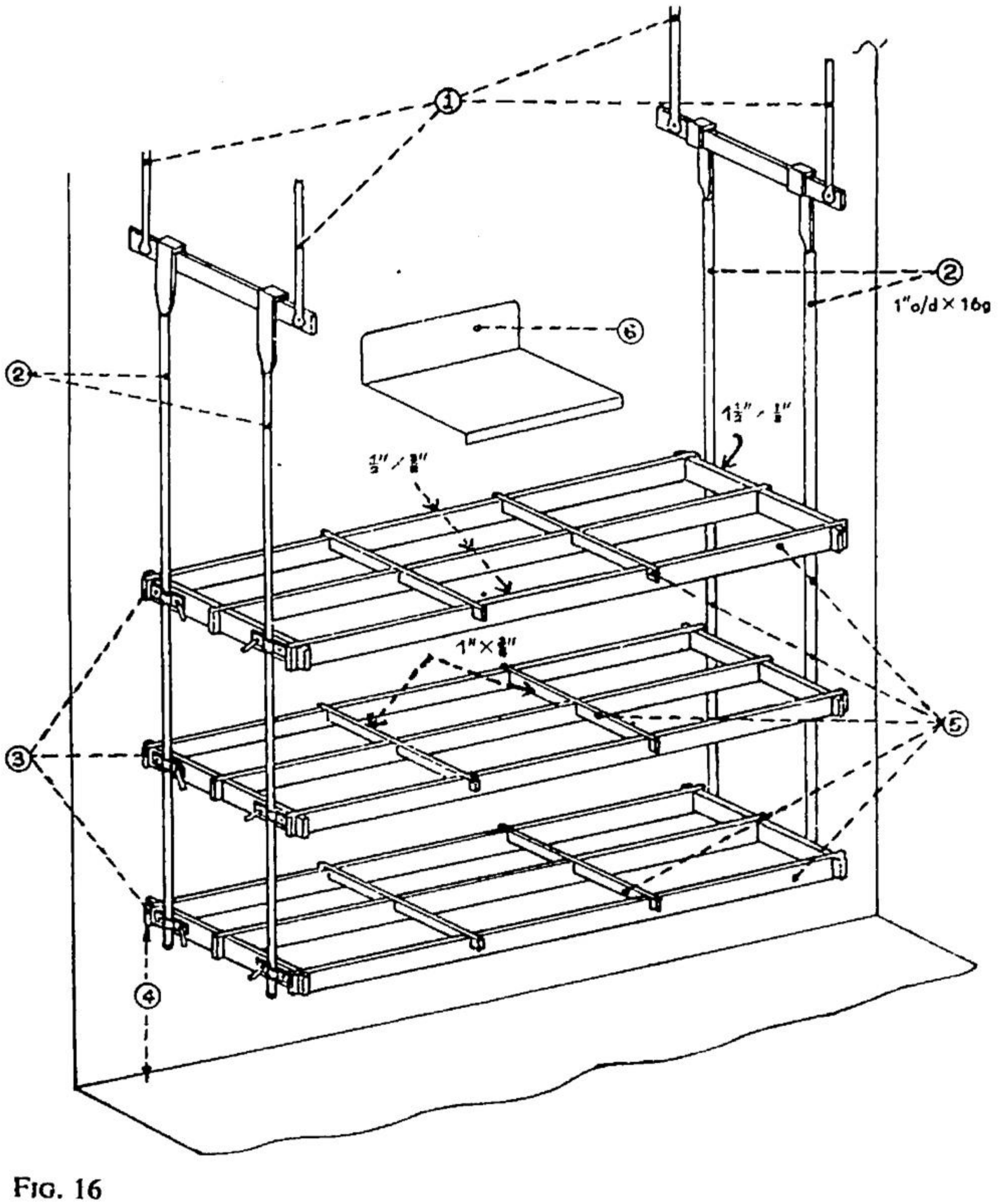

FIG. 16

The plastic caps and drinking-spouts are much cheaper than their metal counterparts, but the metal outlasts the plastic by many years. Fig. 17 shows a selection of water-bottles and spouts.

Lane-Petter (1952), writing about the mechanics of the animal water bottle, said, 'The increasing popularity of the inverted bottle and drinking spout for watering small laboratory animals has brought to light potential drawbacks:

it is well, therefore, to consider the physical (including the mechanical) principles governing this simple system, in order that these drawbacks may be overcome. . . .

'A bottle, which should be sufficiently capacious in relation to the needs of the animals, is fitted with a suitable spout or drinking tube, filled with water and inverted. The animal licks or sucks water from the spout, air entering the bottle to replace the water. The water does not run out of its own accord, provided certain conditions are observed:

'(i) The internal diameter of the tube should be $\frac{1}{4}$–$\frac{3}{8}$ in. (6–9 mm) and constricted at the distal end to a hole of diameter about $\frac{1}{8}$ in. (3 mm.) (Short and Parkes 1949). A smaller tube will be subject to air locks: a larger tube is unnecessary and is liable to spontaneous emptying.

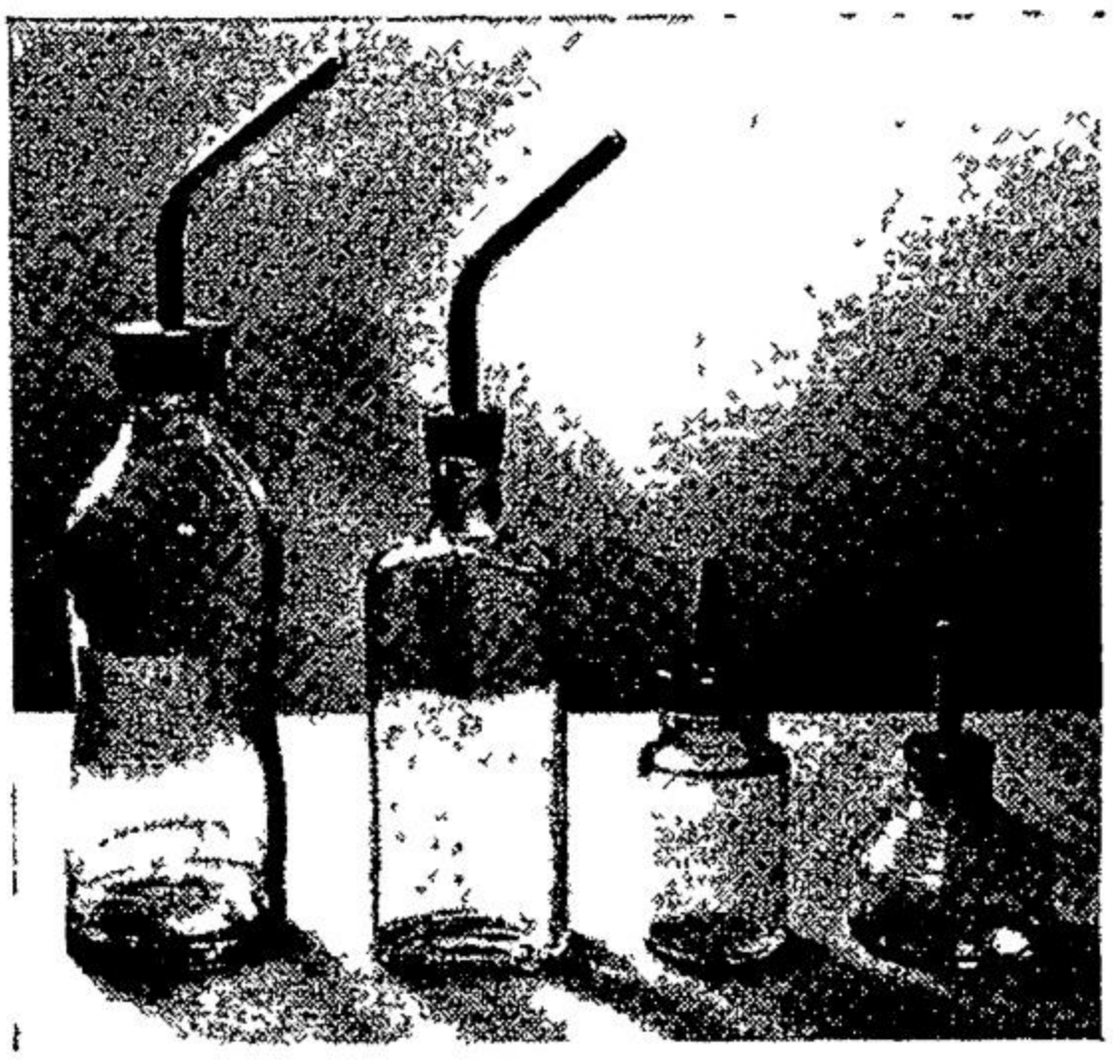

FIG. 17

'(ii) The connexion of the tube to the bottle must be air-tight. The slightest leak will cause the bottle to empty spontaneously.

'(iii) The bottle must not be too large: 500 c.c. is about the limit. An increase in the volume of air inside the bottle will lead to expulsion of water, and there are several ways (apart from leakages around the bung) in which this can happen when the animal is not drinking: (*a*) an increase in temperature of 1°C will cause an increase in volume of approximately 0·3 per cent, that is 0·3 c.c. for every 100 c.c. of air in the bottle. (*b*) A fall in atmospheric pressure of 0·1 in. (2·5 mm) mercury will cause a similar increase of volume; neither of these results in serious leakage normally. (*c*) Shaking the bottle; movement of the bottle may cause the volume of water expelled to exceed the volume of air admitted: a further volume of air will then be drawn into the bottle, and the process may be repeated until the bottle is empty.

'Leakage from any of these causes depends on the increase in the volume of the air in the bottle being greater than the drop of water which can hang on the end of the tube; if the drop does not fall off, a subsequent decrease in volume (such as will immediately follow an increase resulting from shaking)

will draw the drop back into the tube, provided the drop covers the aperture in the end of the tube.

'Drop-size depends on a number of factors, the most important of which are the material comprising the tube and the shape of its extremity. Glass has been found to carry a larger drop than other materials, metal being the least satisfactory: while a small terminal expansion can increase the drop-size considerably.

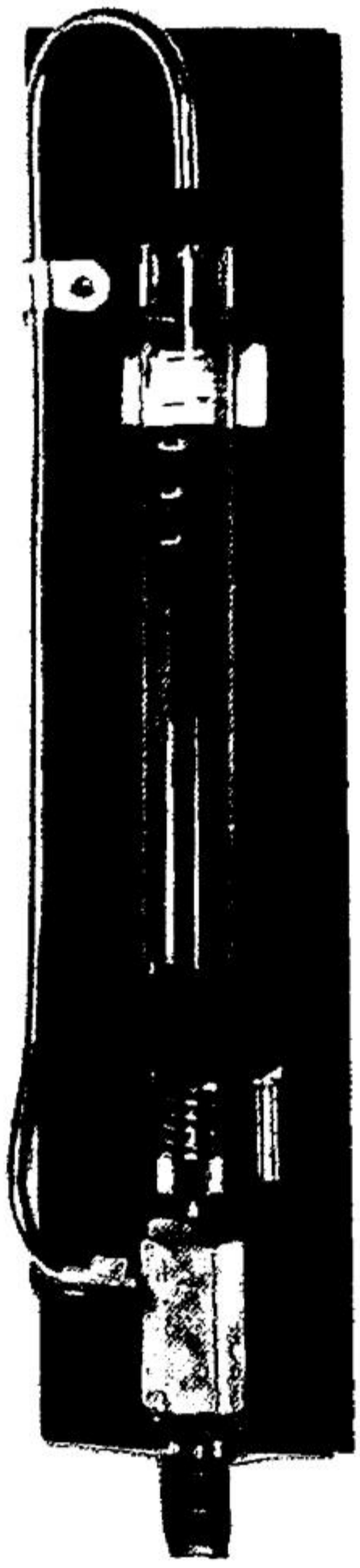

FIG. 18

'If the tube, instead of being vertical, is at an oblique angle (as frequently happens when the bottle is held outside the cage) the drop of water will not cover a centrally placed aperture. It is necessary, therefore, to make the terminal aperture at the most dependent part of the tube, so that contraction of the air inside the bottle will suck back the drop of water, rather than air, (which may lead to spontaneous emptying).'

Watson (1961) described an automatic drinking system for rabbits which, with the aid of Schrader tank valves, was non-leaking and could be used for animals on solid floors. Gray and Carter (1960) described a drinking-fountain for dogs that would: (i) fill automatically to provide fresh water at all times;

(ii) the water-bowl was so designed that it would easily be removed for cleaning; (iii) there would be no back-siphoning into water-supply system; (iv) the valve would be protected against urination; (v) the whole fountain could be fitted into a space bounded by sides of 4–6 in.

Automatic watering has been used for mice, but it is necessary to house the animals on grid floors, because all valve-type watering leaks from time to time. Leakages into mouse boxes might result in drowning the mice.

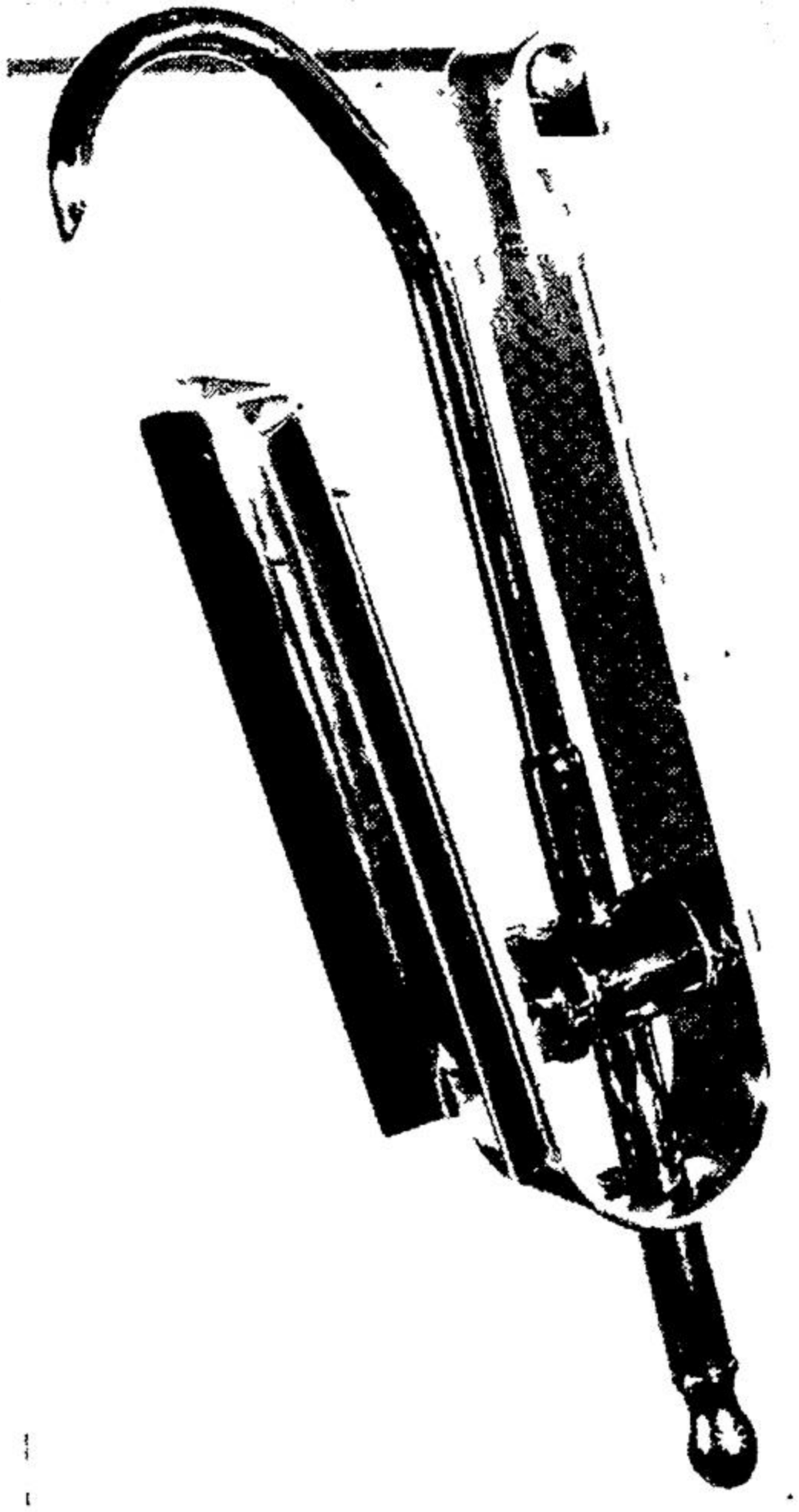

FIG. 19

There are many automatic bottle-filling devices; two are shown in Figs. 18 and 19. They have the advantage that they can: (i) be taken to the cages so that bottles can always be replaced in the cage from which they came, and (ii) the bottles need not come into contact with the filler pipe. The apparatus shown in Fig. 18 was described by Gaunt (1961), and that shown in Fig. 19 has been in use at Mill Hill since 1953.

There are a number of automatic watering devices available which can be connected to the mains water supply through a storage tank. They are fitted

with an internal constant-level device which allows the animal a constant supply of water.

These automatic units require some plumbing and are rather expensive to instal; they soon repay their cost by the labour saved.

FEEDING HOPPERS, BASKETS, AND POTS

These are the common receptacles for food for animals. Diets in the form of dry powders or wet mashes or gruels are presented in open dishes. Hoppers and baskets are used for the cubed (or pelleted) diets, which are now the standard form of diet for stock colonies.

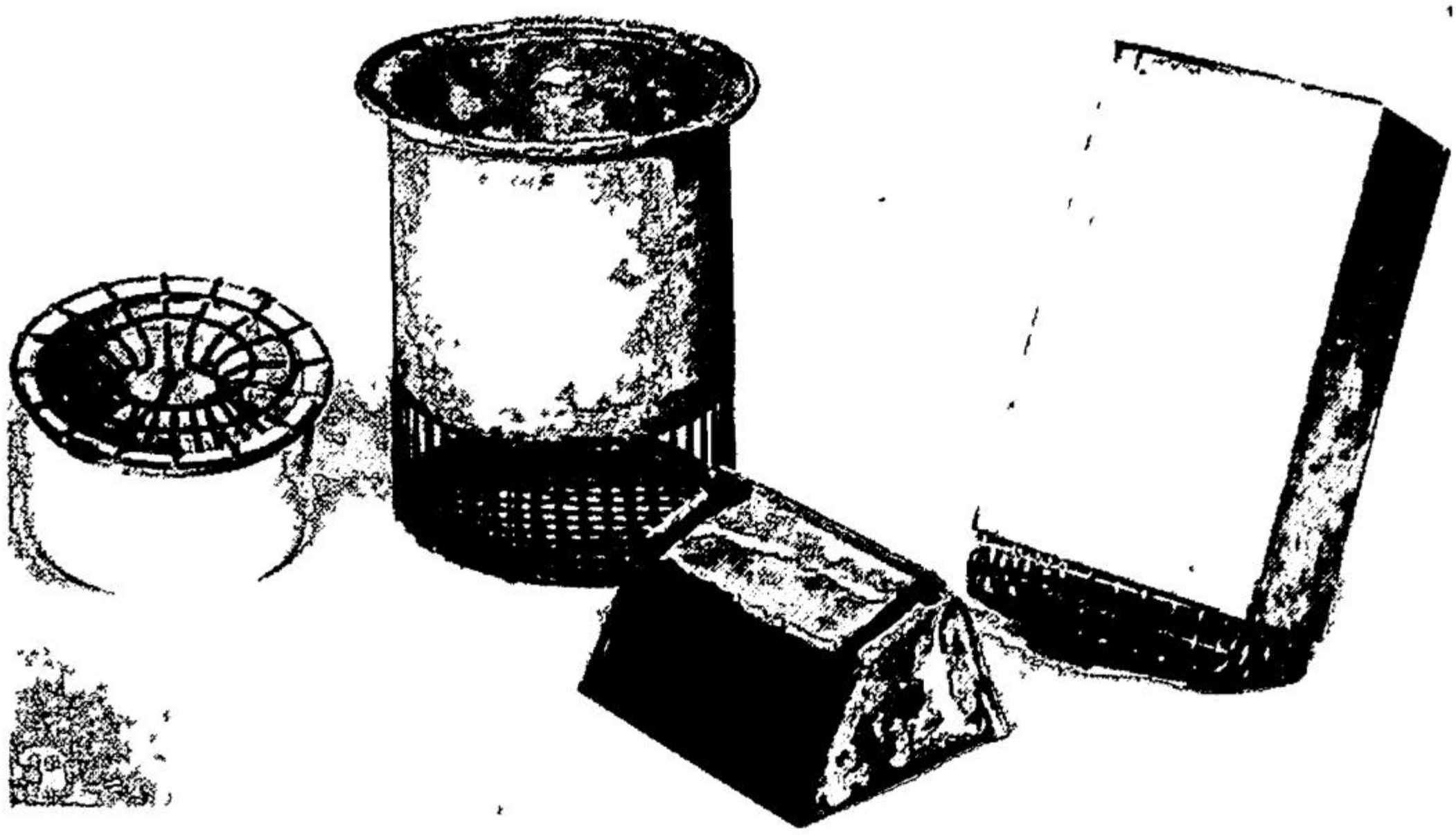

FIG. 20

Hoppers and baskets exist in several forms; some are built into the top of the cages; others hang on the front or sides of the cage. If possible, it should be so arranged that the food containers can be replenished without opening the cage; this saves much time where many animals have to be fed. Hoppers and food baskets should be designed so that the animal can neither get inside them nor climb up the outside and foul the food with their excreta (Fig. 20). They should hang with a clearance of 1 in. above the bedding. The base of a rabbit food hopper (Fig. 21) should be 2 in. above the floor grid; this will prevent the animal from scratching the food out with its front feet.

The materials for the construction of hoppers or baskets are similar to those suitable for cages, and galvanized iron is probably the most popular material.

Rats and mice can be fed with cubes contained in baskets, but the wires of the basket should be arranged to give a long (about 2 in.) vertical opening about $\frac{5}{16}$ in. wide. This makes it much easier for the animal to chew the cubes. The use of hoppers and baskets effects considerable economies in the cost of food and labour.

Small ointment jars or glass or china pots are suitable for feeding powdered diets to small rodents; they can be secured in the corner of the cage by means of a suitably sized 'Terry' spring clip. Spillage can be collected and weighed on a piece of filter-paper placed beneath the pots under the wire grid.

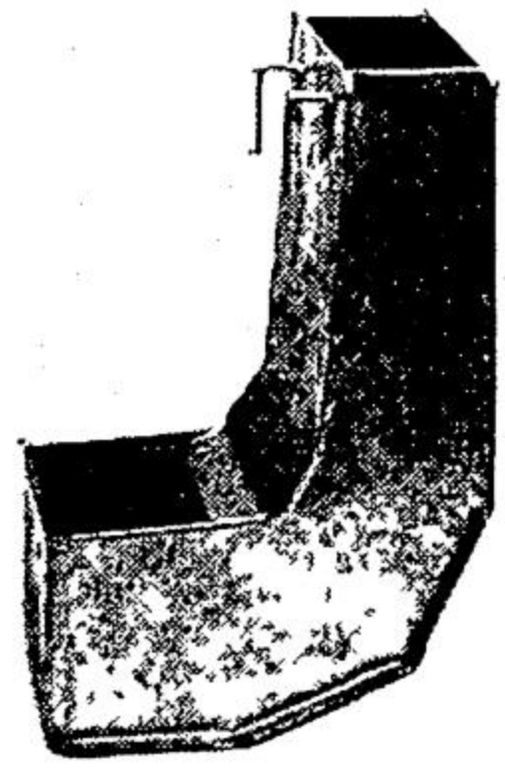

FIG. 21

WEIGHING MACHINES

A balance suitable for weighing such things as small animals and the constituents of diets is a piece of apparatus which should be available in any animal house.

The most suitable type of balance for weighing small animals (such as rats, guinea pigs, and rabbits) is a double-pan, automatic-lever balance. These can be obtained with a scale of 250 gm graduated in 1-gm divisions and weighing up to 5 kg with extra weights. They should have an hydraulic damper to prevent undue hunting of the pointer with every slight movement of the animal. Scales with luminous dials showing the exact weight are available, and when large numbers of animals have to be weighed these are great time savers.

Similar models are obtainable for weighing food, etc., but scoop or helmet shaped, rather than a flat, weighing pan is desirable for a food balance. A scale of 20 or 25 gm graduated in 0·1 or 0·2-gm divisions is useful if its range can be extended with the addition of extra weights. A balance of this type usually has a maximum capacity of about 500 gm.

Balances with larger capacities are readily obtainable to weigh up to 25 or 50 kg, but the scale is usually of 500 or 100 gm, graduated in 5-gm divisions.

The domestic-types of spring balance should be avoided, as they do not stand up to the continual work of a laboratory. On the other hand, it has been found that a relatively cheap pair of 'sweet' scales with a capacity of up to 2 kg will, if used carefully, give very good results with an accuracy of 1–2 gm in 500 gm.

ELECTRIC HAIR CLIPPERS

These are essential in the animal division, and care is necessary in selecting them. They should be suitable for clipping any type of hair from horse to mouse without causing discomfort to the animals. The clippers should be

suitably insulated as proof against electric shock. Most clippers run hot after constant use (e.g. clipping a large batch of guinea pigs), but Fig. 22 shows hair clippers operated by compressed air, either from direct supply or bottle, which may be used for several hours without overheating, and which does not carry the risk of electric shock should the operator be compelled to work in wet conditions.

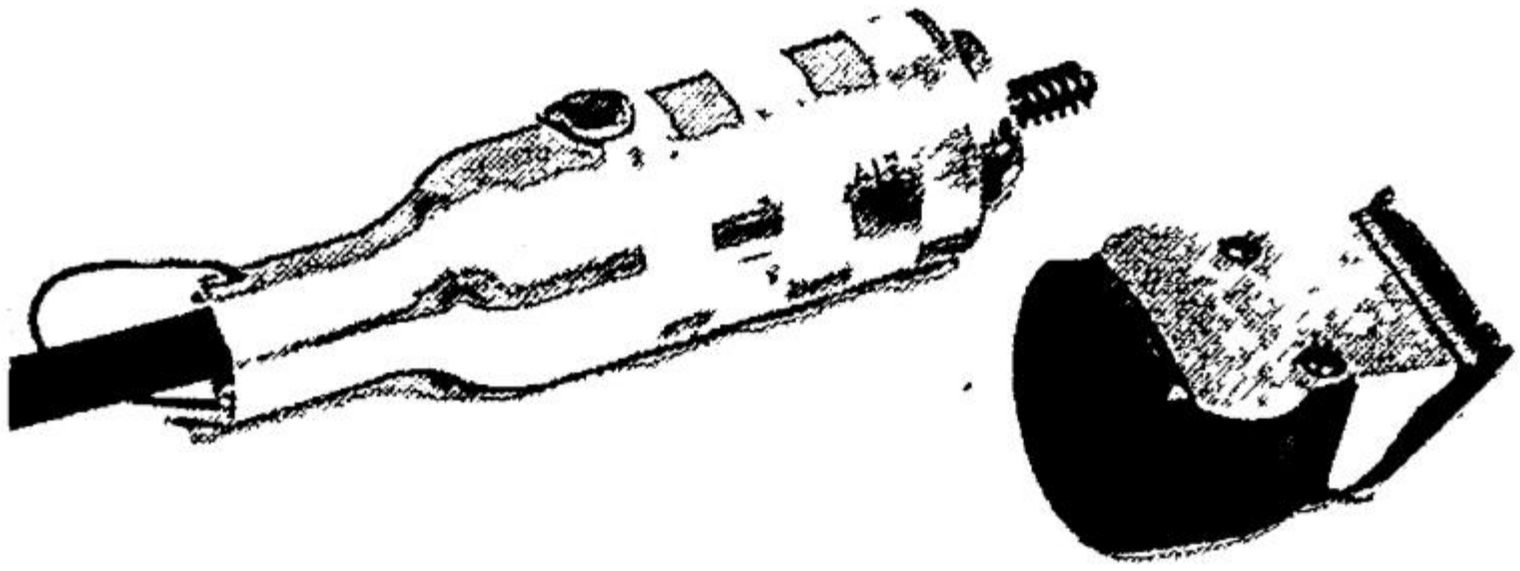

FIG. 22

ROUTINE CLEANING OF DIRTY BEDDING FROM ANIMAL CAGES

This is one of the most time-consuming jobs in the animal house, as well as being the most irksome duty of the animal technician. The traditional dirt bin, cage scrapers, brooms, and brushes can be possible sources of disease unless constantly sterilized. Experience has shown that a central vacuum system could be used for the general cleaning of dirty animal bedding from cages, sweeping floors, brushing cage racks, and walls, thus dispensing with all the items of equipment listed above. Suitable points can be arranged in the animal rooms where flexible hose can be attached, and all dirty cage bedding would be instantly conveyed through sealed pipes to a container situated outside the animal house. The container or hopper could be erected directly over the incinerator and, by the aid of controlled doors, fed directly on to the fire without being touched by human hand. Such installations are already in use, and reports on their usefulness are eagerly awaited. Charles, Poppleton, and Stevenson (1962) reported a cheap flexible vacuum system for cleaning out mouse cages which could be adapted for other animals. They considered that such a system would improve the standard of hygiene and the efficient use of labour in the animal house.

BIBLIOGRAPHY

HOELTGE, E. J., *Proceedings of Animal Care Panel.* **11**, No. 1 (1961).
SPIEGEL, VONA, and GONNERT, R., *Ischr. Versuchstierk.* Bd. 1. 5. 38.46 (1961).
LANE-PETTER, W., *UFAW Handbook* (1957).
SHORT, D. J., *Journal of Animal Technicians Association*, **11**, No. 1 (1960).
BRUCE, H. M. *Journal of Animal Technicians Association*, **1**, No. 3 (1950).
SHORT, D. J. and PARKES, A. S., 'Drinking Spouts for Laboratory Animals', *Nature*, **163**, 292 (1949).

LANE-PETTER, W., 'Soft Plastic caps for Water Bottles', *Journal of Animal Technicians Association*, **2**, No. 3, 13 (1951).
SHORT, D. J., 'Some Items of Animal Equipment', *Journal of Animal Technicians Association*, **2**, No. 4, 13 (1952).
LANE-PETTER, W., *Mechanics of Water Bottles* (1952).
WATSON, S. C., *Journal of Animal Technicians Association*, **1**, No. 4 (1961).
GAY, W. L. and CARTER, J. L., *Journal of American Veterinary Association*, **137**, No. 9 (1961).
GAUNT, W. T., *Journal of Animal Technicians Association*, **1**, No. 4 (1961).
CHARLES, R. T., POPPLETON, W. A. R., and STEVENSON, D. E., *Journal of Animal Technicians Association*, **13**, No. 1 (1962).
BRABY, F. & CO., *Braby News* (November 1961).
LANE-PETTER, W., 'Animal House Equipment', *UFAW Handbook on Care and Management of Animals*, 2nd Edition (1957).

CHAPTER FOUR

Measurement of Temperature and Humidity

The vital processes of the animal body are accompanied by considerable energy changes. The energy produced is used for mechanical work, but a large proportion of the energy is evolved as heat.

The interior of the body must be maintained at a constant temperature, and this is achieved by physiological mechanisms which govern the rates of heat production and heat loss by the body.

Heat is produced by oxidation of food. Heat is lost by *radiation* from the skin to the boundary surfaces of the room, by *convection* from the skin to the air surrounding the body, and by *evaporation* of moisture or sweat from the skin. Increasing the rate of air movement, e.g. by fans, will increase the convective and evaporative but not radiative heat losses.

Although the internal body temperature is relatively constant, the skin temperature varies with the temperature of the environment. When this is low, blood flow to the skin is reduced, the skin temperature falls, and heat loss by radiation and convection is reduced; at high environmental temperatures the reverse occurs.

The object of maintaining suitable external environmental conditions is to minimize physiological stress required to maintain the constancy of the internal environment of the body.

In order to assess the environmental conditions one should measure the temperature and humidity of the air in the room, the air speed, and the temperature of the boundary surfaces of the room.

Animals should not be placed in draughts, and if the ventilation is adjusted so that no draughts are apparent to the attendant the rate of air movement will be low, i.e. not more than 50 ft per minute.

In many rooms there is little difference between the temperatures of the air and the boundary surfaces of a room under steady-state conditions, so that in practice it is usually necessary only to measure the temperature and humidity of the air.

But the effects of radiation must be remembered. For example, in one room when the air temperature was 74°F (23·3°C) the mean radiant temperature (MRT) was 102°F (38·9°C), owing to the sun shining through the windows. Closing the blinds reduced the MRT to 80°F (26·7°C), but away from the window the MRT was only 75°F (23·9°C).

Animals should not be placed close to sources of radiant heat.

THE MEASUREMENT OF TEMPERATURE IN THE ANIMAL HOUSE

A THERMOGRAPH gives a continuous record of room temperature and shows not only the maximum and minimum temperatures attained but also the *duration* of any variation from the desired temperature. A thermograph consists of a bimetallic spiral, having the more expansible metal on the inside,

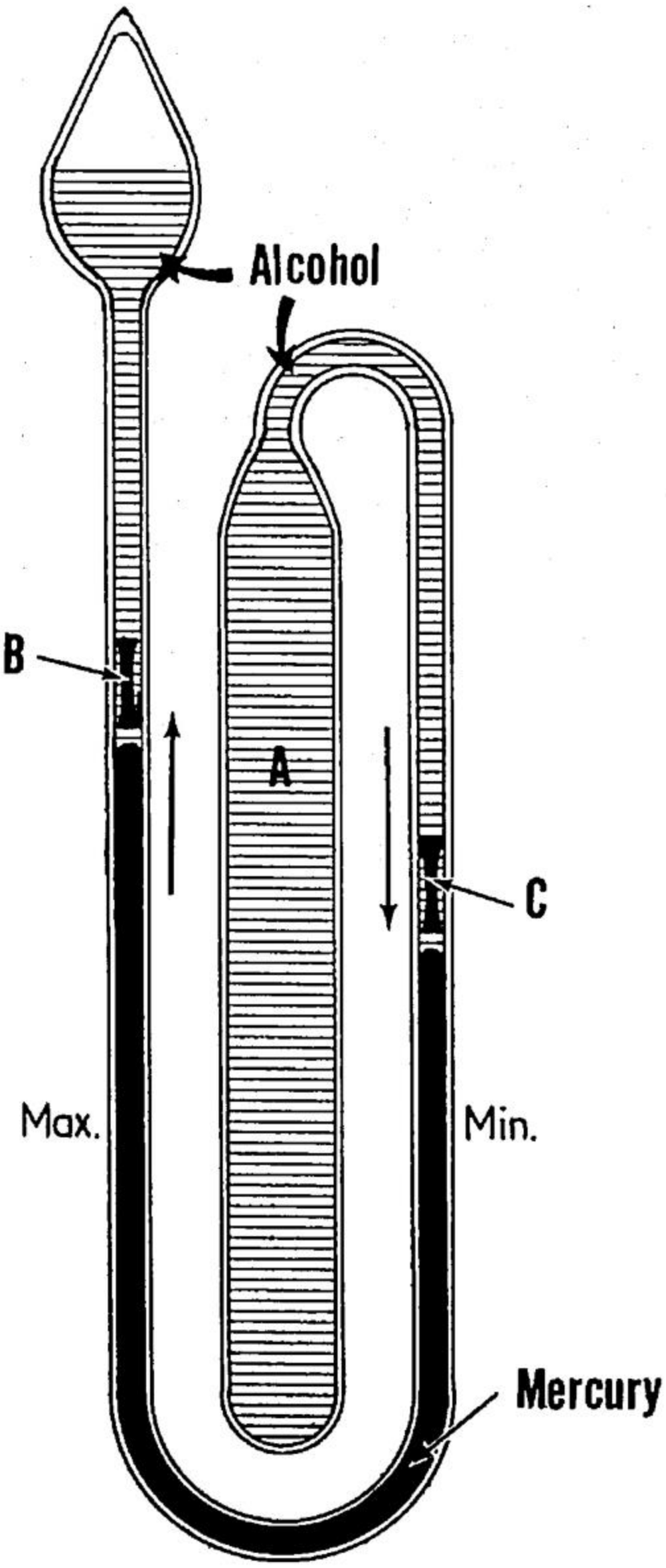

FIG. 1. Diagram to show a maximum–minimum thermometer.

which is fixed at one end and has a lever (for magnifying the movement) connected to the free end. When the spiral is heated it uncoils gently and the temperature is registered, by a pen attached to the lever, on a paper-covered, rotating drum. A good instrument is accurate to about $\pm\frac{1}{2}$°F (0·3°C) over most of the range used.

MAXIMUM AND MINIMUM THERMOMETERS are used to ascertain the extremes of temperature reached over a period of time—usually one day. Various designs are available, but the general principle underlying them is as follows. A large bulb filled with alcohol is connected by a capillary tube containing a short column of mercury to a second, smaller bulb which is partially filled with alcohol. Two small, metal markers are placed in the capillary tube, one on either side of the mercury column. The thermometer is usually bent, often into a U-shape, for compactness, and mounted over two temperature scales.

A rise in temperature causes the alcohol in the large, filled bulb *A* to expand and push the mercury column forward. The mercury column pushes the marker *B* forward. A fall in temperature causes the alcohol in bulb *A* to contract, and the mercury column falls back, pushing marker *C* back along the capillary tube. Marker *B* remains unmoved, as alcohol wets the markers and passes them, which mercury cannot do.

The maximum and minimum temperatures attained over any chosen period can be read from the position on the scales of the ends *nearest* the mercury column of the two markers. The mercury column itself expands with increased temperature, and allowance is made for this when the thermometer is calibrated.

THE MEASUREMENT OF HUMIDITY IN THE ANIMAL HOUSE

The instruments used to determine the humidity of the atmosphere are called HYGROMETERS or PSYCHROMETERS. The simplest *wet- and dry-bulb hygrometer* consists of two similar mercury-in-glass thermometers mounted side by side. The bulb of one thermometer is kept moist by a surrounding cotton wick which dips into a small vessel of distilled water. It is difficult to obtain accurate measurements of humidity with this instrument; to avoid uncertainty it is best to use a ventilateral hygrometer. The *whirling hygrometer* is a wet- and dry-bulb thermometer mounted in a rattle-frame which can be rotated rapidly so that the thermometer bulbs pass through the air at considerable velocity. To obtain accurate readings it is essential that: (i) a ventilated hygrometer is used; (ii) the wet-bulb is indeed wet; (iii) the wet-bulb reading is taken *first*, immediately after whirling the hygrometer for 30 or 40 seconds; (iv) the instrument is whirled and wet-bulb readings taken until two successive readings agree closely; (v) the cotton wick is kept clean; (vi) clean, distilled water is used in the reservoir; and (vii) when readings are taken the instrument is held so that the hands do not warm the bulbs of the thermometer.

The *Assman psychrometer* is a much more expensive instrument in which air is drawn over the thermometer bulbs by means of a clockwork or electrically driven fan.

DEFINITION OF TERMS USED IN THE CALCULATION OF RELATIVE HUMIDITY

(*a*) Dry-bulb temperature

The temperature of the air registered by an ordinary thermometer. An ordinary thermometer with a bulb about 1 in. × ¼ in. in calm air assumes

equilibrium between the mean radiant temperature and the true air temperature. The sling thermometer quickly assumes air temperature. If both thermometers are used one may ascertain if thermal radiation measurements are warranted. In other words, the bulb of an ordinary stationary thermometer is affected by thermal radiation.

(*b*) Wet-bulb temperature

The temperature registered by a thermometer the bulb of which is covered by a wetted wick and exposed to a current of rapidly moving air.

(*c*) Dew-point temperature

The temperature to which air has to be cooled (at constant pressure) before it becomes saturated with respect to liquid water; in saturated conditions the dew point and air temperature are equal.

(*d*) Wet-bulb depression

Difference between dry- and wet-bulb temperatures.

(*e*) Dew-point depression

Difference between dry-bulb and dew-point temperatures.

(*f*) Relative humidity

The relative humidity is defined as the ratio, expressed as a percentage, of the actual vapour pressure to the saturation vapour pressure at the air temperature.

(*g*) Absolute humidity

The absolute humidity is defined as the ratio of the mass of water vapour in the volume occupied by the moist air with which it is associated. It is thus equivalent to the density of the water vapour, and can be calculated from the vapour pressure and the temperature.

RELATION BETWEEN DRY-BULB, WET-BULB, AND DEW-POINT TEMPERATURES AND RELATIVE HUMIDITY

Whenever the air is not completely saturated the wet-bulb temperature is lower than that of the dry-bulb, due to cooling by evaporation. Since this wet-bulb temperature is a function of total heat content of the air including moisture, it follows that if both wet- and dry-bulb temperatures are known, relative humidity and dew point can be determined. Stating it more broadly, if *any two* of the properties of air for a given condition are known—dry-bulb temperature, wet-bulb temperature, dew-point temperature, or relative humidity—the other two may be found.

Relative humidity can be determined when dry-bulb and dew-point temperatures are known. The dew-point temperature determines the actual vapour pressure in the air at a given temperature, and the saturated vapour pressure at this dry bulb temperature can be found, both quantities being obtained from

psychrometric tables. If the air is saturated no evaporation can take place from the wick of a wet-bulb thermometer, so the dry- and wet-bulb temperatures are the same. Under such conditions the dew-point temperature coincides with the dry- and wet-bulb temperatures.

Conversion of these quantities from one to another can be effected by reference to psychrometric tables. For practical work, however, a psychrometric chart is used, because its degree of accuracy is usually acceptable for ordinary calculations. The chart is concise and convenient, and with its aid conditions may be visualized. A large-scale psychrometric chart is supplied in the Supplement to the MRC War Memorandum No. 17, published by HMSO.

EXAMPLE 1:

Given: dry-bulb temperature, 70°F (21·1°C).
wet-bulb temperature, 60°F (15·6°C).
Find: percentage of relative humidity and dew point.

Locate point of intersection of vertical line representing 70°F (21·1°C) dry-bulb temperature with the oblique line representing 60°F (15·6°C) wet-bulb temperature. By interpolation this point indicates the percentage of relative humidity as 56 per cent and by following the intersecting horizontal line to the left to its intersection with the saturation line the dew point is indicated as 53·6°F (12·0°C).

EXAMPLE 2:

Given: dry-bulb temperature, 80°F (26·7°C),
relative humidity, 59 per cent.
Find: dew-point and wet-bulb temperature.

Locate the point of intersection of the vertical line representing 80°F (26·7°C) dry-bulb temperature with the interpolated position of the curved line which would represent 59 per cent relative humidity.

Reading horizontally to the left from this point, the dew point is indicated as 64°F (17·8°C), and reading obliquely upward to the left, between the wet-bulb lines, to the saturation line, the wet-bulb temperature is indicated as 69·3°F (20·6°C).

EXAMPLE 3:

Given: dry-bulb temperature, 75°F (23·9°C),
dew-point temperature, 55°F (12·8°C).
Find: percentage of relative humidity and wet-bulb temperature.

Locate the point of intersection of the vertical line representing 75°F (23·9°C), dry-bulb temperature with the horizontal dew-point line intersecting the saturation curve at 55°F (12·8°C). By interpolation, this point indicates the relative humidity as 50 per cent and the wet-bulb temperature as 62·6°F (17°C).

HAIR HYGROMETERS which are commercially available are unsuitable as standard instruments for measuring humidity, but as recording instruments they are simple and efficient, provided they are regularly checked and kept in good condition.

PAPER HYGROMETERS may be used as indicators of the humidity.

SPECIES	RECOMMENDED		RANGE OF	
	TEMPERATURE, °F	RELATIVE HUMIDITY, PER CENT	TEMPERATURE, °F	RELATIVE HUMIDITY, PER CENT
Mouse	72	50	68–74	45–55
Rat	72	50	65–75	45–55
Cotton rat	72	50	65–75	45–55
Mastomys	72	50	65–75	45–55
Gerbils	72	50	65–75	45–55
Hamster*	72	50	65–75	45–55
Guinea pig	70	50	65–75	45–55
Ferret	63	50	60–65	45–55
Rabbit	65	50	62–68	45–55
Cat	63	50	60–65	45–55
Dog	60	50	55–65	45–55
Monkeys:				
From Africa—				
Cercopithecidae*	80	55	78–85	50–60
Baboons*	75	55	65–78	50–60
Chimpanzees*	80	58	78–82	55–60
From India and Far East—				
Rhesus	70	50	68–72	45–55
Cynamolgus*	80	58	78–85	55–60
From the New World—				
Spider monkey*	80	55	78–82	50–60
Capuchin*	80	55	78–82	50–60

* The temperature and humidity must be closely controlled if these species are to be kept in good health.

THE CLINICAL THERMOMETER is a mercury-in-glass thermometer with a short working range of 95–110°F (35–43·3°C). Each degree division is subdivided into five equal parts. There is a small constriction in the bore of the thermometer between the bulb and the lowest graduation. When the bulb of the thermometer is heated the mercury expands and rises up the bore, past the constriction. When the thermometer is cooled the mercury contracts, but the mercury which has risen above the constriction cannot fall back into the bulb, thus the highest temperature recorded by the thermometer may be read at leisure. The thermometer is reset by shaking, so that the mercury trapped above the constriction is jerked back into the bulb.

Clinical thermometers are made with small bulbs and narrow bores, so that they may be quick to respond to changes in temperature. Such a thin column

of mercury is difficult to see, so the glass casing of these thermometers is made to give a convex surface through which a magnified image of the mercury thread may be seen.

STERILIZATION OF CLINICAL THERMOMETERS

Clinical thermometers may not be sterilized by boiling, because the maximum temperature they are designed to measure is some 100°F (37·8°C) below that of boiling water; nor may they be washed in water from a hot tap. These thermometers may be sterilized by immersion in 70 per cent alcohol, and they should then be rinsed thoroughly in cold, running water before use. A clinical thermometer should not be used without first checking that it has been reset and that the top of the mercury column is ½ in. below the lowest graduation on the thermometer.

FURTHER READING

SPENCER-GREGORY, H. and ROURKE, E., *Hygrometry*. Crosby Lockwood, London (1957).

BODY TEMPERATURES OF COMMON SPECIES

Figures obtained from daily measurements taken over a fortnight. No appreciable difference was found between the body temperatures of males and females

SPECIES AND NUMBERS USED	AVERAGE TEMPERATURE °F	AVERAGE TEMPERATURE °C	MAXIMUM AND MINIMUM TEMPERATURES RECORDED °F	MAXIMUM AND MINIMUM TEMPERATURES RECORDED °C
Cockerels (6)	104·9	40·5	103·1–106·4	39·5–40·5
Dogs (2 m.)	101·2	38·3	100·5–102·2	38·1–38·9
Ferrets (3 m. 8 f.)	101·9	38·8	100·0–104·1	37·8–40·0
Guinea pigs (14 m. 14 f.)	101·0	38·3	99·3–103·0	37·4–39·4
Hamsters, Golden (6 m. 6 f.)	99·3	37·4	97·0–102·3	36·1–38·9
Mastomys (25 m. 25 f.)	99·1	37·2	95·9–102·6	35·6–39·1
Meriones (7 m. 7 f.)	99·4	37·4	96·3–102·8	35·8–39·0
Mice, Albino (60 m. 60 f.)	98·8	37·1	95·0–102·6	35·0–39·0
Monkey, Rhesus (entire stock) over several months	103·4	39·6		
Rabbits (18 f.) (strain variation insignificant)	101·1	38·3	99·1–102·9	37·2–39·3
Rats				
Cotton (12 m. 12 f.)	100·8	38·2	98·4–103·6	36·7–39·6
Hooded (12 m. 12 f.)	99·2	37·3	96·8–102·1	35·6–38·9

CHAPTER FIVE

Animal Handling

'The importance of handling animals in the correct manner cannot be over-rated. Improper handling may result in injury to the animal, to the technician himself, or, most important of all, to the animal–man relationship' (Short, D.J., *UFAW Handbook*).

The proper method of handling animals cannot be taught by lecturing or learned by reading; and the most satisfactory method of teaching is by practical demonstration, and of learning is by constant practice.

Forceps or leather gloves should never be used for handling animals unless circumstances warrant it, e.g. with infected animals, where there is a danger of the technician becoming infected from a bite.

The smaller laboratory animals quickly become docile if they are handled properly and frequently. Animals should always be held firmly, but not tightly. If an animal feels insecure or uncomfortable it will struggle to get free, and in doing so may injure itself.

Pregnant animals should be handled with great care and only when absolutely necessary.

It is necessary to employ a different technique with each animal, but the handler must always be relaxed, and must approach the animal with quiet confidence.

SPECIES

(*a*) Rabbit

To take a rabbit out of a cage, hold the ears steady with one hand facing in the direction of the operator, and place the other hand underneath the belly of the animal. Lift both hands together gently but firmly.

To carry a rabbit, place the index finger between the ears, the thumb and other fingers will then control the head. The other hand, which takes all the weight, is placed round and under the tail (Fig. 1).

For artificial insemination, place the animal facing the handler on a non-slippery table. Its head should be tucked into the handler's body, and a hand run down each side of the rabbit's body to grasp the hind-legs, which are held by the first and second fingers of the hand, the other two fingers being placed round and underneath the hind-legs. Hold the legs apart and lift them slightly.

When sexing a rabbit always hold the legs facing *away from operator* (Fig. 2).

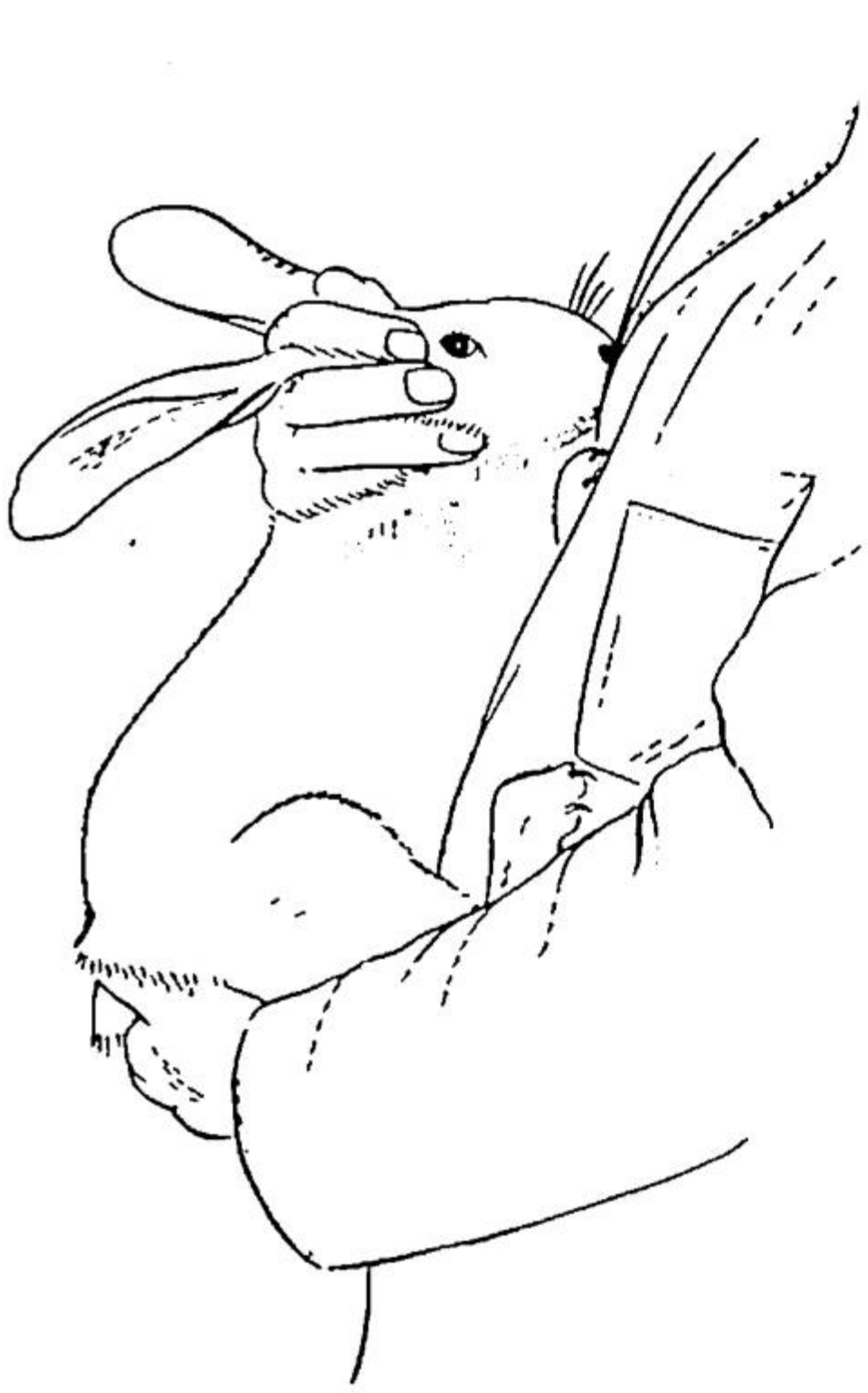

FIG. 1

FIG. 2

(*b*) **Rat**

Do *not* pick a rat up by the tail. Place the hand, palm downwards, high up over the animal's back with the thumb round the neck and under the mouth. Grasp the rat firmly, but not tightly (Figs. 3, 4, and 5). When approaching rats never hesitate or fumble or frighten the animals by waving the hands about.

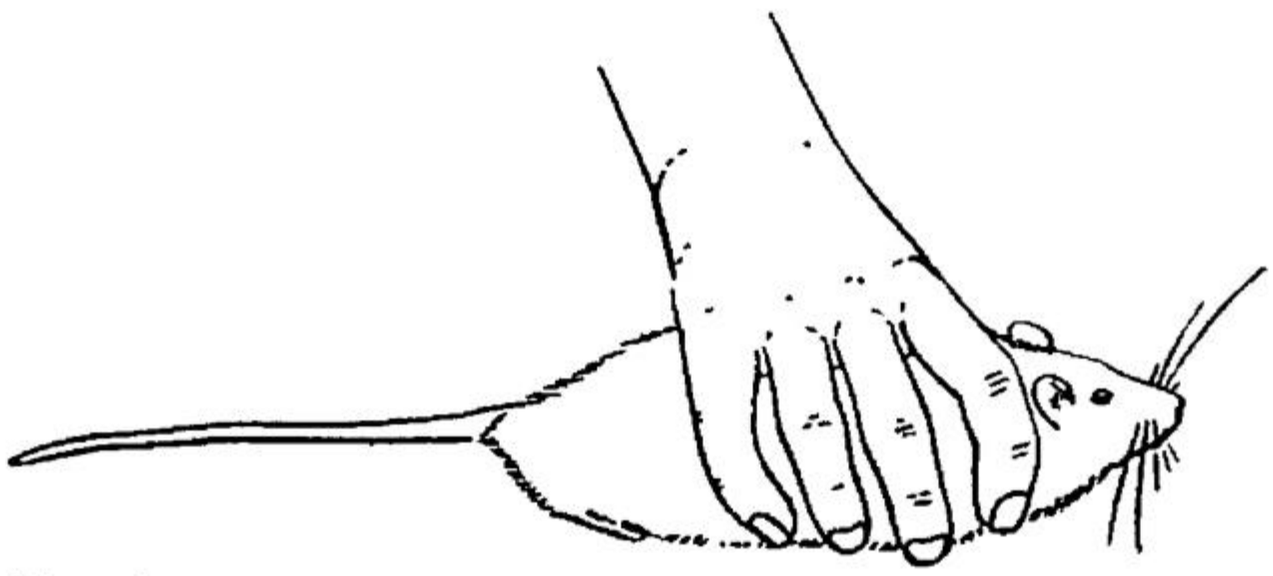

FIG. 3

(*c*) **Mouse**

The mouse, alone among the laboratory animals, may be lifted by its tail. The base of the tail is held between a thumb and forefinger, and the weight of the body is supported by letting the mouse stand on the top of the cage top (Fig. 6). Mice may be sexed easily and quickly by holding them in this way. If a mouse is held by the tip of the tail it may climb up its tail and bite the handler.

of the hand is then placed between the animal's left hind-leg and its body. This leg is held firmly between the fourth and fifth finger. This position allows complete freedom of one hand (Fig. 12).

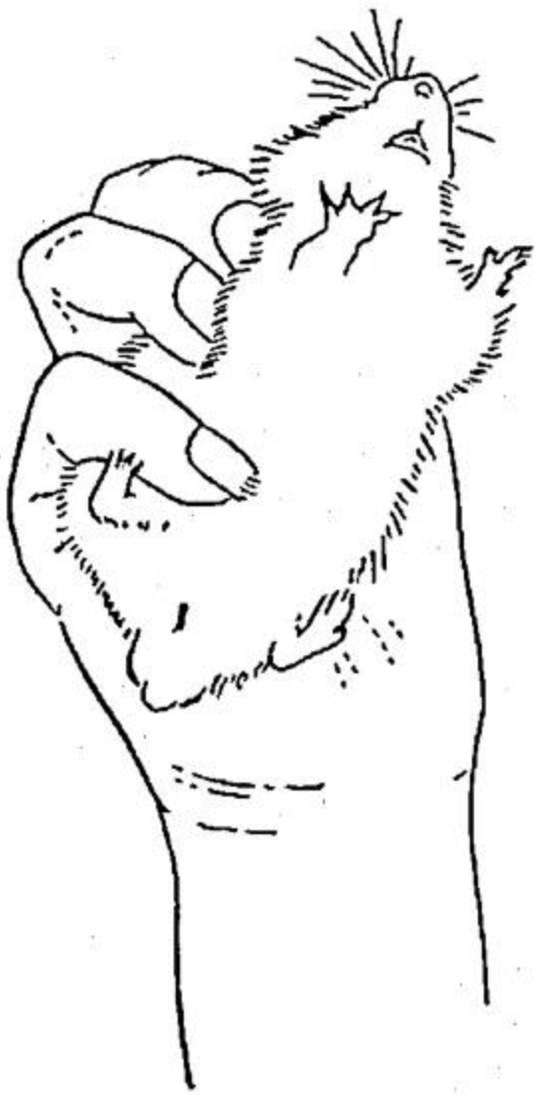

FIG. 12

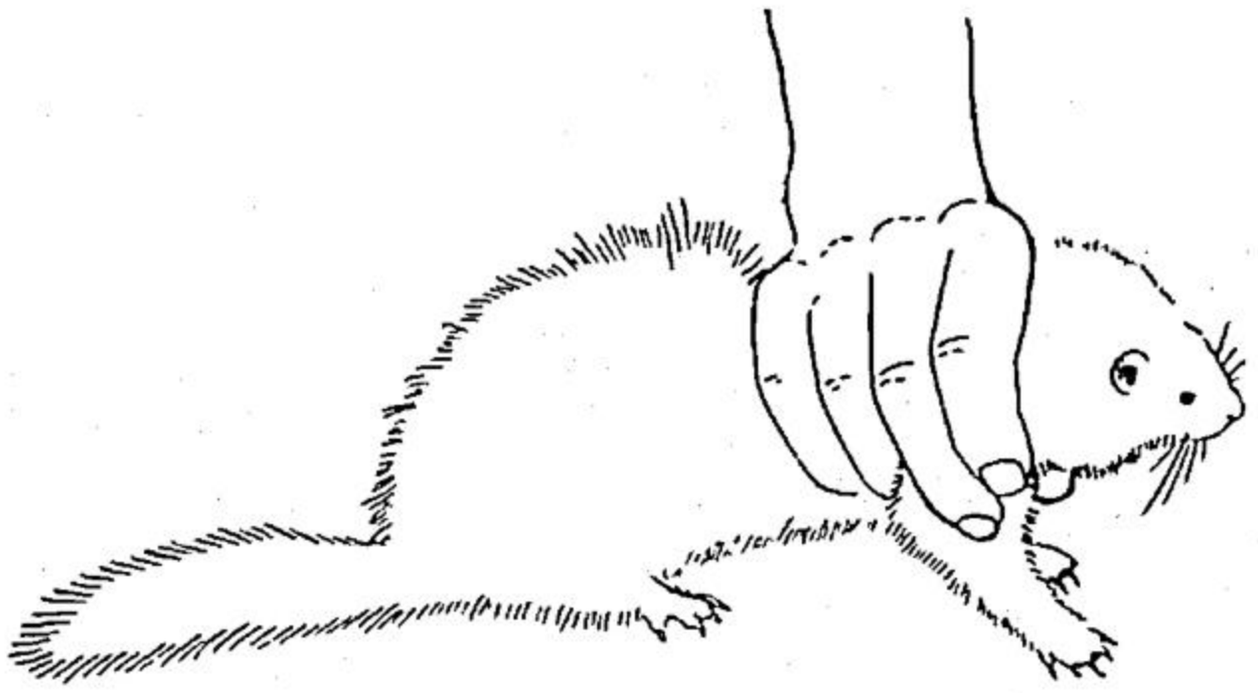

FIG. 13

(*f*) Ferret

When handling ferrets do not hesitate. Pick the animal up firmly but gently by placing the thumb underneath the mouth and the fingers round the neck (Fig. 13). If the animal does attempt to bite the thumb can be used to close its mouth. If necessary, support the weight of the animal with the free hand. On

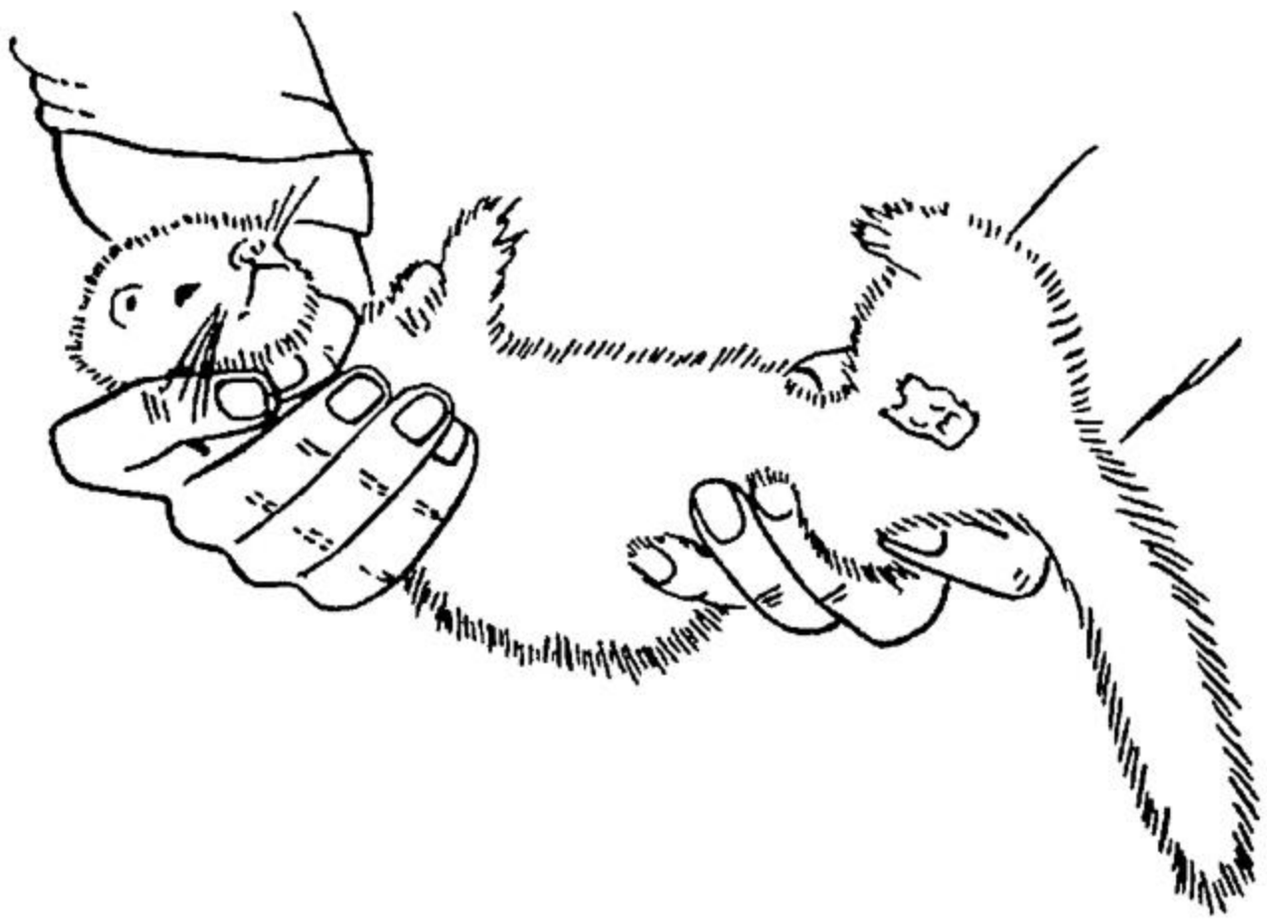

FIG. 14

no account use stiff leather gloves or tongs to handle ferrets. These animals should be handled frequently if they are to remain docile (Fig. 14).

(*g*) Cotton rat

These animals are not vicious, but they do resent being handled. They are very agile, and can jump 2 feet into the air from a standing start. Never catch

FIG. 1

FIG. 2

(*b*) **Rat**

Do *not* pick a rat up by the tail. Place the hand, palm downwards, high up over the animal's back with the thumb round the neck and under the mouth. Grasp the rat firmly, but not tightly (Figs. 3, 4, and 5). When approaching rats never hesitate or fumble or frighten the animals by waving the hands about.

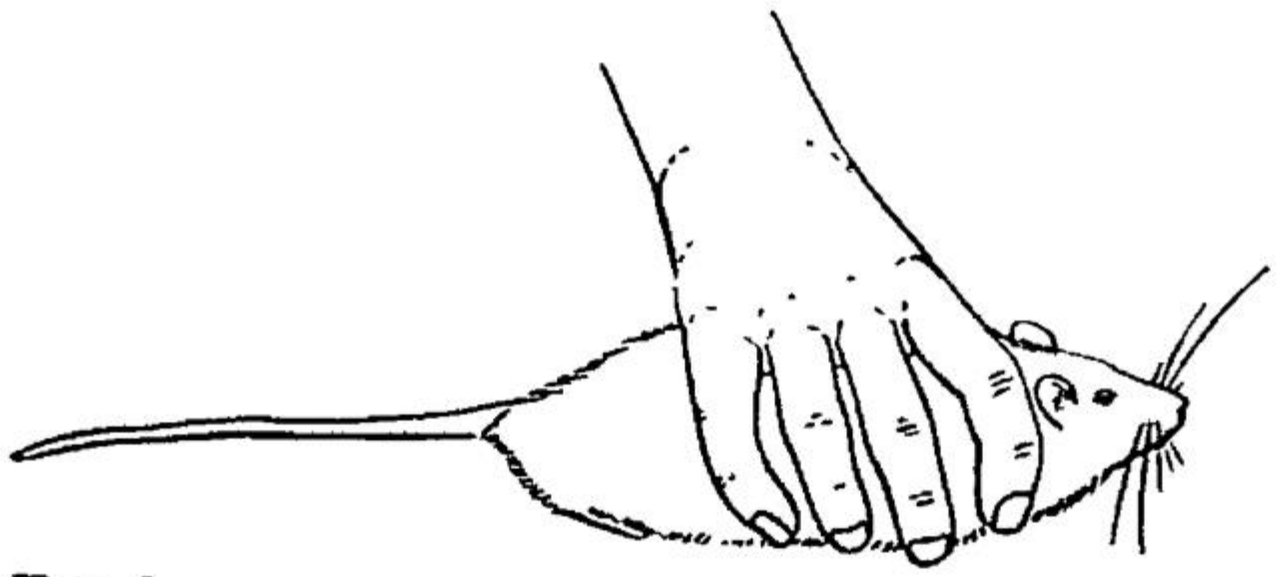

FIG. 3

(*c*) **Mouse**

The mouse, alone among the laboratory animals, may be lifted by its tail. The base of the tail is held between a thumb and forefinger, and the weight of the body is supported by letting the mouse stand on the top of the cage top (Fig. 6). Mice may be sexed easily and quickly by holding them in this way. If a mouse is held by the tip of the tail it may climb up its tail and bite the handler.

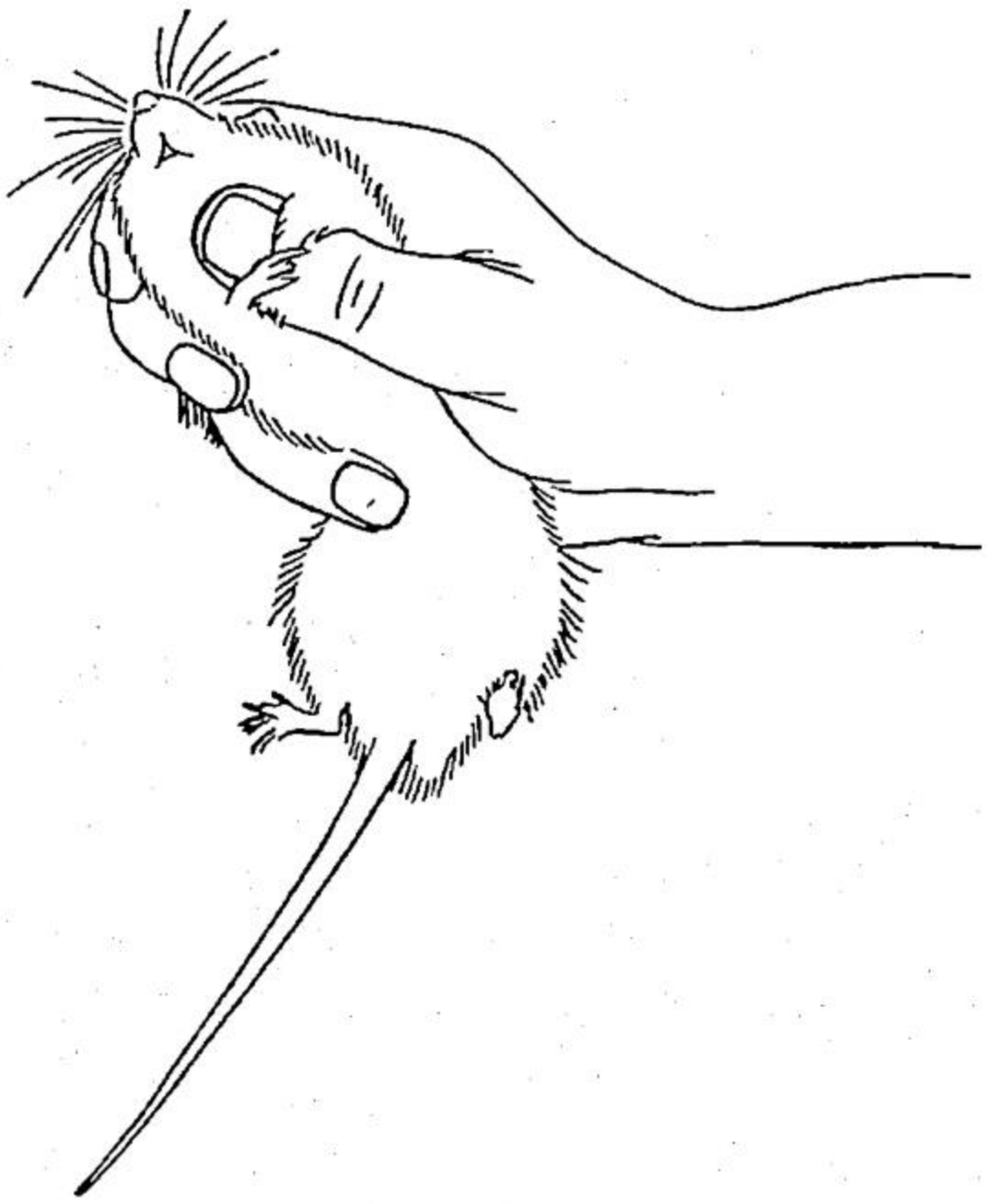

FIG. 4

FIG. 5

To immobilize a mouse for a manipulation, take the loose scruff of the neck between the thumb and forefinger, turn the hand so that the mouse lies, belly uppermost, in the palm and grip the tail between the third and fourth (or fourth and fifth) fingers (Fig. 7).

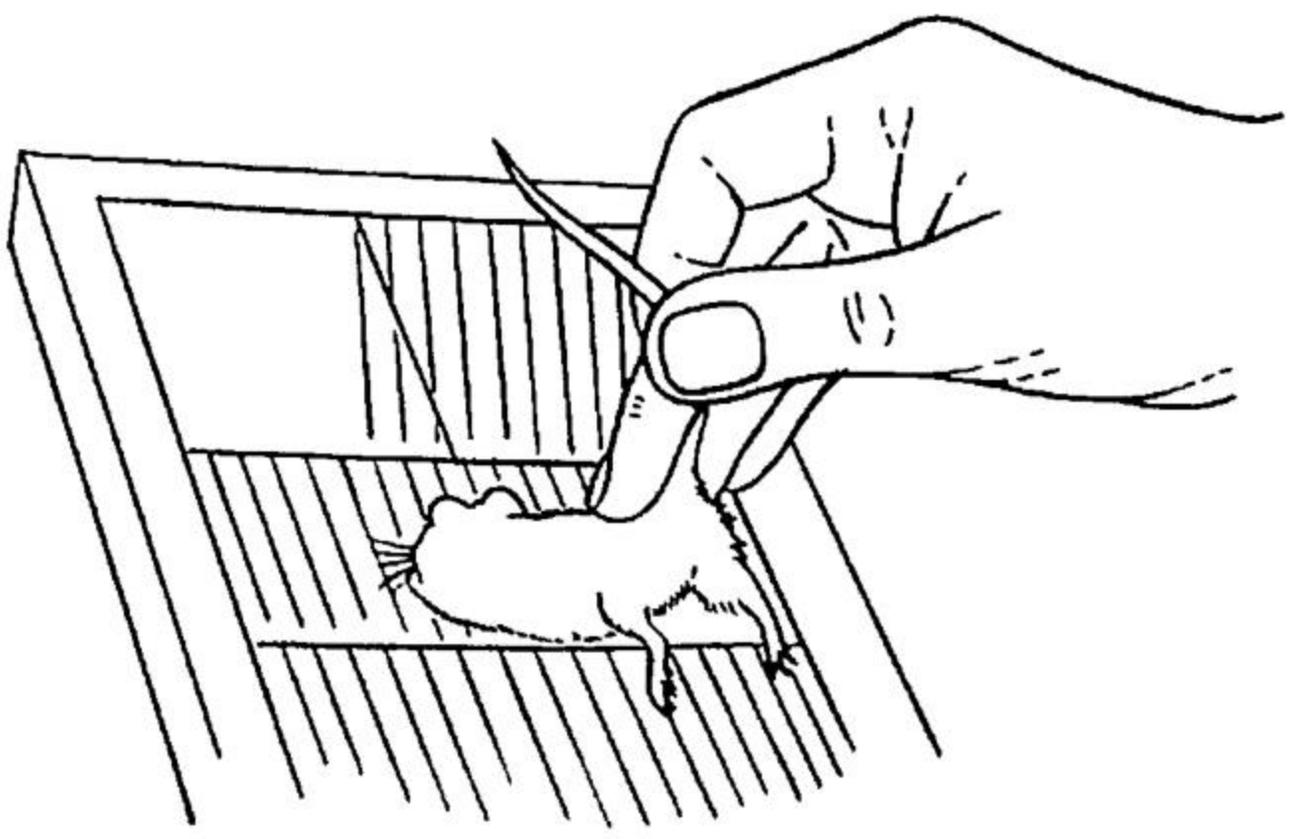

FIG. 6

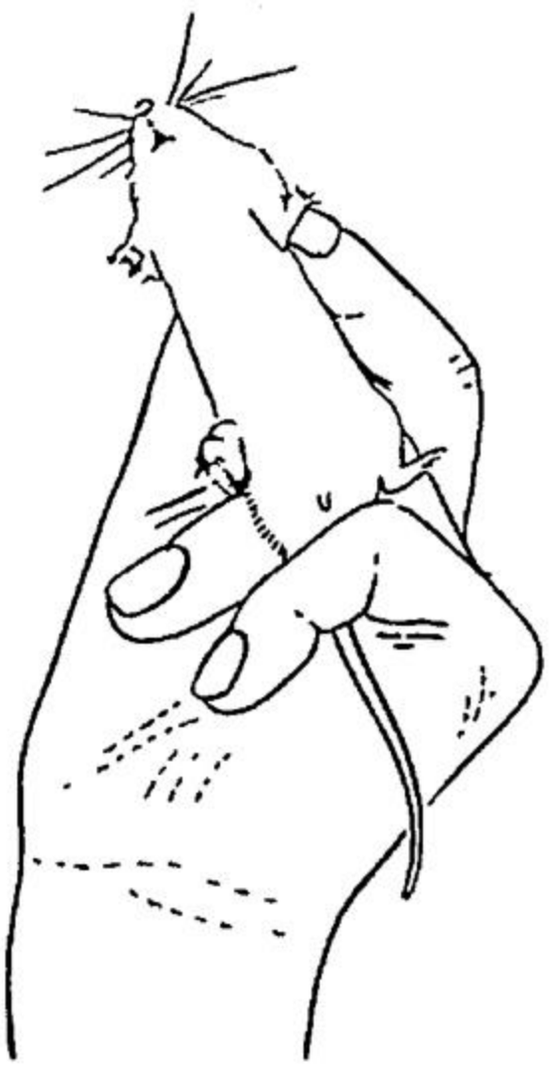

FIG. 7

(*d*) Guinea pig

These inoffensive animals are much more shy of being handled than are rats, although, when under control, they are completely docile. Handle as for rats, but support the weight of the animal with the free hand. Great care is needed with pregnant animals (Figs. 8 and 9).

FIG. 1

FIG. 2

(*b*) Rat

Do *not* pick a rat up by the tail. Place the hand, palm downwards, high up over the animal's back with the thumb round the neck and under the mouth. Grasp the rat firmly, but not tightly (Figs. 3, 4, and 5). When approaching rats never hesitate or fumble or frighten the animals by waving the hands about.

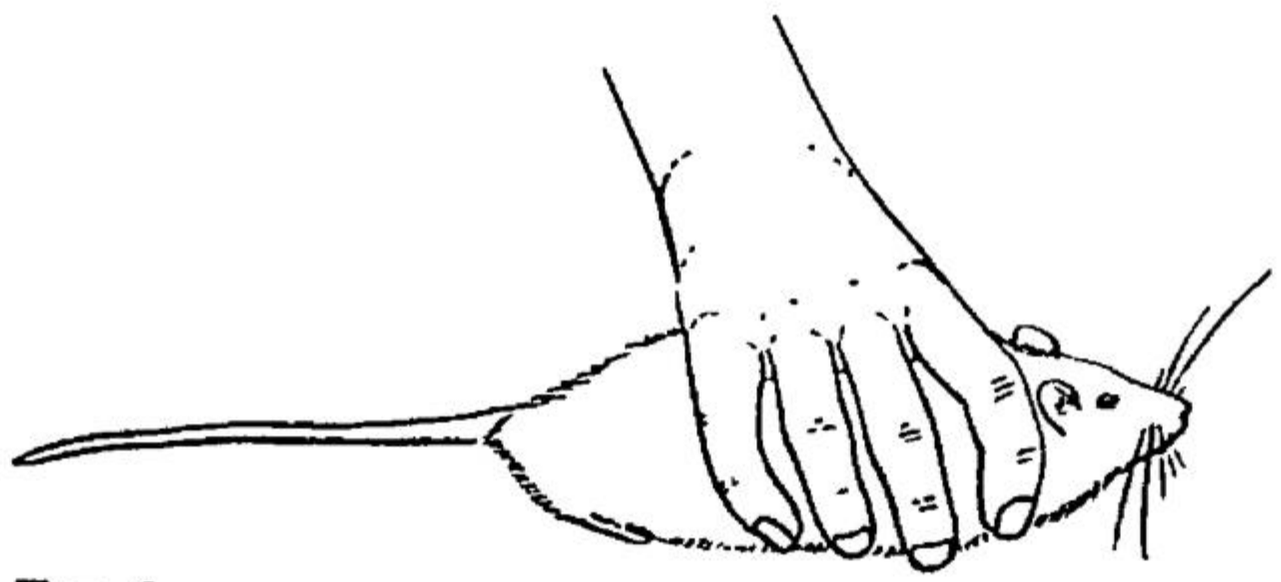

FIG. 3

(*c*) Mouse

The mouse, alone among the laboratory animals, may be lifted by its tail. The base of the tail is held between a thumb and forefinger, and the weight of the body is supported by letting the mouse stand on the top of the cage top (Fig. 6). Mice may be sexed easily and quickly by holding them in this way. If a mouse is held by the tip of the tail it may climb up its tail and bite the handler.

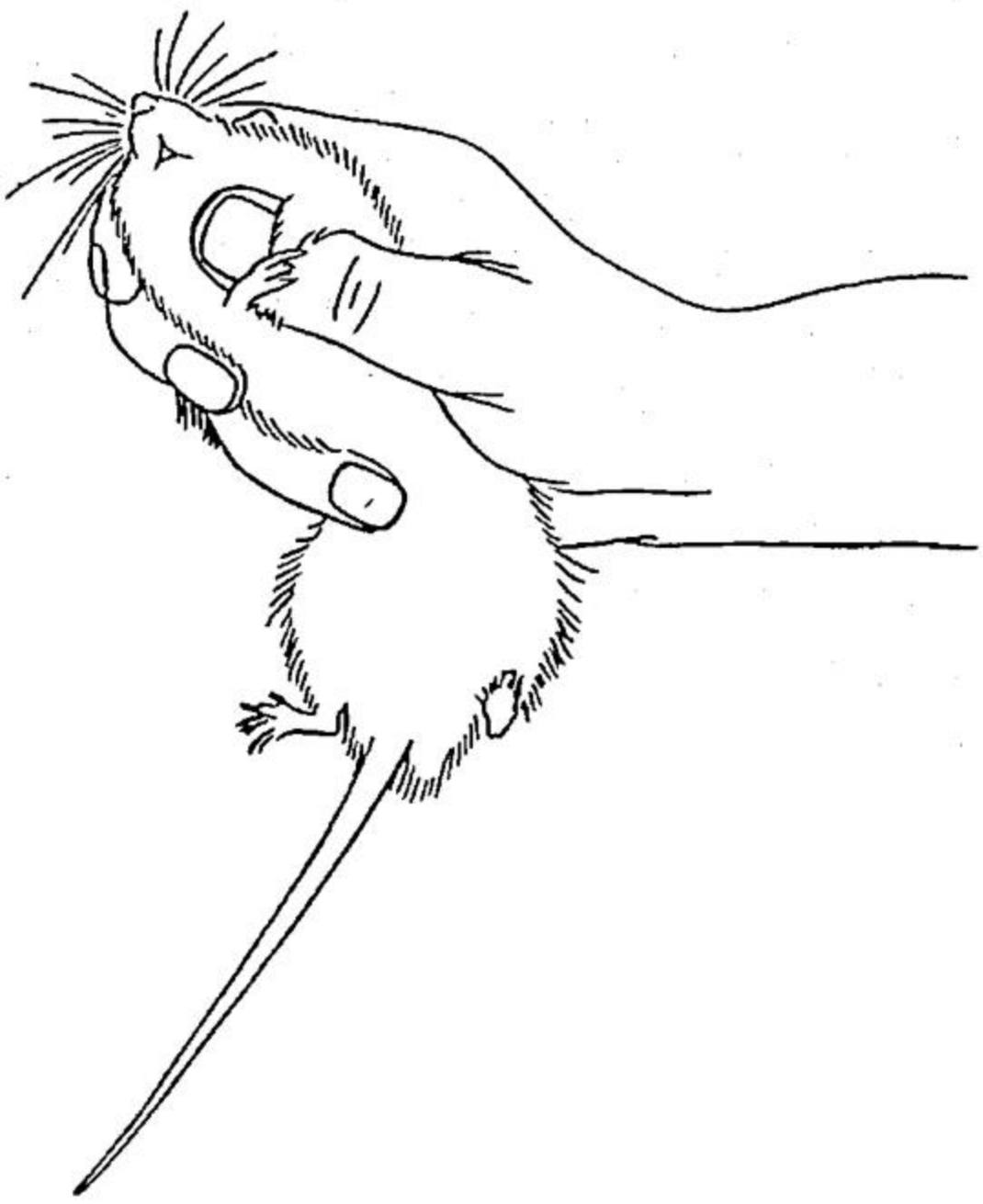

FIG. 4

FIG. 5

To immobilize a mouse for a manipulation, take the loose scruff of the neck between the thumb and forefinger, turn the hand so that the mouse lies, belly uppermost, in the palm and grip the tail between the third and fourth (or fourth and fifth) fingers (Fig. 7).

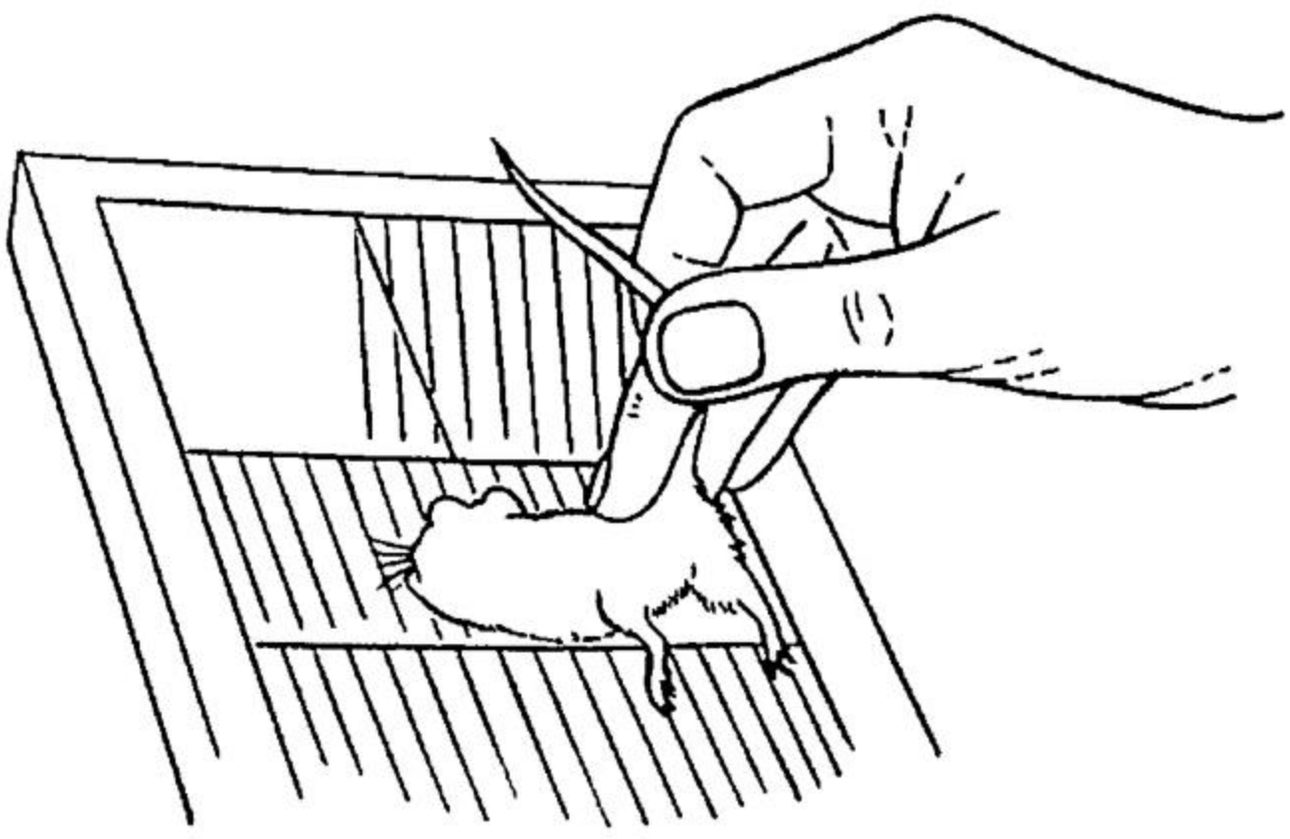

FIG. 6

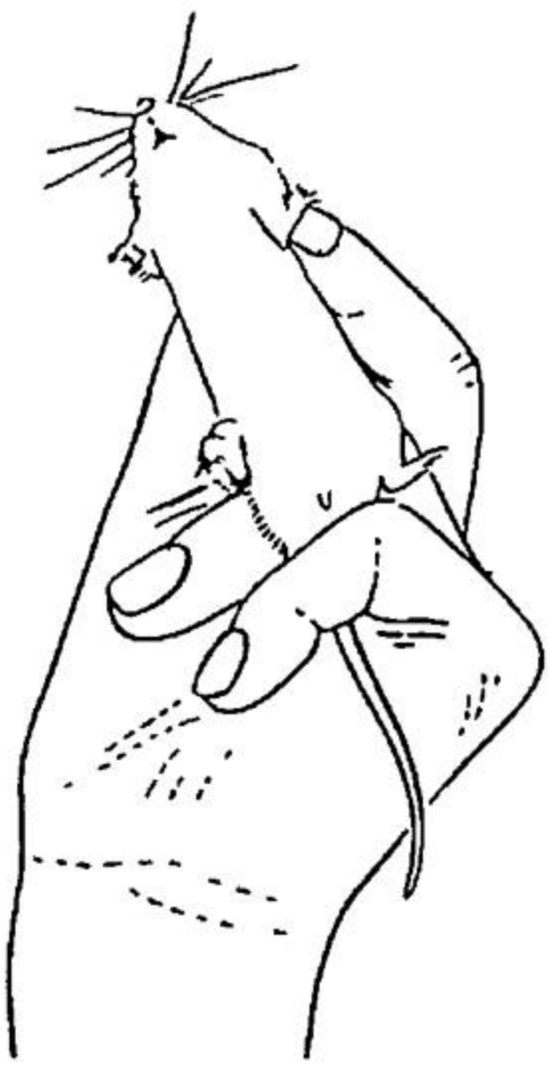

FIG. 7

(*d*) Guinea pig

These inoffensive animals are much more shy of being handled than are rats, although, when under control, they are completely docile. Handle as for rats, but support the weight of the animal with the free hand. Great care is needed with pregnant animals (Figs. 8 and 9).

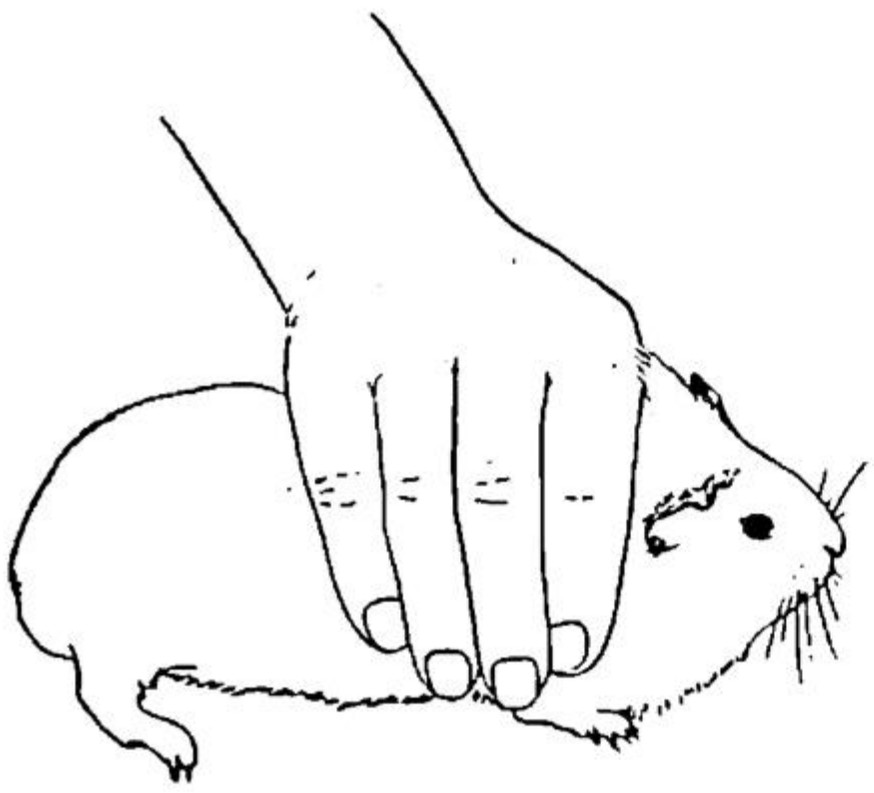

FIG. 8

FIG. 9

(*e*) Hamster

If hamsters are handled frequently they present no problem. Hamsters may be lifted by cupping both hands under the animal. Do not grip them too tightly (Figs. 10 and 11). If the animal must be held firmly for manipulation or

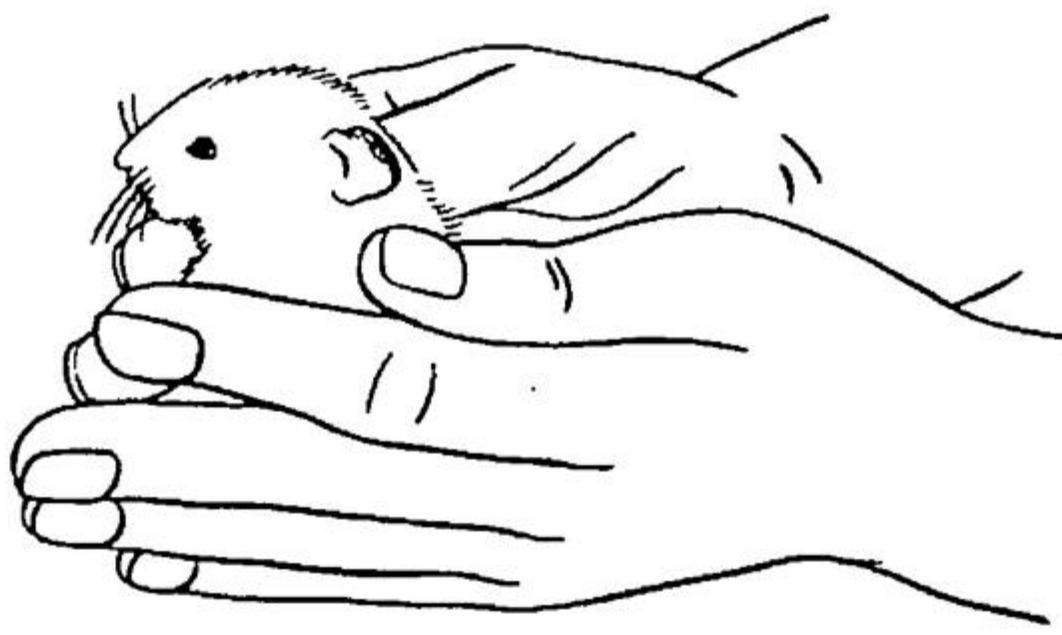

FIG. 10

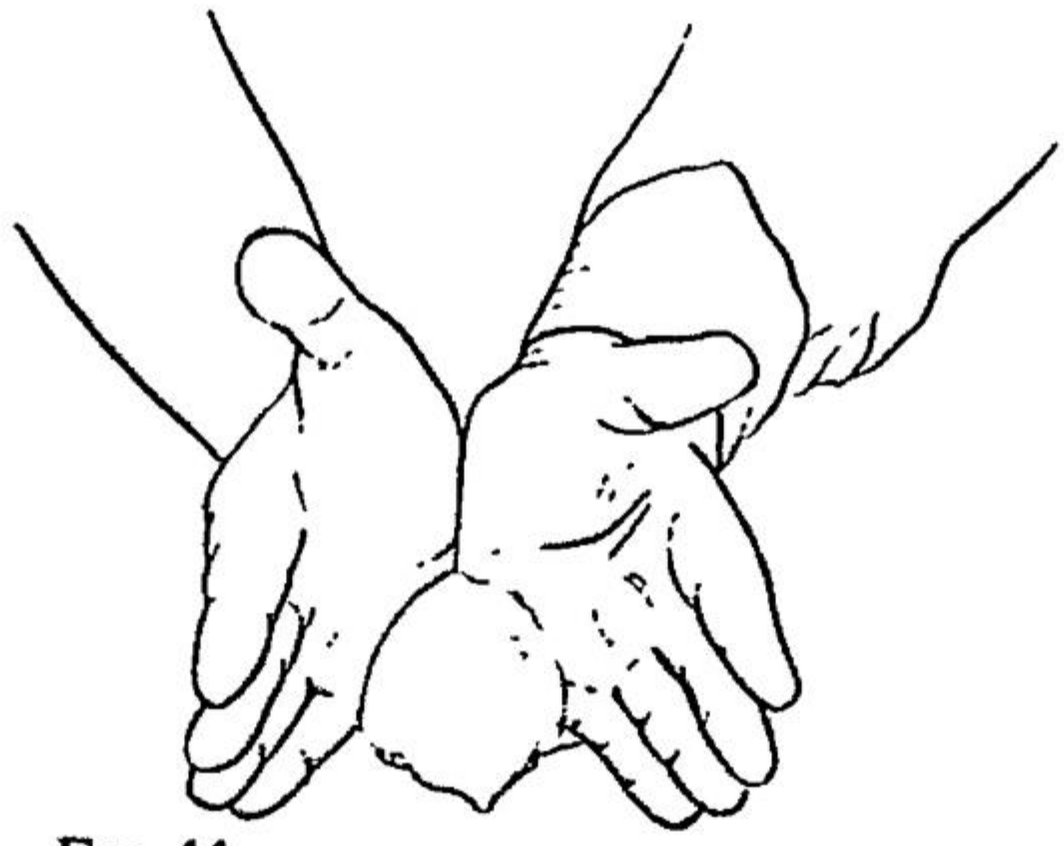

FIG. 11

inoculations grasp the loose skin at the back of the neck with the forefinger and thumb of the right hand and lift the animal, turn the wrist over, and the animal's back will then be resting in the palm of the hand; the fourth finger

of the hand is then placed between the animal's left hind-leg and its body. This leg is held firmly between the fourth and fifth finger. This position allows complete freedom of one hand (Fig. 12).

FIG. 12

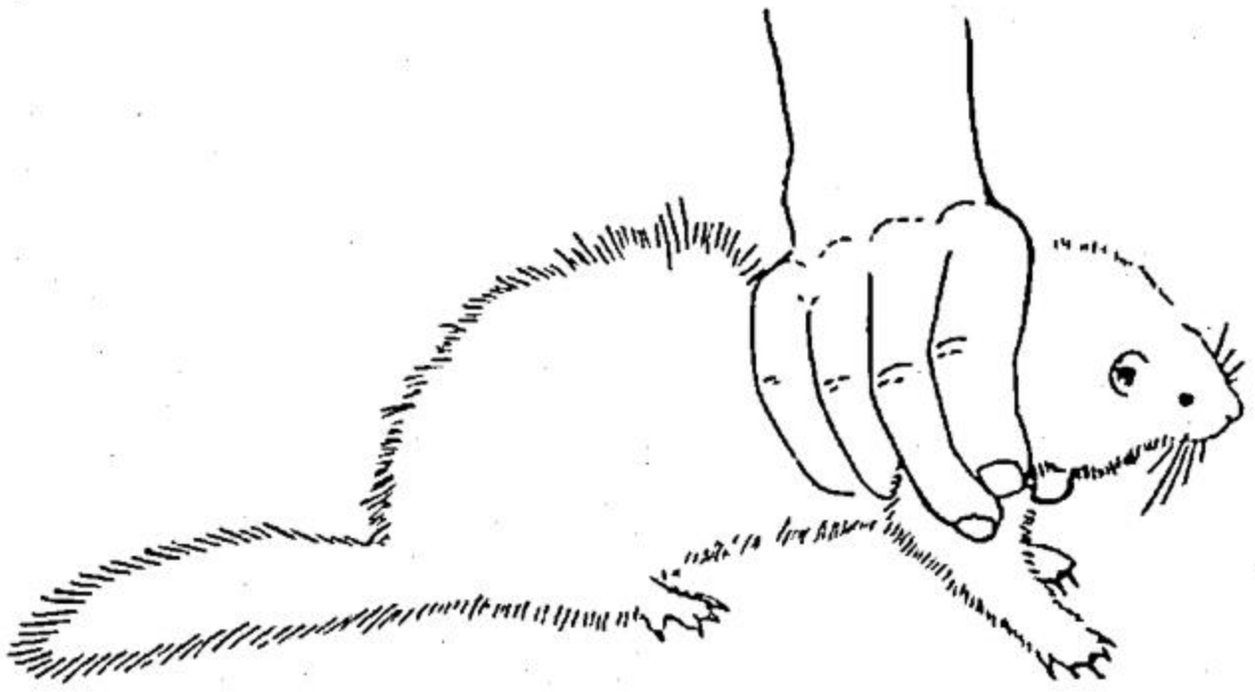

FIG. 13

(*f*) Ferret

When handling ferrets do not hesitate. Pick the animal up firmly but gently by placing the thumb underneath the mouth and the fingers round the neck (Fig. 13). If the animal does attempt to bite the thumb can be used to close its mouth. If necessary, support the weight of the animal with the free hand. On

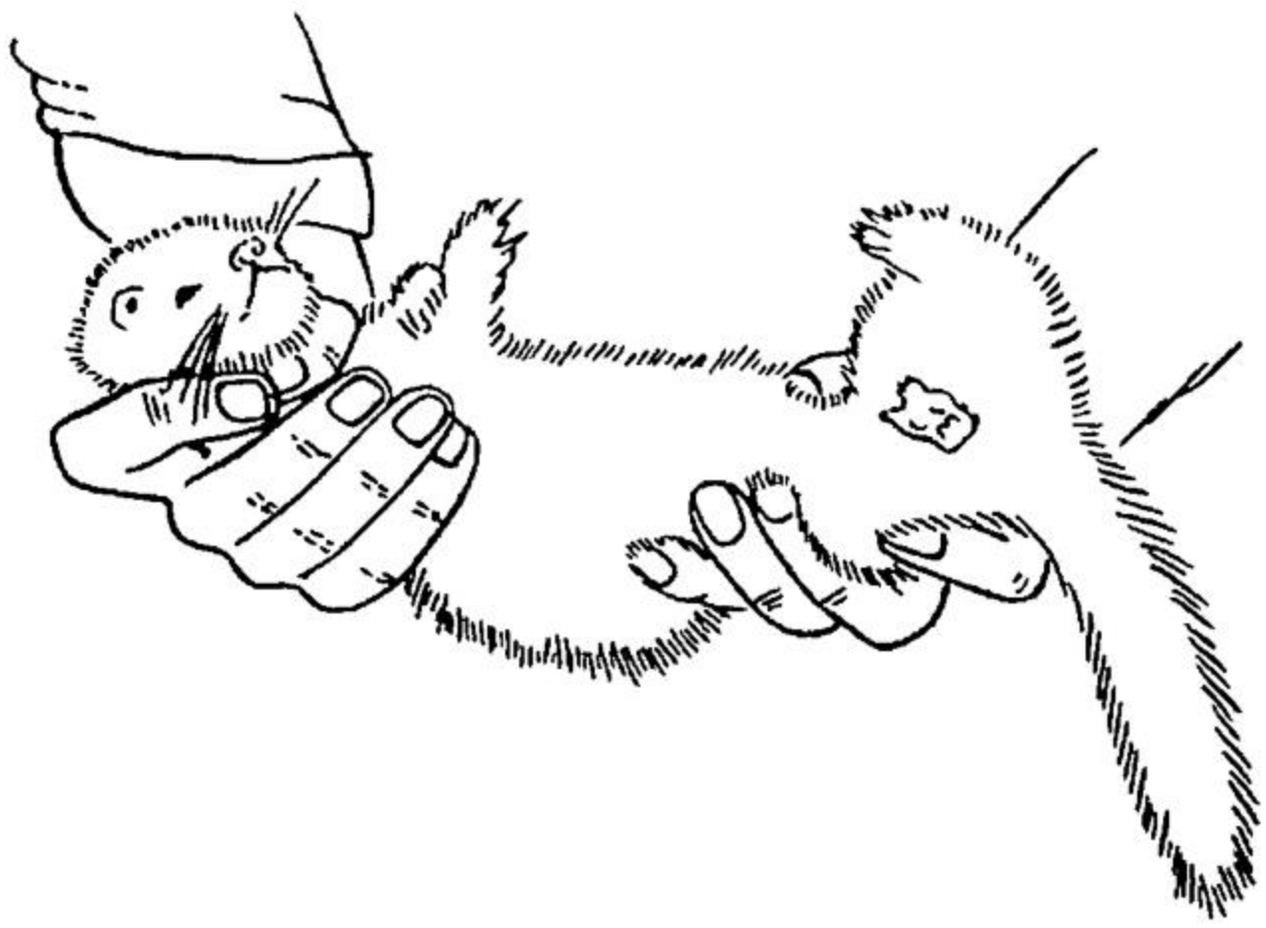

FIG. 14

no account use stiff leather gloves or tongs to handle ferrets. These animals should be handled frequently if they are to remain docile (Fig. 14).

(*g*) Cotton rat

These animals are not vicious, but they do resent being handled. They are very agile, and can jump 2 feet into the air from a standing start. Never catch

a cotton rat by the tail; put its cage into a sanitary bin, or other deep receptacle, and let the animal come out; place the palm of the hand over the rat's back and grasp the skin at the back of the head with the thumb and forefinger. Much practice is necessary to perfect this technique (Figs. 15 and 16).

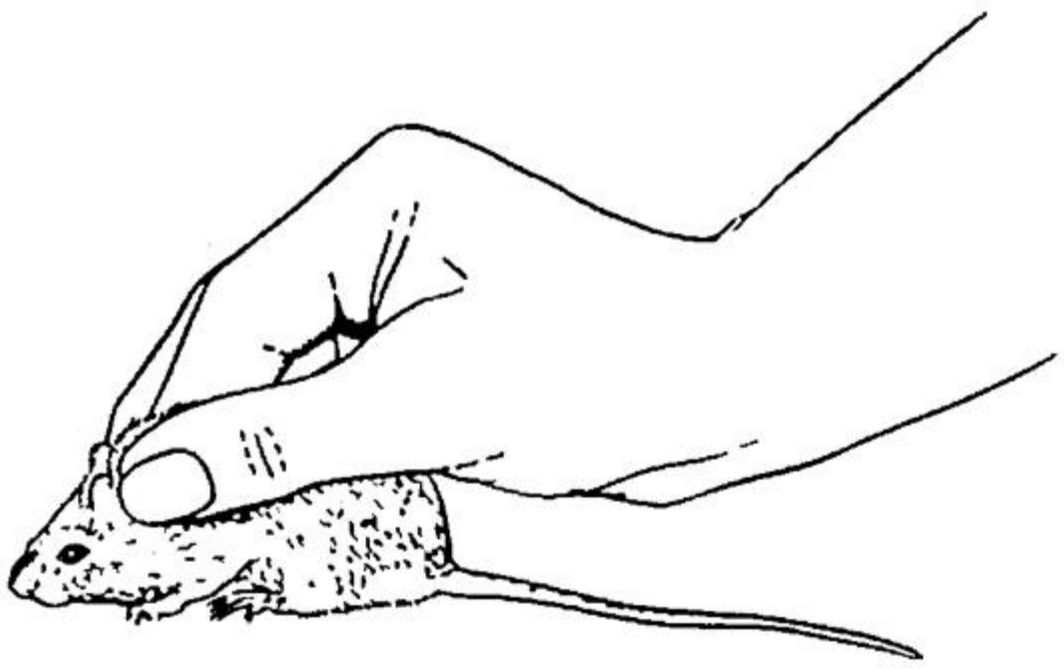

FIG. 15

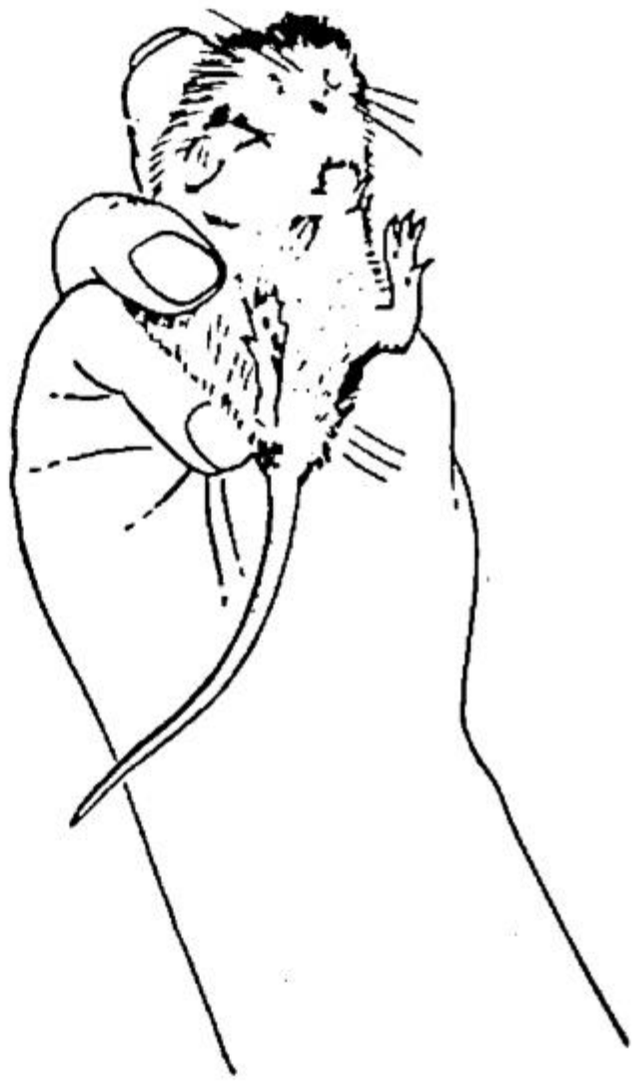

FIG. 16

(*h*) Fowl

Always keep the wings close to the bird's body to prevent fluttering. Face the bird and slide both hands, with fingers outspread, down and under the breast, placing the first and second fingers of one hand between the bird's legs. Grip both thighs and wing tips with the thumbs and third and fourth fingers, and then lift bird. Be calm and deliberate, and do not make quick movements (Figs. 17 and 18).

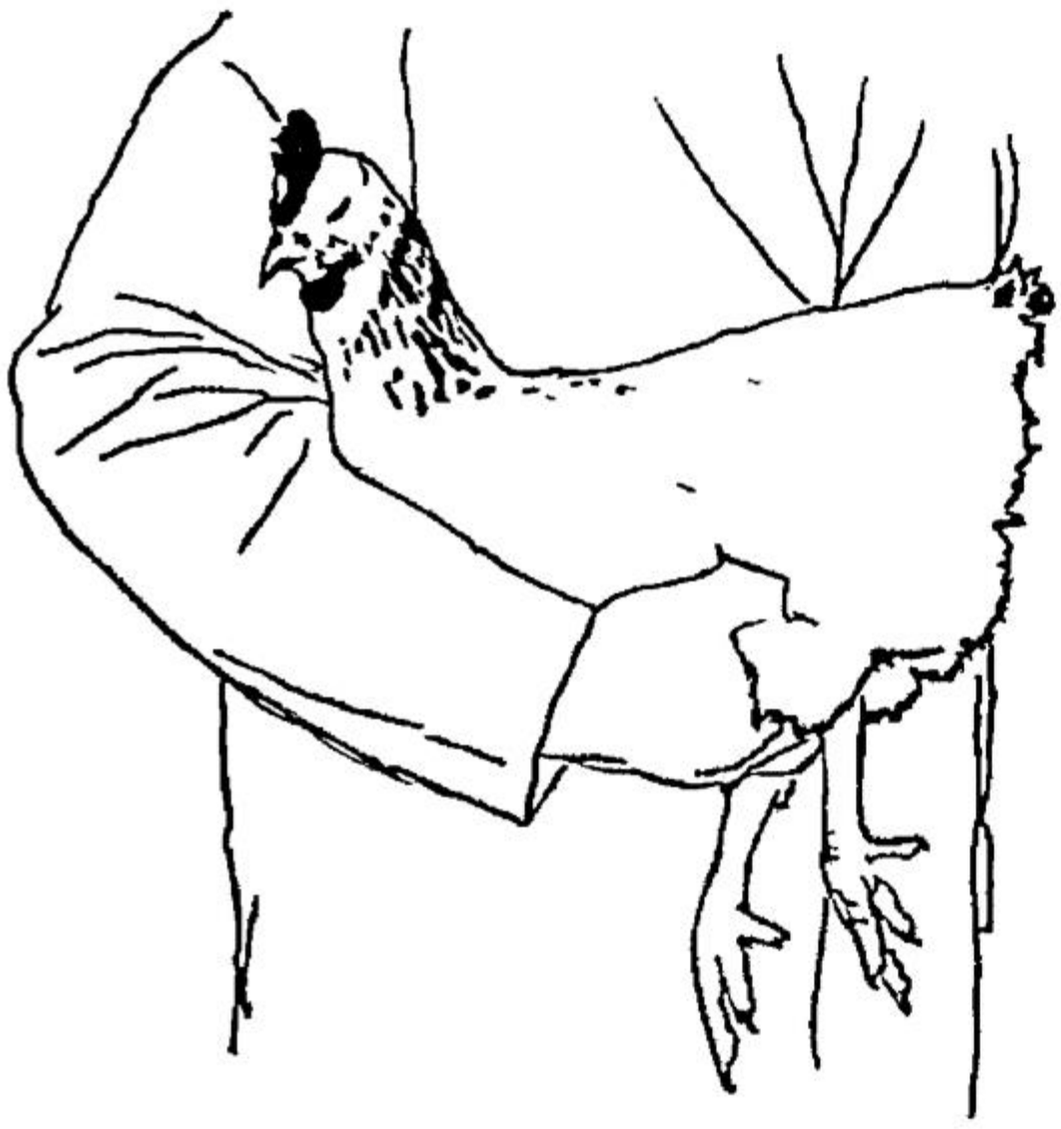

FIG. 17

FIG. 18

(*i*) Frogs and toads

With the fingers pointing towards the rear of animals, place a forefinger between the hind-legs and close the hand round the animal's body (Fig. 18 [*a*]).

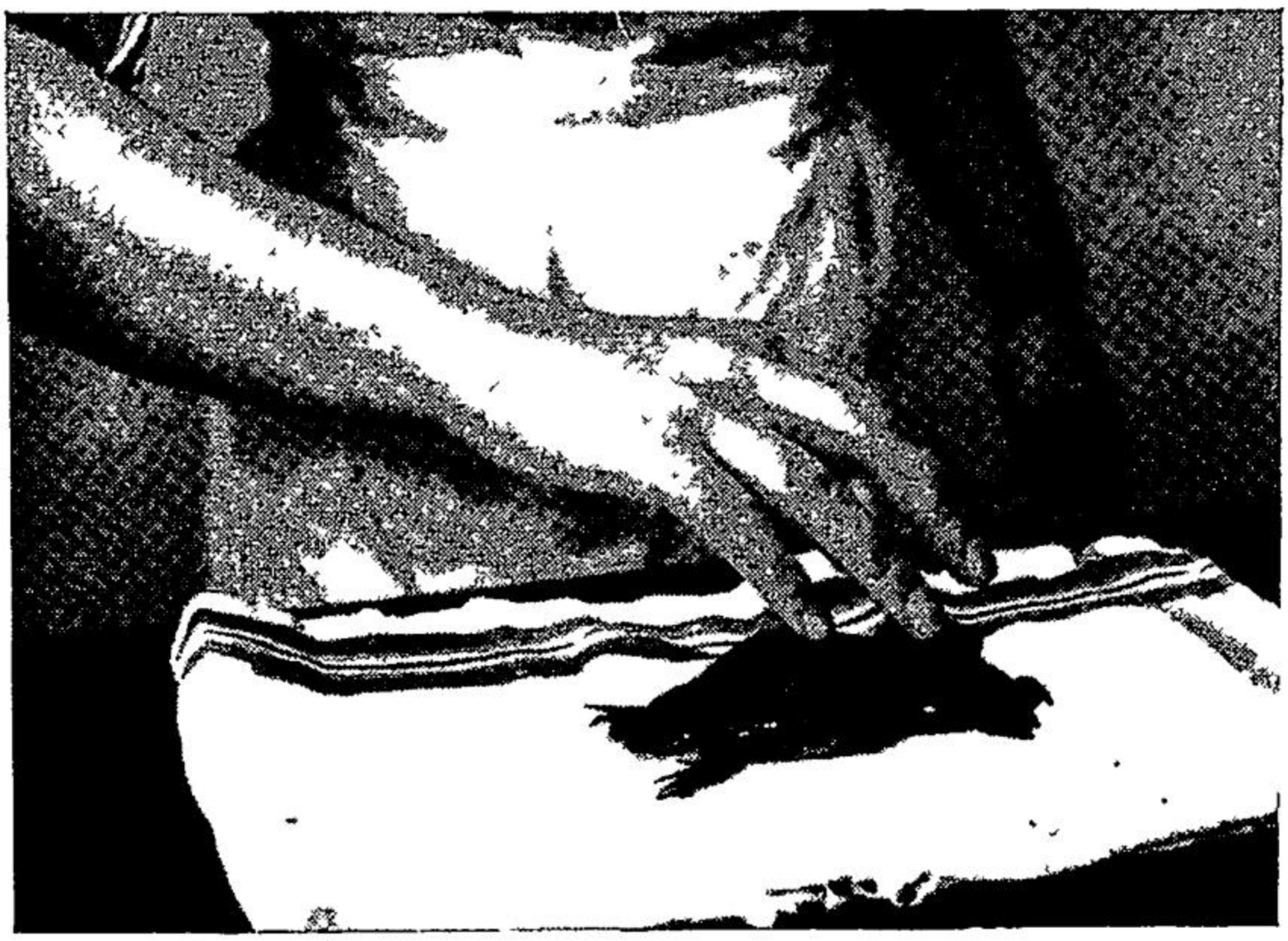

FIG. 18 [*a*]

(*j*) Dog

The approach to a strange dog must be quiet and confident. Nothing arouses a dog's suspicions more rapidly than diffidence on the part of the handler, and from suspicion it is but a short step to active resentment. Make the dog aware of your presence by sight as well as sound before attempting to handle it. Let the dog see your hand; place the back of the hand in front of and below the muzzle and gradually work along the side of the face until the animal has general confidence.

Do not make a sudden grasp for the collar or loose skin of the neck. Whichever is grasped, the movement should be slow and deliberate, and the holder's forearm should be kept in line with the dog's spine to avoid bites (Fig. 19).

If it is impossible to approach the dog use a dog stock, but with care.

To prevent the dog biting during manipulations it may be necessary to tie a tape round the muzzle. This is done by winding a 2-in. bandage round the jaw once or twice and tying it at the back of the neck. This method is useful for nervous dogs.

When applying a tape muzzle it may also be necessary to restrain the fore-legs, as the dog may try to pull the tape off with its feet.

(*k*) Monkey

Because of the possibility that newly imported monkeys may be infected with some disease which is transmissible to man, great care must be observed if the

FIG. 19

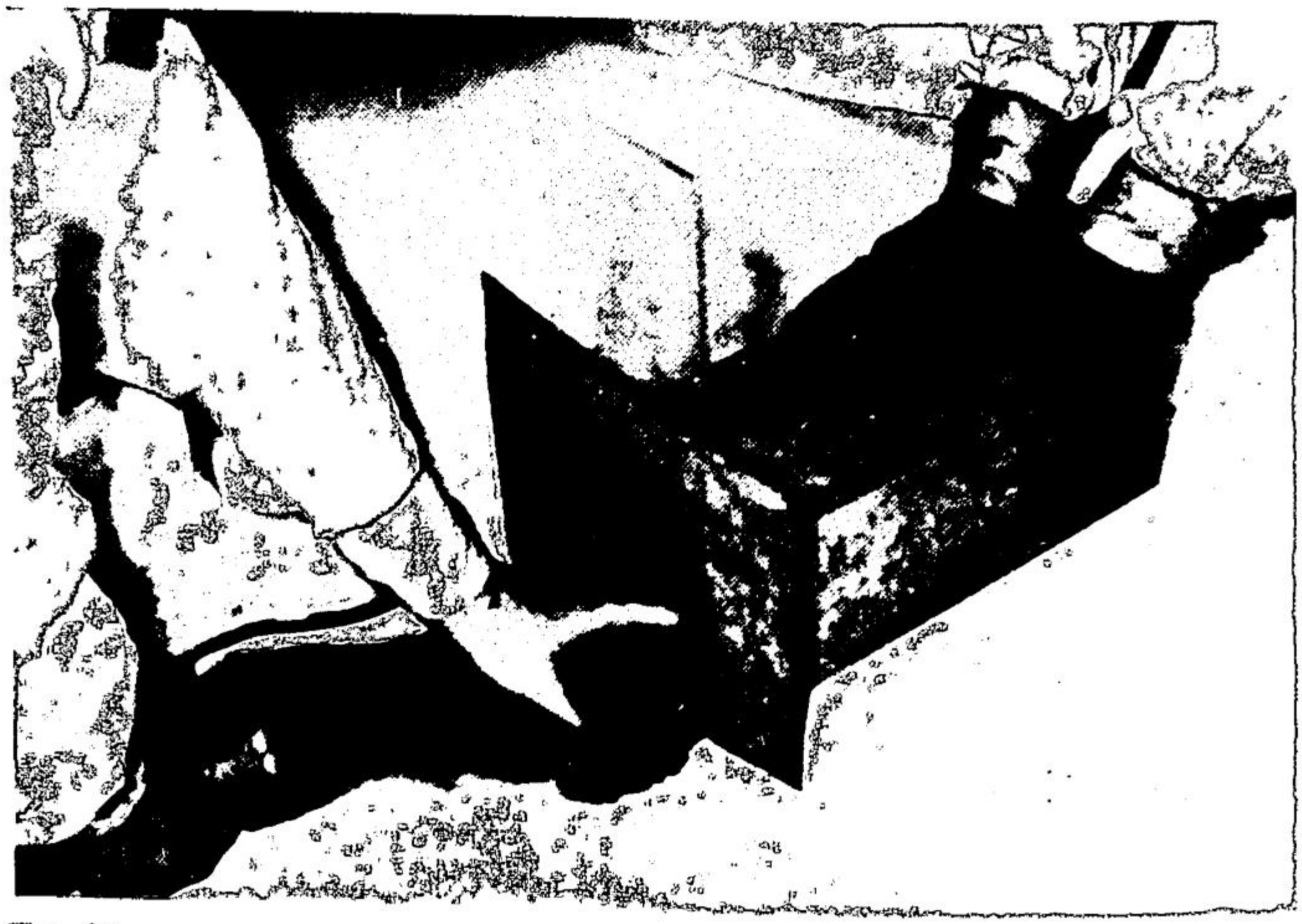

FIG. 20

animals have to be handled, especially during the first few weeks. Face masks and protective gloves must be worn. It may sometimes be necessary to anaesthetize newly imported monkeys before handling them, in which case sodium pentobarbitone or nembutal may be given by the intraperitoneal route.

Method I. Use a box, preferably metal, large enough for the monkey to get into, but not big enough for it to turn round in. The box should be fitted with a sliding door at each end, one door being of transparent Perspex. Induce the

FIG. 21

animal into the box. To remove the monkey, pull up the sliding door at the tail end of the monkey, grasp the animal around the loins, and pull it gently out of the box until its shoulders emerge; then grasp the two arms together behind the monkey, which is then under perfect control. This method is recommended for the smaller monkeys weighing 3–9 lb. (Fig. 20).

Method 2. This method requires a larger box of light wood, 16 in. × 13 in. × 20 in. high fitted with a sliding door at the top and bottom, and a net fitted to a stout iron frame 13 in. × 10 in. with a handle attached. The frame and net fit snugly into the box. Hold the box, with one end open, up to the cage and slide open the cage door. When the monkey has entered the box replace the sliding end and put the box, with the monkey in it, on the floor. The monkey may have to be encouraged to enter the box by pulling forward the movable back-panel of the cage. The net, on its frame, is then placed over the top sliding door and that door removed, so that the net may be pushed down into the box and over the top of the monkey. The rim of the net is then resting on the bottom sliding door, which is removed. The remaining four sides of the box are lifted away over the net handle, leaving the monkey trapped in the net. To take the monkey out of the net, face the monkey's head away from the handler, put a hand under the net, and run it up the back of the monkey until the arms can be grasped firmly high up and behind the animal (Fig. 21). The net may then be drawn from over the monkey.

Monkeys of 50–60 lb in weight can be handled easily by this method (Figs. 22 and 23). The handler has perfect control, without any emotional upset between the animal and the handler.

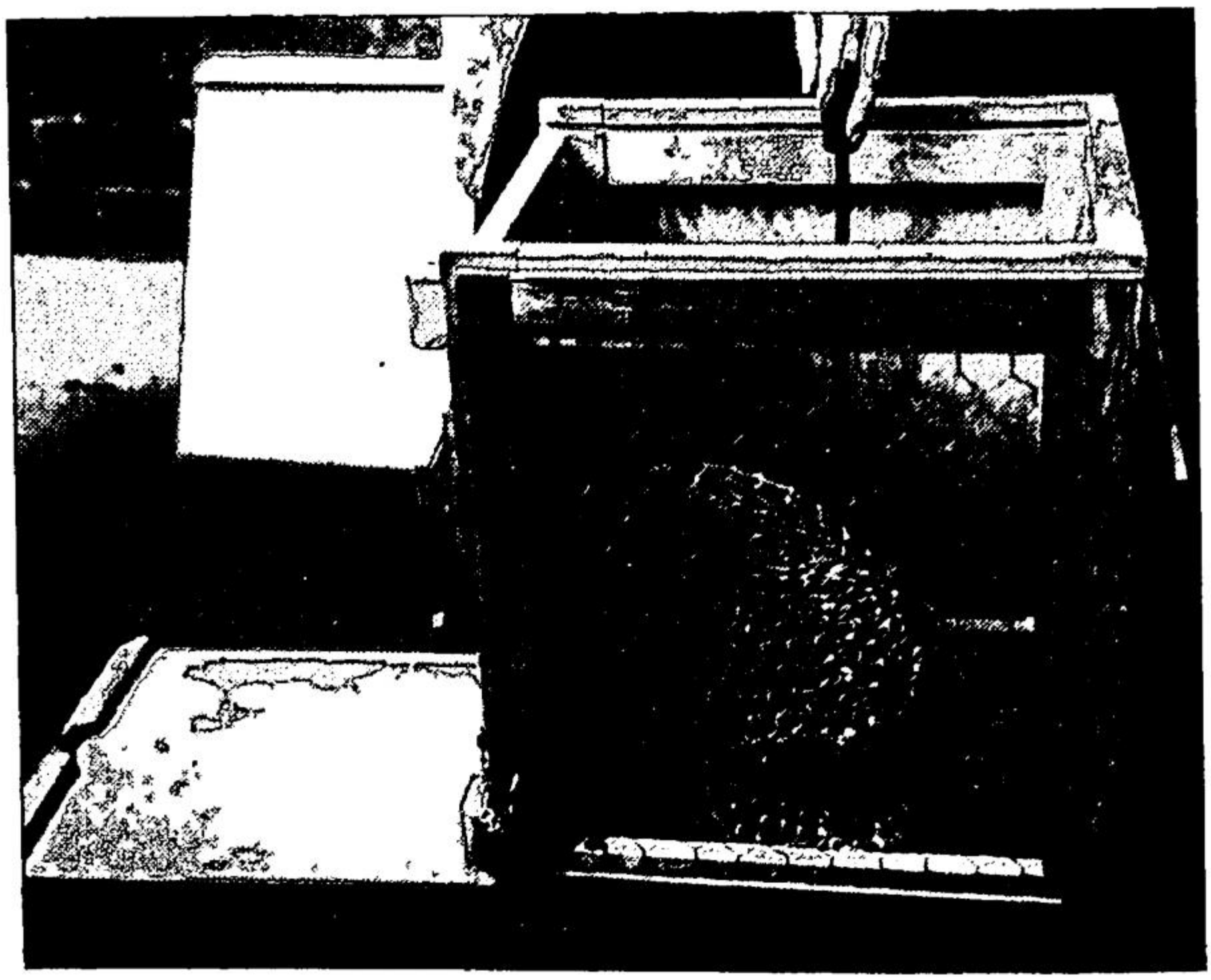

FIG. 22

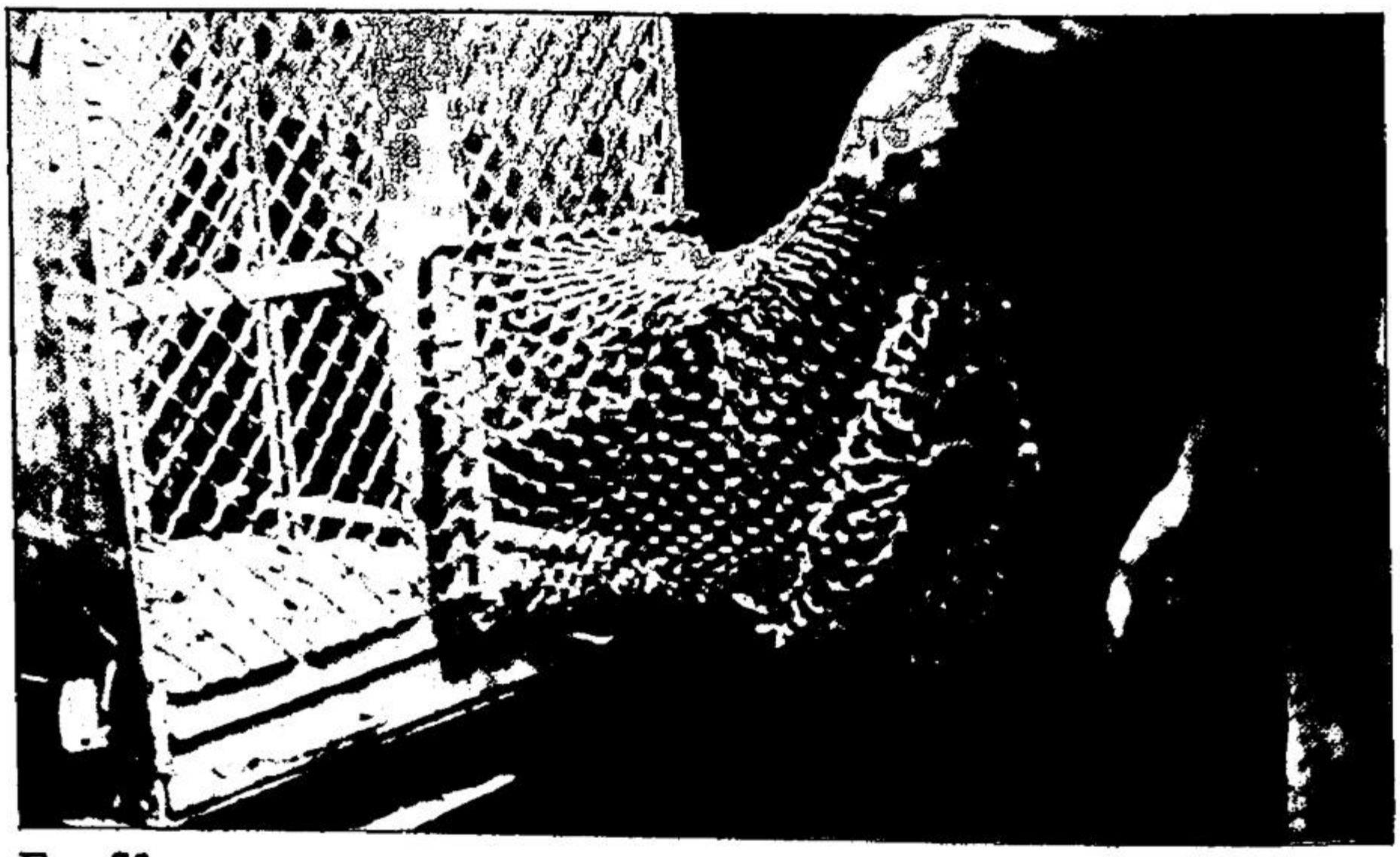

FIG. 23

It is difficult to recapture an escaped monkey by using a net. It is better to keep the animal moving until it tires, it will generally end by catching hold of a cage door or window guard. It is then an easy matter to grasp the animal by the neck and tail and replace it in its cage.

Make sure the monkey's head is facing away from its handler at all times.

(*l*) Cat

The cat can be the most difficult of the small laboratory animals to handle. The typical wild-cat temper can be provoked in many animals as a result of ordinary handling and restraint. The cat has four sets of claws and a set of teeth with which it is always ready to defend itself. For these reasons it is desirable at all times to have complete control over the cat, even when merely lifting it or placing it in a basket. Lift a cat by taking a firm grip of the scruff of its neck high up, being careful to keep the forearm in line with the cat's spine.

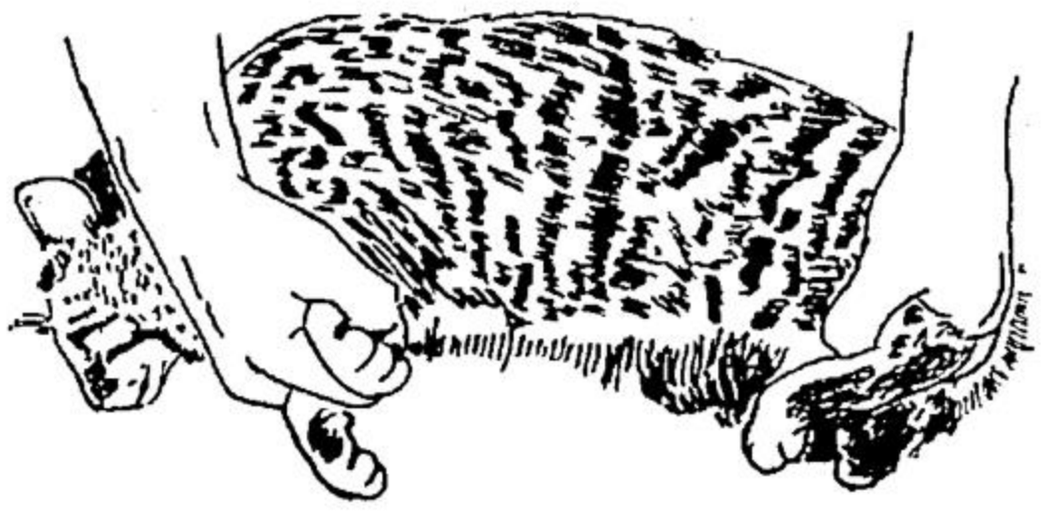

FIG. 24

To carry a cat for a short distance, tuck its head and the right hand holding the scruff under the left arm, and use the left forearm to hold the cat's body close to the handler's body.

To give the cat treatment it is often necessary to control all danger points, i.e. four sets of claws and the teeth. In this case place the cat on a table with its left flank towards the handler. The left hand is placed on the front of the fore-legs with the fingers gripping them just above the middle joint. The right hand does the same with the hind-legs. This immobilizes the four sets of claws. The left arm, which now is resting against the neck of the animal, is pressed firmly into the handler's leg or body. This prevents the animal moving its head and using its teeth. The animal may be held down on a table in a similar manner. When releasing the animal let go with both hands at the same time (Fig. 24).

(See *UFAW Handbook* for more detailed chapter on this subject.)

CHAPTER SIX

Methods of Identification

When it is necessary to identify individual animals some method of marking them has to be adopted. All methods should be simple, permanent, quickly applied, easily deciphered, and harmless to the animal. Some of the methods in general use are described below.

STAINING

The following stains, which are soluble in alcohol at a temperature of 78·8°F (26°C), are generally used:

COLOUR	STAIN
Yellow	Saturated picric acid or chrysoidin
Red	Fuchsin (acid, basic or carbol)
Violet	Methyl violet (gentian violet)
Green	Brilliant green, ethyl green, or malachite green
Blue	Trypan blue

Histological dyes should be used and made up as 3–5 per cent solutions in 70 per cent alcohol, except the yellow dyes, which are made up as saturated solutions. Stock solutions should be made up in bulk, as the efficiency of the stains increases with keeping.

Before applying the stains to the animal, consideration should be given to the side effects of staining on the coat and skin of the animal, and compatibility with the experiment for which the animal is being used.

A small area deeply stained is preferable to a large area lightly stained; reapplication at regular intervals is necessary. There is on the market a proprietary stain ('Darafur') specially prepared for animal marking. This preparation gives good durable results.

EAR PUNCHES

Surgical instrument manufacturers supply ear-marking pliers of various designs for use on large animals. For some small animals a chicken toe-punch may be used; these may be obtained from poultry-equipment suppliers. The ear-marking pliers and the chicken toe-punch are designed to cut holes right through the ears or toes, or in a series of notches round the edges of the ears.

Before marking the animals by this method, a well-defined code should be drawn up, and it is advisable to display a code chart wherever animals are being handled. By this method it is possible to mark individual animals in a

colony. The method cannot be applied to animals, such as the rabbit, having large marginal ear veins.

EAR-MARKING STUDS

These studs are designed on the 'batchelor button' principle, and are made of brass or aluminium. The studs are lettered, numbered, or codified to requirement, and are supplied with a combined punch and stud forceps which pierces a hole in the ear and closes the stud in position.

TATTOOING

Surgical-instrument manufacturers supply tattooing outfits in several sizes and with a variety of interchangeable numerals and letters—see table for size of numeral recommended for each species. The colouring medium should be black for non-pigmented animals and green or red for heavily pigmented animals. The skin should be cleaned with spirit before it is tattooed.

A surgical, triangular cutting needle and marking ink may also be used for tattooing and can sometimes be less laborious than using forceps, which involve the continual changing of numerals. An electro-vibro tattoo may also be used.

The inner surface of the ear is the usual site for tattooing.

RING AND LEG-BANDS

For the identification of birds and poultry two types of leg bands are used—plastic split-rings, of different size and colour, and light metal, adjustable leg bands numbered or codified to requirement.

Codified or numbered metal leg-rings of a different size for each strain of rabbit are available. (See table for size of band and age at which rabbits may be ringed.)

WING-BANDS AND WING-CLIPS

Wing-bands are adjustable, and they are numbered or coded to requirement. These bands are clipped through the wing above the radial feathers in a manner unlikely to impede the bird's movements.

The wing-clip in general use resembles a small gilt safety pin. These bands and clips may be obtained from any poultry equipment supplier.

COLLARS, CHAINS, AND NECK-BANDS

Collars or neck-chains with alloy discs (numbered or coded) may be used on dogs and cats. Puppy collars may be used for cats. Monkeys may strangle themselves with a neck-band, therefore any chain or collar used on a monkey should be fixed around the waist-line.

TAMPERPROOF WING TAG ('*Ketchum tags*')

This is a wing tag made of a light alloy, numbered or coded to requirement. It consists of a thin metal band doubled over and pointed at one end, and specially designed pliers are required for clamping the tags in position. This tag may be used for marking all species of birds; it is also a convenient method of ear-marking rabbits and guinea pigs, providing the tags are applied close to the head. These tags are also available in sizes suitable for farm animals.

ANIMALS

SPECIES	MARKING EQUIPMENT	METHODS OF APPLICATION
Day-old chicks, ducklings, and fowls	Wing-band	Clipped round the wing, above the radial, close to the body; must not impede movement
,,	Wing-clip	Clipped through the skin and neatly closed, so that no discomfort is caused to the bird
,,	Leg-band	Fit closely but comfortably round the leg
,,	Leg-ring	Fit closely but comfortably round the leg
Pigeons	Leg-band	Fit closely but comfortably round the leg
,,	Leg-ring	Fit closely but comfortably round the leg

ALL LEG-BANDS AND RINGS MUST BE INSPECTED REGULARLY FOR TIGHTNESS

Cats	Chain or collar and disc	Collars or chains should fit comfortably
Dogs	Tattoo	Tattoo the ears with forceps and numerals $\frac{3}{4}$ in. $\times$ $\frac{1}{4}$ in.
,,	Chain or collar and disc	To fit comfortably round the neck
Ferrets	Stain	On the back or head
,,	Tattoo	Tattoo the ears with forceps and numerals $\frac{3}{16}$ in. $\times$ $\frac{1}{4}$ in., or with triangular needle and marking ink
Frogs	Fine nylon braided suture thread. Small coloured glass beads. Sewing needle to take thread	The coloured beads are sewn to a fold of the skin covering the dorsal sac. A code of colour combinations can be used
Goats	Tattoo	Tattoo the ears with forceps and numerals $\frac{7}{16}$ in. $\times$ $\frac{1}{4}$ in.
,,	Ear punch	Use a well-defined code and cut a series of notches or holes in the ears
,,	Collar and disc	Fit comfortably round the neck
Guinea pigs	Stain	On the back or head
,,	Natural coat colour	Have, on the cage label, an outline drawing of the animal and mark on it the distribution of natural colouring
,,	'Ketchum Tag'	The sharp point is pushed through the ear close to the head, and is locked in position with special pliers
Hamsters	Tattoo	Tattoo the ears with forceps and numerals $\frac{3}{16}$ in. $\times$ $\frac{1}{4}$ in., or with a triangular needle and marking ink
Mice	Stain	On the back
,,	Tattoo	Use special mouse-ear tattooing forceps
,,	Chicken toe-punch	Use a well-defined code and cut a series of notches or holes in the ears (Figs. 1, 2, and 3)

ANIMALS (continued)

SPECIES	MARKING EQUIPMENT	METHOD OF APPLICATION
Monkeys	Tattoo	Tattoo the chest, upper lip, or forehead by using a triangular needle and marking ink, or an electro-vibro tattoo. *Monkeys should be anaesthetized for tattooing the lip*
,,	Chain and disc	Thin chains with discs securely fixed round the waist
Pigs	Tattoo	Tattoo the ears with forceps and numerals $\frac{7}{16}$ in. × $\frac{1}{4}$ in.
,,	Ear punch	Cut a series of notches or holes in the ears
,,	Ear studs	Pierce a hole in the ear into which the stud is clamped
Rats	Stain	On the back
,,	Tattoo	Tattoo the ears with forceps and numerals $\frac{3}{16}$ in. × $\frac{1}{4}$ in.
,,	Chicken toe-punch	Use a well-defined code and cut a series of notches or holes in the ears (Figs. 1, 2, and 3)
Rabbits	Tattoo	Tattoo the ears with forceps and numerals $\frac{3}{8}$ in. × $\frac{1}{4}$ in.
,,	Leg-ring	Choose the size of ring to suit the breed. Place the ring above the hock on a hind leg. INSPECT LEG RINGS AT FREQUENT INTERVALS
,,	Ear stud	Aluminium studs should be used for rabbits (see method described under pigs)
,,	Stain	On the back
,,	'Ketchum Tag'	As described for guinea pigs
Sheep	Tattoo	Tattoo the ears with forceps and numerals $\frac{7}{16}$ in. × $\frac{1}{4}$ in.
,,	Ear punch	Use a well-defined code and cut a series of notches or holes in the ears
,,	Ear studs	As described for pigs

Rabbit leg-rings

Rabbit rings are placed above the hock joint on a hind-leg. Small breeds may be ringed at six to seven weeks of age; larger breeds at nine to twelve weeks of age. Though it is easy to slip a ring on a young rabbit, the hock joint soon grows, so that the ring cannot be removed unless it is cut off. Provided the correct size of ring is used for each breed, the rabbits suffer no discomfort from the rings.

Recommended ring sizes are:

Dutch and Himalayan	Internal diameter		$\frac{18}{32}$ in.
Chinchilla	,,	,,	$\frac{20}{32}$ in.
Half Lop	,,	,,	$\frac{26}{32}$ in.
New Zealand White	,,	,,	$\frac{28}{32}$ in.

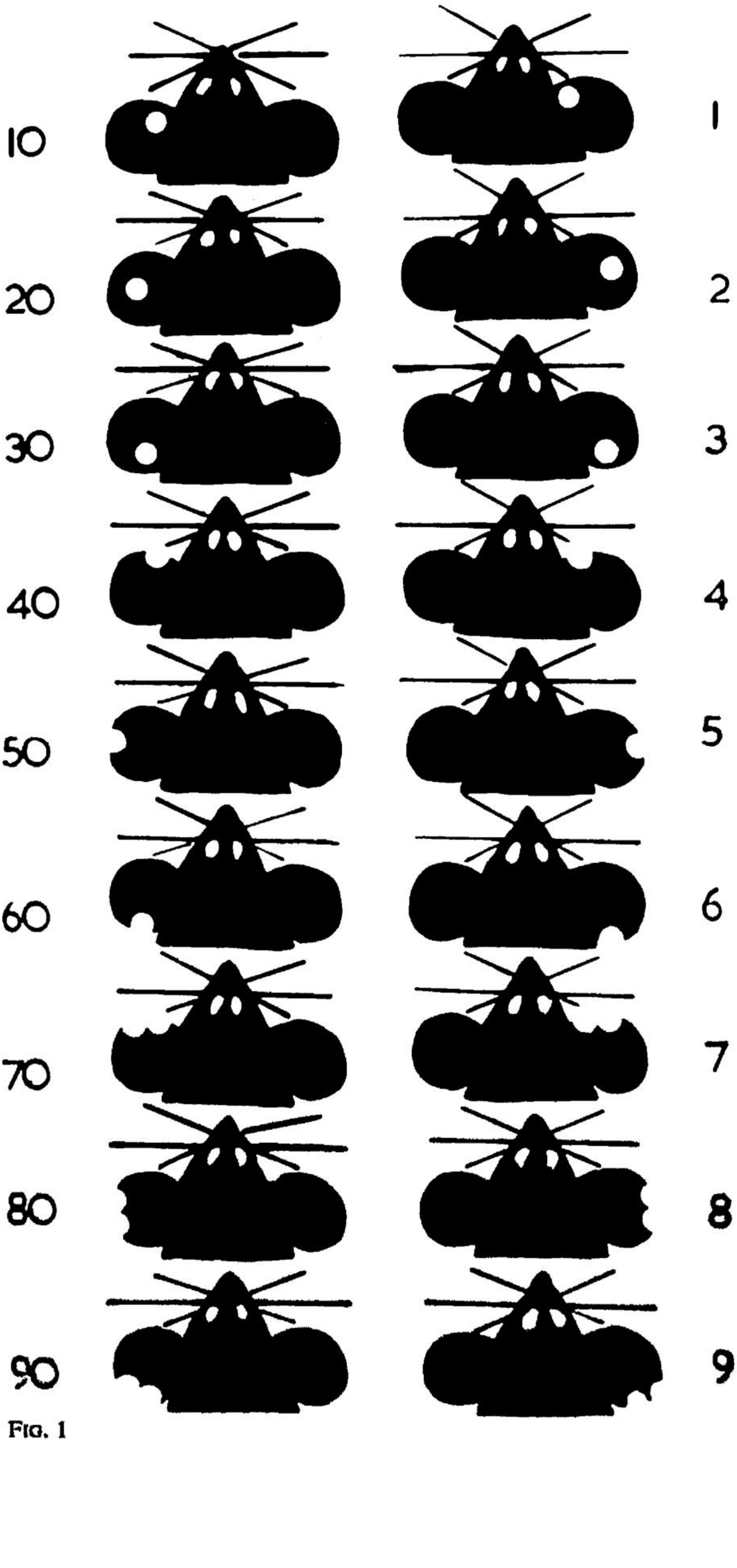

FIG. 1

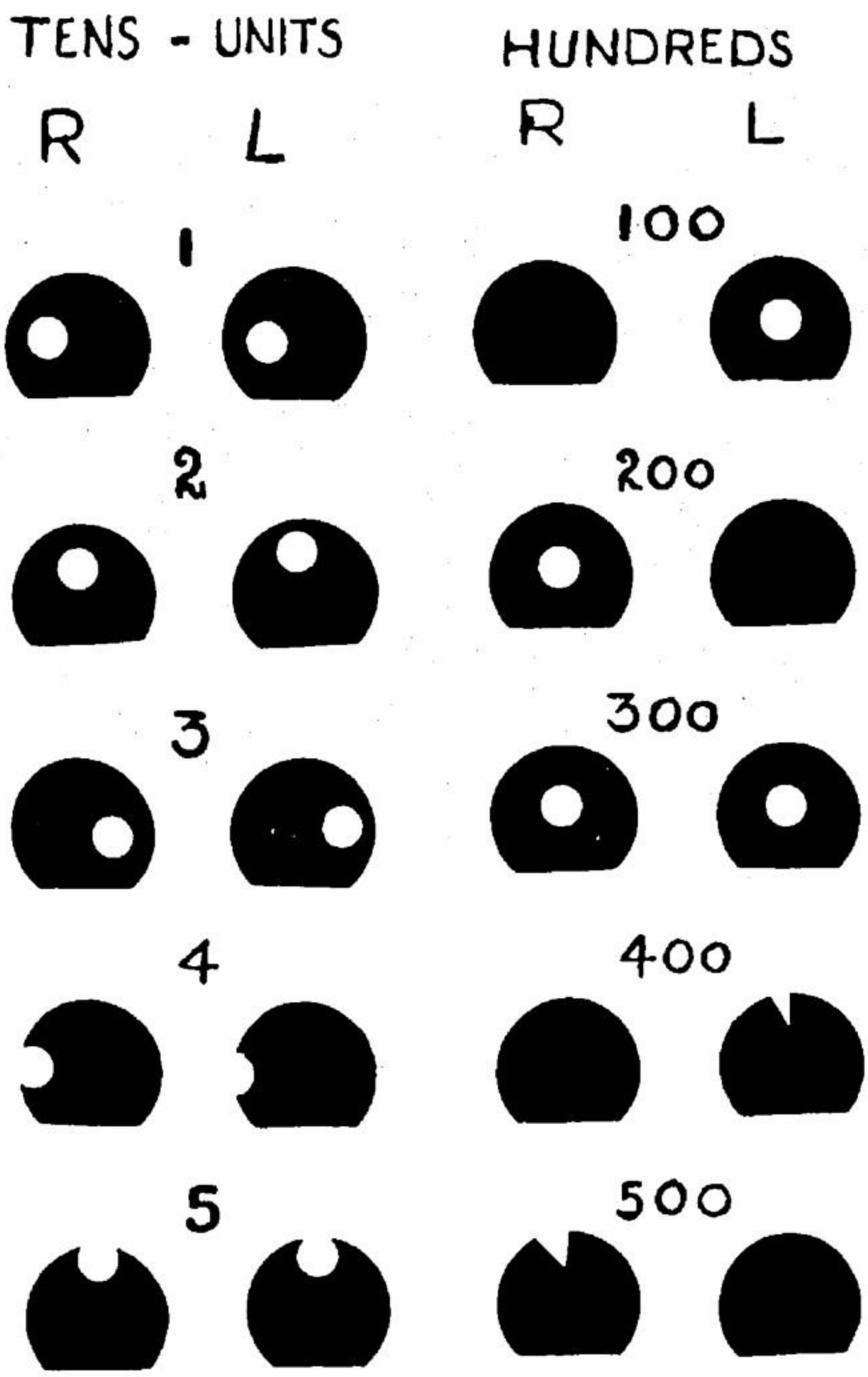
TENS - UNITS
R
L
HUNDREDS
R
L
1
100
2
200
3
300
4
400
5
500
6
600
7
700
8
800
9
900

FIG. 2


999

FIG. 3

CHAPTER SEVEN

Bedding

Bedding has to be considered in relation to the method of husbandry adopted in the laboratory and the type of equipment installed. In the field of animal husbandry, bedding must be regarded as one of importance. Little has been published on the subject, and its use in practice is largely a matter of individual preference.

The ideal bedding material should be:

(1) Harmless to animals—

(*a*) non-toxic and non-staining;
(*b*) non-edible;
(*c*) free of pathogenic organisms and parasites.

(2) Absorbent.
(3) Disposable.
(4) Readily available and easily stored.
(5) Relatively cheap.

It should be understood that most bedding materials are exposed to contamination by humans, animals, birds, and insects, all of whom may serve as carriers of disease-causing organisms. This places most users in a dilemma, as bedding, by its bulk, is a difficult material to sterilize. The safest and most efficient method is to autoclave all bedding in containers which are not too big (so that the bulk is not too large) before the bedding is allowed into the animal room. Autoclave temperature of 250°F (121·1°C) obtainable by leaving the material in for 1 hour at 15 lb per square inch will destroy all pathogenic organisms, but it is important to make sure that this temperature reaches every part of the bedding. This moist heat provides the best method of destroying bacteria and parasitic ova in bedding, whereas chemical disinfectants have not yet been proved either safe or practical for this purpose.

Small laboratory animals may be maintained on wire-mesh grids above trays or shelves on to which all faecal matter drops. This method is quite justifiable and generally accepted as the most hygienic way of maintaining stock animals, providing the mesh is of the correct size for the species concerned. Breeding animals must, however, be given some sort of material with which to make a nest.

Sawdust

Sawdust is most commonly used, but it must be from white soft wood and obtained from a reliable source where the sacks are filled directly from the sawbench through the sawdust extractor chute, because it is less likely to be contaminated by cats, dogs, or other wild rodents than when it is obtained from a mill dump. Wet, dirty sawdust should never be used, because it is a possible source of infection.

Sawdust from resinous hardwoods, such as teak and mahogany, may contain phenolic substances that would be harmful to the animals. It is equally important to ensure that the sacks are not contaminated, and where possible they should be sterilized before being sent for refilling. Multiple paper sacks are recommended.

Trays below wire grids should be covered with a good layer of sawdust; for small rodents $\frac{1}{2}$-in. thick, for larger animals $\frac{3}{4}$-in. thick. Shelves or trays may be covered with removable paper or automatically washed.

For animals kept on solid floors, cover the floor of the cage up to $1\frac{3}{4}$-in. thick. For cats place up to 2 quarts in one corner or in the dirt tray. The cost is low.

Softwood shavings

Softwood shavings can be used with great success as a bedding for mice, rats, hamsters, cotton rats, mastomys, and other small animals. Good nests for breeding animals can be made with this material; the absorbency is fair to good, and the shavings are easily disposable. As softwood shavings can be used as both a bedding and nesting material, the cost is low. The shavings should be baled or bagged at the mill and delivered direct to the animal house. A good deodorizer is cedar-wood shavings.

Peat-moss litter

The smell in an animal house can be considerably reduced by the use of peat-moss litter, because it has a high acid content which delays the decomposition of faecal matter and the release of ammonia.

Peat moss is obtainable in two forms—(1) compressed in bales, (2) granulated in sacks—the latter is easier to handle, but the former impervious to infestation. Use as sawdust. The cost is high.

Sterolit

A new type of animal bedding processed from a naturally absorbent mineral found in the south-east of the United States. It deodorizes, self-sanitizes, and is exceptionally absorbent. Trays below wire grids should be covered with a thin layer of Sterolit; for small rodents $\frac{1}{4}$-in. thick and for larger animals $\frac{1}{2}$-in. thick. For animals kept on solid floors, cover the floor of the cage up to 1-in. thick. For cats place up to 2 quarts of Sterolit in a corner of the cage or in the dirt tray.

Sterolit reduces labour; trays require cleaning less frequently. Remove the trays at regular intervals and shake gently in order to mix the dry material with the soiled. Clean out the trays when the Sterolit has lost its absorbency or when the odour level has increased.

Sterolit will not burn, therefore *it cannot be incinerated*; *nor can it be spread on farm land.* The cost is high.

Straw (chopped)

Straw is seldom used as a bedding material for small animals, but for guinea pigs in floor pens, chopped straw with an underlay of sawdust makes a fine bedding. Wheat or oat straw should be used.

Cut chaff and cavings (oat husks)

These materials are sometimes used on top of sawdust or peat moss litter for rabbits and guinea pigs on solid floors. There is the ever-present danger of introducing infection into the colony unless this form of bedding is sterilized, and sterilization presents many difficulties. It is therefore advisable not to use these materials unless absolutely necessary. The cost is low.

Meadow hay

Generally used as a fodder, it does, however, make a good bed for breeding rabbits, especially during cold weather, as soft hay makes a close and warm nest for the young.

Wood wool

Fine woodwool, Grade No. 1, as used for fruit packing is good nesting material for rodents. Some workers have, however, found that woodwool gives off a fine dust which is irritating to rats. Woodwool is normally delivered in bales weighing approximately one hundredweight. They are packed by a high degree of pressure, which makes the bale impervious to attacks by vermin. The baling process is carried out as the wood is cut, therefore the possibility of contamination is reduced to a minimum, and may be completely eliminated if sterilization facilities are available. The cost is medium.

Shredded paper

Clean shredded paper is highly recommended for nesting material. Shredded paper is obtainable from printers at a very reasonable rate. A special clean shredded paper as used for food packing is also obtainable, although slightly more expensive. The former should be sterilized if possible, the latter can be used without sterilizing.

BEDDING FOR ANIMALS IN TRANSIT

To ensure their arrival in good condition animals in transit must be treated with special care. In all travelling boxes place a layer of at least 2 in. of soft-wood sawdust or peat moss litter, wood shavings with a liberal covering of woodwool, shredded paper or soft hay, depending on the species of animal. Travelling boxes should contain sufficient bedding to counteract shock caused by handling and to keep the animals dispersed throughout the box, also to provide privacy from interested spectators. Sufficient bedding must be included to absorb moisture and to ensure that the animals arrive in a dry, clean condition.

BEDDING FOR EXPERIMENTAL ANIMALS

Paper, cotton wool, and certain other materials can be used for experimental animals.

Post-operative animals may be given a liberal supply of sterile cotton wool or sterile shredded paper. The sterile materials minimize the danger of introducing infection.

Animals on nutritional experiments may be deprived of any form of bedding, but they should be placed in a sterile cage with a wire grid with a mesh of the correct size for the species concerned.

In parts of the world where the above-mentioned materials are not readily available, dry inland sand may be used.

Storing

All bedding materials must be stored in a vermin-proof building, either in racks or in large bins. Surplus bedding once taken out of the store should never be returned; undetected contamination may have occurred. Brooms and other pieces of equipment necessary for cleaning should be retained for use in the bedding store only.

Sterilization

As an added precaution, and where facilities are available, all material should be sterilized directly it is received, or at least before it is placed in the store.

Disposal

Sterilization of all infected bedding along with the cage must be regarded as a necessary routine. After sterilization the soiled bedding should be removed and conveyed to the incinerator by vacuum or a closed bin. Uninfected bedding may not generally be regarded as a source of danger, it should nevertheless be handled carefully and burned in the manner described above. All bins and utensils used in the process of cage cleaning should be sterilized after use.

Summary

1. Procure bedding from a reliable source.
2. Sterilize all bedding before use.
3. For sawdust or shavings use only softwoods.
4. Peat moss litter or Sterolit may be used in place of sawdust.
5. Dry inland sand may be used in certain circumstances.
6. Fine woodwool and shredded paper is recommended as a nesting material for rodents.
7. Sterile cotton wool or shredded paper is recommended for bedding for post-operative animals.
8. Store bedding in rodent-proof building.
9. Adequate bedding in travelling boxes.
10. Sterilize all infected bedding before handling and burning.

CHAPTER EIGHT

Routine Care of Laboratory Animals

A clean and tidy animal house is the hall-mark of a good animal technician. Much time and energy is spent on everyday chores in an animal house, but the effort is well worth while when it results in a healthy stock of animals maintained in surroundings in which it is a pleasure to work.

No hard-and-fast rules can be given for the conduct of any animal house; each must work out its own routine according to the demands made upon it. But whatever scheme is devised there are some aspects of the routine care of stock animals which are inescapable, and it is these points which are discussed here.

Environmental conditions

Room temperature and relative humidity are checked and recorded first thing each morning. The setting of time switches for lights should be checked. Female rats kept in windowless rooms and exposed to more than 15 hours artificial light in every 24 hours come into continuous oestrus and will mate, but fail to conceive.

Litter and bedding

Sawdust, wood shavings, and peat moss are the commonest litter materials. They are put in trays or pens to absorb the excreta in which the animals would otherwise run. Generally speaking, litter is renewed twice, if not three times, each week—usually on Mondays, Wednesdays, and Fridays. Animals which produce a large volume of urine (monkeys, dogs, cats) require fresh litter daily; rabbits infected with coccidia must be given fresh litter daily if the level of infection is to be held to a minimum. When peat moss is used the litter may need renewing only once or twice weekly, depending on the number of animals in the boxes or cages. Though peat moss is more absorbent than sawdust, it should be remembered that animals running on it do not excrete less urine or faeces or fewer infective organisms than they do when running on sawdust. Also, peat moss litter is as good a breeding place for flies and fleas as is sawdust.

Bedding (e.g. woodwool) is supplied to animals for nesting. It is renewed when it is dirty and/or damp. The frequency with which fresh bedding must be given is determined by the number and age of the animals. However, bedding should be renewed once each week whether or not it appears soiled.

The relative merits of different litters or bedding are discussed in the chapter on 'Bedding'.

Customarily, soiled litter is scraped from the trays into a waste bin. If a metal scraper is used the galvanizing of the trays is damaged (thus accelerating the rusting and ultimate decay of the trays), and the process is a noisy one. Wooden or plastic scrapers do not damage galvanizing and are quieter in use, but they are more troublesome to sterilize. The same comments apply to galvanized and plastic waste bins.

Disposable paper bags can be used instead of waste bins, and are especially useful for infected or contaminated waste. Triple paper bags, having considerable wet-strength, may be hung from portable frames which are designed to hold open the necks of the bags.

Litter can be removed from trays and boxes by a specially designed vacuum-cleaner-like apparatus (Charles *et al.*, 1962). Waste is drawn off through pipes into a sealed container (which may be situated outside the animal building), from which it is fed, automatically, into an incinerator. The nozzle of the vacuum pipe is fitted with baffles which admit the passage of litter and excreta, but not animals. This is an important feature when mouse boxes are cleaned by vacuum. The advantages of this system are obvious—waste bins, scrapers, brushes, brooms, floor sweeping, and, above all, dust are eliminated. Vacuum cleaning is widely used in the United States, and has been installed in at least one large Institute in this country, where the cost of the complete apparatus, fitted with six vacuum pipes which may be used simultaneously, was about £2,000.

Cages

Animals should be given clean cages as often as is practicable; the frequency is usually determined by the number of spare, clean cages available and the speed with which dirty cages may be sterilized. The aim should be to give clean cages at least once a month. It is desirable to give a clean floor grid once a week. Ferrets kept on mesh are best given clean floor grids each day because of the sticky nature of their faecal matter. Rabbit cage floor grids can become clogged with trampled faeces, and if a rabbit is left on such a grid the animal will certainly develop sore hocks.

When cages are changed the tray, food container, and water bottle should also be changed. The rack on which the cage stands and the wall behind the rack should be washed thoroughly with hot, soapy water. Any 'disinfectant' used in this washing process must be chosen with care. All disinfectants are toxic to a greater or lesser degree (see chapter on 'Sterilization and Disinfection'). Lysol should *never* be used in the presence of living animals, to whom even a small dose of this substance can be fatal.

Cages may be moved, so it is useless to rely on cage position as a guide to which batch of cages is due to be changed. When clean cages are put up they should be marked to indicate the date. The mark should be a semi-permanent one, and should be visible when the cage is in position. Suitable marking can be achieved by the application of strips of coloured adhesive tape; different colours and/or positions indicating the month and week of the month.

For long-term experiments it is advantageous to use removable cage labels and to transfer them, with the animals, to clean cages, thus obviating the need

for new adhesive labels. Removable labels must be fitted securely to the cages; the term 'removable' meaning 'removable by humans' and not 'removable by animals'.

Each new experiment (and each animal counts as one experiment) must be set up in a clean cage. No cage may be used twice for two different experimental animals. When an experiment ends the cage must be sterilized, even if it has been in use for only ten minutes.

It is customary to combine cage changing with routine cage cleaning. Thus, Mondays, Wednesdays, and Fridays become the days when the heaviest work is done.

Water bottles and food containers

All water bottles should be emptied and the bottles and fittings washed and sterilized once each week. Three or four days later in the week every bottle should be emptied, rinsed, and refilled. On the remaining five days the bottles may be topped up with water. Water bottles must be filled from a mains water supply, as infections may be introduced through contaminated water from storage tanks.

Water bottles for monkey cages must be fitted with metal drinking spouts which cannot be bitten through. The bottle must be fixed securely to a solid metal part of the cage so that it is: (i) out of the monkey's reach, and (ii) cannot be dislodged by the monkey pushing against the spout which protrudes into the cage.

It is important that a bottle which has been only rinsed or topped up is returned to the cage from which it came. Care in this matter eliminates one of the routes for cross-infection in an animal house.

Water bottles should be wide-necked and without sharp shoulders and corners which are difficult to clean. Round, wide-necked bottles may be quickly and efficiently cleaned by using a bottle-washing machine. The drinking spouts can be tiresome to clean if they are allowed to get very dirty and greasy. Metal spouts can be cleaned with ordinary domestic scouring powder applied with a pipe-cleaner or a miniature test-tube brush, but all such powders are abrasive and will scratch chromium plating. Glass spouts are easily cleaned by treating them in the same way as laboratory glassware, i.e. by immersing them for a few hours, or overnight, in 'chromic acid'.

Food baskets and hoppers containing pelleted diets should not be topped up daily unless that is necessary to supply sufficient diet to satisfy the animals' needs. Rather, these food containers should be allowed to run low before refilling them with fresh diet. Baskets and hoppers do not have to be filled to the brim, and are best filled only to a level which will supply sufficient food for three or four days. Pelleted diets which are left uneaten in baskets and hoppers constitute an excellent medium for mould growth, which can proceed rapidly in the warm, damp atmosphere of an animal house. Not all food baskets are designed so that the animals can eat only from the lower layers of pellets, and mice and rats will, if given the opportunity, climb the sides of food baskets and contaminate the diet with excreta.

Some animals cannot eat from food baskets or are fed diet which cannot be pelleted. Moistened diets must be freshly prepared each day, including the week-ends. They should not be prepared in bulk and stored, even in a cold

room. Uneaten food must be discarded and not offered again the next day, when it will be sour and, consequently, refused by the animals.

Farm animals, dogs, cats, and ferrets cannot drink from bottles, and must be given open dishes of water. Guinea pigs can and will drink from water bottles, though it is customary to offer them water in open dishes or in constant-level water troughs. When food and water is supplied in open dishes these dishes must be washed daily, for they always become fouled with excreta and litter unless they can be placed where the animals can reach them but not walk over them.

Dishes, baskets, and hoppers always become greasy in the places where the animals rub against them, so they have to be scrubbed with hot, soapy water to clean them.

Open-air exercise runs for larger animals

Animals in exercise runs should at all times have access to fresh drinking-water and to shelter from strong sunlight and rain. Solid rubbish (e.g. fallen leaves, faeces, sawdust) which may choke drains must be removed before the runs are hosed down daily. The wire netting and fencing runs should be inspected regularly for rusted or weak places which animals could break through.

The care of rooms, equipment, and fittings

WASTE BINS must be kept covered when not in use, and all waste should be incinerated as soon as this can conveniently be done. Bins should be sterilized and washed out once each week. Waste bins are easier and quieter to move if they are fitted with castors, but if the castors are to have a long, useful life they must be carefully cleaned and oiled when the bins are cleaned.

TROLLEYS should be dismantled and cleaned at weekly intervals, special attention being given to the cleaning and oiling of the wheels.

ANIMAL BALANCES and the weights should be washed thoroughly each week, care being taken to prevent water penetrating to the mechanism. The balance pan should be cleaned immediately after each use. When a balance is moved it must be set level in the new position. Most balances are fitted with spirit levels so that this may be easily accomplished.

DUST must not be allowed to accumulate in an animal house, where it would form an excellent breeding place for pests. Dust is best washed away, or at least removed with a damp cloth. Dry dusting tends only to move dust from place to place. Besides 'dusting' obvious places, such as tables, chairs, and window ledges, dust and dirt should also be removed from service pipes, light fittings, doors (including the handles), underneath sinks and fixed benches, racking, and solid-topped cages.

The washing of walls and ceilings is a tedious task, but it should be done as often as possible, and certainly not less often than twice per year. The floors of animal rooms and corridors have to be washed on at least six days of each week. On one day of the week hot water and a disinfectant should be used, and on this occasion all drain covers should be removed and the drains and gulleys cleaned. Lysol may be used only in the corridors. On other days floors may be

washed with hot water only. Floors may be dried off by the use of a squeegee. If water is left lying on the floors the humidity may increase to an uncomfortably high level.

FOOD BINS should be kept in a cool place and not in an animal room. The lids should be kept closed when the bins are not in use. Empty bins should be washed out and sterilized before they are refilled. On no account should fresh diet be put into a bin containing old diet crumbs and dust.

BROOMS, brushes and dustpans, pails, mops and swabs used in the business of cleaning, themselves become dirty, and should be washed at least once each week.

PROTECTIVE CLOTHING AND FOOTWEAR must be worn at all times in an animal house. When not in use these garments are kept at the entrance to the animal house. High-necked gowns which fasten at the back afford more protection to the wearer than coats. Short gum boots are more comfortable to wear for long periods than are full-length Wellington boots. It is possible that within a few years all colonies will be of Specific Pathogen Free animals, in which case it will be necessary for all animal technicians to strip, bathe, and dress in clean clothing before entering the animal house.

FOOTBATHS at the entrances to animal houses must be large enough and so placed that they cannot be stepped across or walked round. A large sponge rubber mat soaked with disinfectant solution may be substituted for a footbath. A 3 per cent Lysol solution is used as the disinfectant for both mats and baths. Fresh disinfectant solution must be used daily. Footbaths must be lined with non-slip mats. These devices are used to prevent the carriage of infective organisms on footwear, but they have the additional advantage of detering unnecessary traffic.

INSPECTION OF ANIMALS. Technicians must observe animals in addition to cleaning, feeding, watering, and breeding them. Animals housed in captivity require regular inspection if they are to be kept in perfect condition and free from minor ailments. Animals can only be helped by technicians who care *about*, as well as care *for* them.

THE RABBIT

Examinations should proceed in logical order so that nothing is missed. Start at the head and work down to the tail. The rabbit should be placed on a flat, non-slippery surface, as this animal becomes panic-stricken if it loses its foothold. The eyes should be bright and alert; if the rabbit looks as if it had been crying the fact should be reported. In most cases bathing with 2 per cent boric acid solution or the application of penicillin eye ointment will effect a cure.

Any discharge from the nose should be reported at once. The animal should be isolated immediately if the ailment is 'snuffles', as this disease is infectious.

The teeth should be inspected carefully. The front teeth of a rabbit are of an interesting construction. The two front upper teeth (incisors) are grooved, giving the impression that there are four, rather than two teeth. Immediately

behind the main teeth are two small incisors, only about $\frac{2}{5}$ in. of which are visible. When, through accidental displacement, the lower incisors do not meet they grind against the small upper incisors: the lower incisors continue to grow like tusks (preventing the animal from eating properly), and may even grow up through the mouth (see Fig. 1). When this occurs it is necessary to cut the tooth off to its correct length with bone forceps, but continued attention is necessary, as the tooth will continue to grow wild.

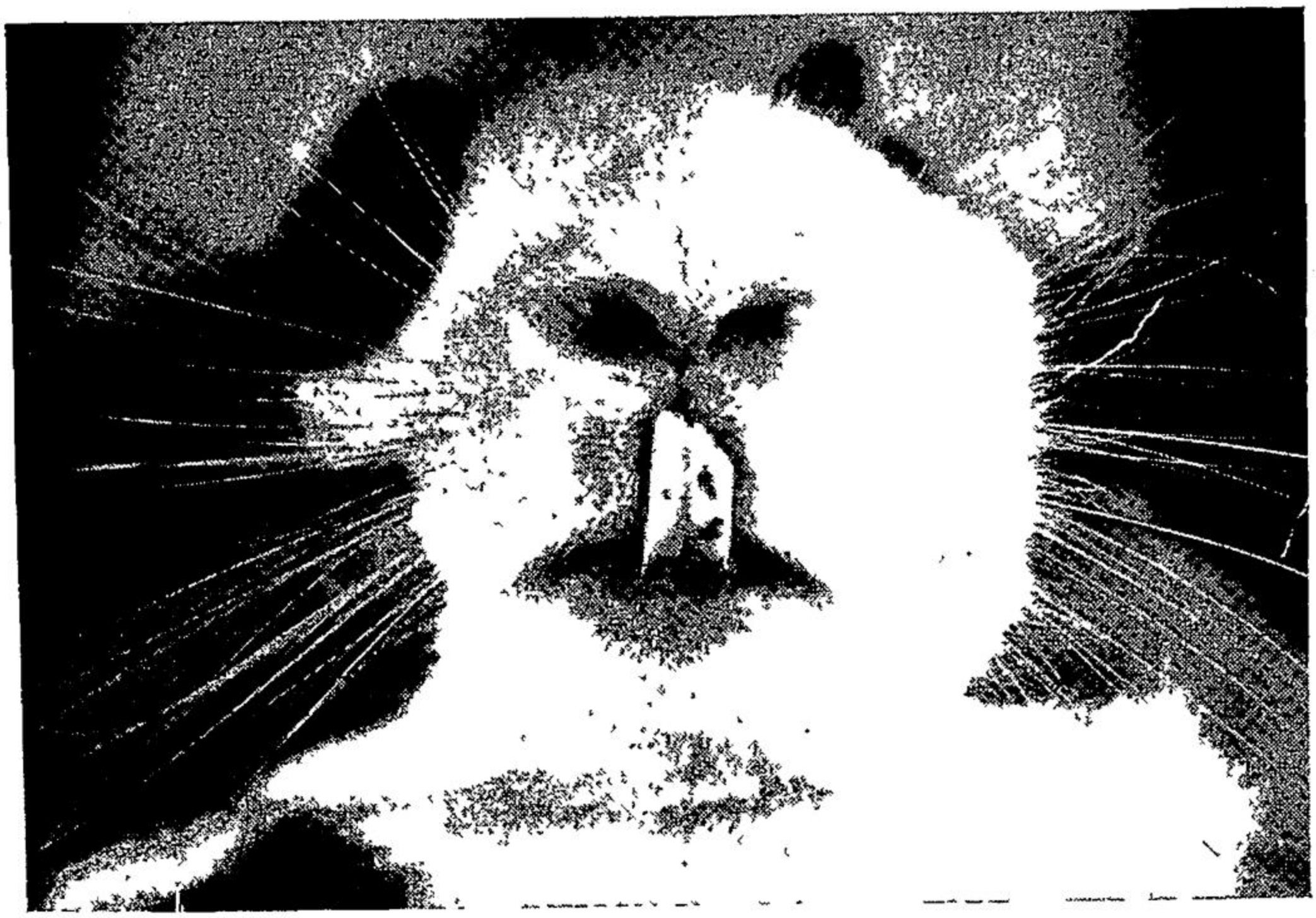

FIG. 1

The ears should be examined inside as well as outside, for it is inside the ear that ear canker (caused by a mite) first appears. It is important that rabbits' ears are inspected regularly, as ear canker, although easy to cure, can be both troublesome and painful if not treated in the early stages. Any vegetable oil will help to soften the scabs, but the application of 2 per cent of phenol in liquid paraffin or of benzyl benzoate is recommended. Great care should be taken if the canker is bad, as treatment can be very painful if carried out clumsily. The following technique is recommended:

If possible, get a second person to hold the rabbit. If the task has to be done single-handed place a duster flat on a table, place the rabbit on the duster, tie the two front corners of the duster round the rabbit's neck (taking care its front feet are inside) and tie the other two corners on the top of the rabbit's back (seeing that both hind feet are inside the duster). Have ready some cotton wool, a pair of blunt-ended forceps, and a supply of medicant.

Pour a little of the oily substance into each ear and leave for at least 5 minutes so that the scabs will loosen. Then, with forceps and using great care, lift off the scabs and place them in a burnable bag. This operation must be done very gently, for much pain can be caused by rough treatment. When all

the scabs have been removed wipe the ears out with a pad of cotton wool soaked in either benzyl benzoate or phenol in liquid paraffin. This treatment should be repeated after ten days.

The nails of the front feet should be inspected, and if necessary cut with a small pair of bone forceps. It is on the inside of the front legs that evidence of snuffles can be found, because when the rabbit sneezes it wipes its nose on its front legs.

Examine the length of the nails of the hind feet. Rabbits living in cages, and having no opportunity for digging, often grow long nails. Place the rabbit on its back and examine the pads of its feet. If there is any soreness (sore hocks) remove the floor grid from the cage and place the rabbit on a bedding of sterilized wood wool or hay and paint the sore hocks with iodine solution of the following formula:

Iodine (resublimed)	10 gm
Potassium iodide	6 gm
Distilled water	10 ml
Ethyl alcohol (industrial)	100 ml

When this condition is found in rabbits the cage grids must be sterilized and scrubbed at least once a week. If the skin on the foot is broken the fact must be reported at once. If the lesion becomes infected the animal will lose condition rapidly.

A healthy rabbit's coat shines and looks attractive; a sick rabbit has a dull and unattractive appearance. Run a hand down from head to the tail of the rabbit, feeling the skin for lumps or scratches. The backbone of a healthy rabbit is smooth and firm to the touch. The bones of the spine are palpable only when the muscles of the back have wasted.

The importance of good hygiene in the rabbitry cannot be overstressed, and the cleaning routine should on no account be allowed to lapse. This is illustrated by the fact that the life cycle of the protozoa, coccidia, may be broken by removing all faeces from the cage each day.

Coprophagy (eating of faeces) in rabbits

Coprophagy in rabbits has been attributed to a lack of B-vitamins in the diet. Blount (1957) studied this nocturnal habit and concluded that the swallowing of specially formed faecal pellets is a normal feature in rabbits. It does not represent a depraved appetite, but is, in fact, a specialized process. Several theories, none of which is conclusive, have been advanced to explain this phenomenon.

It should be understood that the stomach of a rabbit does not empty after every meal. Observation has shown that the stomach of the rabbit, like that of the horse, has no great powers of contraction except at its exit—the pylorus. No matter when a healthy rabbit is killed, its stomach will always be found to be more than half full of food. By nature the rabbit eats little and often, but never takes a large feed. If a rabbit becomes hungry but cannot, or does not, eat, bulk must be provided to push onward the stomach contents, and coprophagy is resorted to.

The points of interest are:

(i) Coprophagy is a normal occurrence in rabbits of all ages and both sexes, except in baby rabbits receiving only milk.

(ii) The formation of the special faecal pellets takes place about six hours after the last meal, usually at night.

(iii) These pellets are swallowed voluntarily an hour or so later, and sufficient are taken to fill about one-third of the stomach. They are taken direct from the anus and are not picked from the floor.

(iv) These pellets remain intact in the stomach for a number of hours, after which they undergo a gradual softening and disintegration.

(v) The formation of these pellets is not stopped by feeding large quantities of a vitamin B complex-rich foodstuff (Marmite) or by adding to the diet of the rabbit a proportion of normal rabbit or bovine (dairy cow) faeces daily.

(vi) It is suggested that coprophagy is developed as a compensatory habit during the natural life of the rabbit, when it remains in the burrow for long periods to avoid its enemies.

THE GUINEA PIG

In good health the smooth-coated guinea pig should feel compact and firm to handle, with the flesh evenly distributed. Any suggestion of 'lightness' when the animal is handled should be regarded with suspicion. The coat should be shiny, dense, and smooth; the eyes bright; the nose clean; and respiration regular.

Evidence of diarrhoea should be reported at once, as it may be the first sign of a salmonella infection in the colony. Slobbering from the mouth is unusual, and indicates either trouble with the teeth or a more serious complaint which must be investigated. Any rise above the normal death rate of a guinea pig colony should be carefully investigated by the veterinary officer, and, routinely, post-mortem examination should be made of all animals found dead or moribund, and adequate records of the causes of death should be kept. If necessary, laboratory reports should also be filed. These steps will ensure that serious, epidemic diseases are checked before they obtain a hold on the colony. This will call for close co-operation between the chief animal technician and the veterinary officer.

Guinea pigs are most susceptible to changes in temperature or humidity, and draughts. The actual temperature at which guinea pigs are kept is not so important as the maintenance of an even environmental temperature. As a guide, 62–65°F (17–18°C) room temperature and 50–60 per cent relative humidity is suggested.

Guinea pigs will eat and do well on a pelleted diet provided that vitamin C (ascorbic acid) is present in the diet, either in the form of a green food supplement or added to the pellets when they are made. Evidence of the latter must be available, as ascorbic acid decomposes quickly in a pelleted diet unless great care is taken with the compounding of the pellets.

Signs of vitamin C deficiency (Scurvy) can be detected after eleven to fourteen days, when the guinea pigs will exhibit difficulty in walking, steady

loss of weight, staring coat and, later, swollen joints and bleeding from the gums around loosened teeth.

It is difficult to breed and rear guinea pigs for long periods without the addition of top-quality meadow hay to the diet. The exact reason for this has yet to be determined. Clean water is also necessary.

It is important to examine the hair and skin of guinea pigs from time to time, as they are parasitized by two species of lice (see Tuffery, 1960). The treatment suggested is by the installation of an aerosol containing gammexane or by dusting the animals with Pybuthrin.

For further reading the *UFAW Handbook* is recommended, and also see Paterson (1957).

THE RAT

Paratyphoid disease (Salmonellosis) is the only serious, common, specific INFECTION of all laboratory rats. However, about 75 per cent of all adult rats are affected with chronic, pulmonary disease, and middle-ear disease is quite common in some stocks of rats.

The first signs of paratyphoid disease in rats are acute diarrhoea and loss of condition. Prompt and ruthless action by the technicians and veterinary officer is necessary if the stock is to be saved (see chapter on Animal Diseases). Great attention must be paid to hygiene. Precautions against the contamination of food by wild rats and mice must be strictly enforced. One source of paratyphoid infection may be a 'carrier', that is, an animal which carries and transmits a disease without exhibiting signs of infection. Bacteriological screening should be instituted to detect carriers. It may be necessary to obtain a fresh colony with rats from a pathogen-free stock (see chapter on Breeding).

Sniffling at the nose (*Murine pneumonitis*) is present in most stocks of rats. The disease is transmitted from adults to offspring during the first few days of life. Childs and Rees (1958) recommend controlling the infection with sulphonamides, but to eliminate the infection it is necessary to take rats by caesarian section and raise them in a specific pathogen-free colony (see chapter on Breeding).

Fifty per cent of all rats, wild or domestic, carry *Streptobacillus moniliformis*, the causal organism of 'infective arthritis' in mice.

Ringtail sometimes occurs in newborn rats. The skin of the tail, and sometimes also the feet, appears scaly, and the tail becomes corrugated by rings of constriction. In severe cases the part of the tail distal to one of the constrictions becomes necrotic and falls off. Occasionally one or more toes may be similarly affected. The necrosis is accompanied by inflammation of adjacent tissues, probably due to secondary infections. Ringtail is established during the first few days of life, after which it retrogresses spontaneously. It does not appear to distress the rats. The condition has been attributed to a deficiency of essential fatty acids in the diet, but it is more probably due to exposure of the newborn pups to cold and draughts. The use of solid-bottomed, rather than mesh-bottomed, litter cages greatly reduces the risk of exposure for the pup, and colonies kept in such conditions rarely exhibit ringtail.

Scabies of the ear and tail is caused by mites (*Chorioptes cuniculi* or *Psoroptes*

cuniculi). Such infestation causes dark, scaly lumps on the tail and 'cauliflower' ears. Affected areas should be treated with benzyl benzoate (a specific cure for scabies), the bedding burnt, and the cages sterilized.

THE MOUSE

Laboratory mice are subject to many bacterial and virus diseases, most of which can be avoided by strict attention to routine care and inspection by the technician in charge. Mice brought in from an outside colony should not be mixed with an existing colony until after they have been quarantined for at least four weeks and have been subjected to bacteriological screening.

Mice and rats should not be kept together in one room. Most rats carry *Streptobacillus moniliformis*, which can be transmitted to mice. The feet and tails of mice should be examined at least once a week. Swollen feet might indicate infection with *Streptobacillus moniliformis*, or, more serious still, *Ectromelia* (Mouse Pox).

Infestation with mites should not be tolerated; apart from lowering the animals' condition and vitality, certain mites carry diseases which they can transmit from mouse to mouse and stock to stock, e.g. Eperythrozoon coccoides. Treatment with DMC (see table on ecto-parasites), or Westcodyne (obtainable from West Chemical Co., 42 West Street, Long Island, New York, U.S.A.), or the installation of continuous-flow aerosols is recommended.

Bald patches on mice may be caused by Ringworm fungus, which can be transmitted to humans. Encourage all technicians to wash their hands after touching mice, and to report immediately any unusual skin conditions appearing on either themselves or the mice.

Chloroform must not be used in the mouse room, because this substance has a harmful effect on mice, and may render the male mice infertile. Recommended environmental conditions are room temperature 72–74°F (22–23°C) and a relative humidity of 50–60 per cent.

THE HAMSTER

This animal requires sympathetic treatment. Lack of care and attention can lead to the production of unsatisfactory and even sick animals. Gentle but firm and regular handling is essential (see section on Handling).

In spite of their usefulness in the study of experimentally produced diseases, golden hamsters are generally free from spontaneous disease. Both tapeworm and roundworm may infest a colony, and routine checks should be made for these parasites. Mites and fleas may be the intermediate hosts for worms, so it is essential that the hamster be kept free from ecto-parasites. These measures together with an adequate diet are the best means for disease control.

Hamsters dislike temperature variation and draughts. A sudden drop in room temperature in winter-time will send hamsters into hibernation. This is not an ailment but a natural phenomenon common to many animals. The hamster curls up tightly in its nest and is quite cold and rigid to the touch. Breathing is very slow and shallow, and the heart beats faintly. This condition

can be mistaken for death. No attempt should be made to waken a hibernating hamster; it should be given gradual warmth until signs of life return.

Running eyes, high-pitched bronchial wheezes, and sniffling are all indications of a cold which, if not checked, may lead to a fatal pneumonia. Rooms should be controlled at a temperature of 68–70°F (20–21°C) and kept at a humidity of 50–60 per cent.

Overgrown claws are seen occasionally in hamsters. These should be clipped with a pair of sharp scissors or small bone forceps. Hold the claw up to the light, notice the red blood-supplied portion of the nail and clip $\frac{1}{8}$ in. distal from this point.

Sometimes the teeth grow inwards spontaneously, and natural wear is prevented. Overgrown teeth can be a problem because there is no cure except to keep cutting the teeth with bone forceps.

Hamsters will eat any cubed diet suitable for rats and mice, and do not require any supplement other than fresh, well-washed greenfood.

THE CAT

Cats suffer from at least two virus diseases, one of which, infectious feline enteritis (Panleucopenia) may be fatal. This disease can be controlled by a vaccine which only provides protection for a few months (see section on Diseases).

Early diagnosis, isolation, and good nursing are very important to the recovery of cats from diseases. Particularly is this so in the case of feline pneumonitis, a disease caused by a virus of the psittacosis group. This disease manifests itself in both mild and severe forms (Scott, 1952), and kittens, both weaned and unweaned, are very susceptible. In the mild form the kitten has a discharge from the eyes together with inflammation of the conjunctiva. It is vital that this condition should be detected as early as possible, and the technician must be prepared to stop all other work to attend to the kitten. The eyes should be bathed with boracic solution and penicillin eye ointment smeared on the eyes. Scott (1953) has suggested that aureomycin is the most suitable antibiotic to administer. Short and Lamotte (1958) found aureomycin capsules given orally as the most effective remedy. The technician should make certain that sufficient liquid is given to the patient during illness. In most cases the kitten can be returned to the litter or group of kittens within a few days. It has also been suggested that injections of combined penicillin and chloromycetin should be given when the animal is suffering from the severe form of this disease.

Tapeworms and roundworms are common in cats. Healthy adult cats appear to tolerate worm infections quite well, but kittens and pregnant and lactating cats are adversely affected by the presence of worms. Three species of tape worm infect the cat, one of which, *Diplidium caninum*, can be transmitted to man. Strict care with personal hygiene should be taken after handling dogs or cats and their bedding.

The roundworm requires no intermediate host, as the eggs, if ingested, mature and reproduce in the duodenum of the cat. The contamination of rooms, food, water, bedding, and equipment by faeces from infested animals should be prevented.

Proprietary tablets are available for the treatment of round and tapeworm infections in the cat. The treatment is effective, and cats do not have to be starved prior to administration of the tablets.

The ears of cats and kittens should be inspected regularly for the presence of mites. These parasites may be eliminated by treatment with benzyl benzoate.

Ringworm can be troublesome in cats. La Touche (1957) reported that numbers of cats, kittens, and dogs and puppies examined in Leeds were found to be infected. Almost all these animals were from homes inhabited by people suffering from ringworm caused by *M. Canis*, the so-called animal type microsporum, presumably transmitted to them by their pets.

The most frequently affected areas are the bridge of the nose, and inside the ears and the back, although all parts of the hair-bearing surface may be affected.

It is essential that imported cats are carefully screened for ringworm and internal and external parasites before they are admitted to an existing colony. Maintain a high standard of personal hygiene.

THE DOG

Most laboratories rely on dealers and breeding kennels for supplies of dogs. It may be assumed that such dogs, on reaching the laboratory, have already been exposed to various infections and changes in diet. They may be infested with endo- and ecto-parasites, and will certainly be suffering from considerable nervous strain (see chapter on Receiving Animals).

Where dogs have to be exercised with other dogs it is important that the group is watched to see that all the animals behave well towards one another. Persistently quarrelsome dogs should be isolated before other dogs contract the habit of quarrelling.

THE FOWL

The outward signs of ill health are loss of appetite, dullness of plumage, paleness of comb and wattle, running nose and paralysis of either a leg or wing. (Any of these signs of illness should be reported.) Handling the birds will give a good general indication of their health, and will also enable the technician to examine them for external parasites, which can be troublesome in cage-kept birds.

There are several species of lice which parasitize poultry; some attack the head and neck, some the wings, and others the body, especially the abdomen and tail. The body louse is the commonest species. It is about $\frac{1}{12}$-in. long, and is pale yellow with dark spots. When the feathers are parted the lice may be seen running over the surface of the skin. The eggs (or 'nits') are laid in clusters at the base of the feathers.

The wing louse establishes itself in the primary and secondary feathers of the wing. The head louse is most injurious to young chickens.

Lice are easily and effectively erradicated by the use of an aerosol containing gammexane or dusting the animals with DDT powder, or the application (with a camel-hair brush) of two spots only of a 40 per cent solution of nicotine sulphate (perch paint) to the breast feathers of each fowl.

Red mites, unlike lice, do not live on the birds but only visit them to feed. During the day the mites secrete themselves in clusters in the vicinity of the roosting place. Treatment should not be delayed, as these parasites cause great irritation when feeding on the fowl's blood while the bird is roosting.

The mite is about 1 mm in length, and is light grey or yellowish in colour, but appears red when gorged with the fowls' blood. As these mites congregate in masses, they are easily destroyed by dressing their hiding-places with paraffin or by installing an aerosol.

The hen-flea should never be allowed to establish itself, because it is a difficult insect to eradicate. The eggs are laid among the dust or dirt of any crevice. It is useless to apply insect powder to the birds and cages only. To eradicate fleas the cages should be autoclaved or sprayed with a coal-tar disinfectant; the whole room and its contents must be sprayed and each bird dusted with DDT powder. An aerosol should be installed.

THE MONKEY

Monkeys are an accepted laboratory animal, and may be kept in cages for several years provided that the cages, if they have mesh floors, also have a wood or metal seat, so that the animal can sit or stand on a solid surface and is not forced on to the grid. Failure to provide this simple seat can result in the monkey becoming paralysed in one or both hind-legs.

Monkeys do well on the cubed diets which are satisfactory for breeding mice and rats (Short and Parkes, 1949), but a daily supplement of greenfood (or some other source of vitamin C) must also be given. Monkeys deprived of vitamin C develop scurvy. It is known that the young monkey requires a *minimum* of 2 mg vitamin C daily.

Most monkeys bite, so it is important to learn how to handle them. Newly imported monkeys must be handled with special care.

The Rhesus monkey (*Macaca Mulatta*) is a fairly hardy creature and may be housed at a room temperature of 68–72°F (20–22°C). Monkeys from Africa and the Far East require much higher temperatures, 78–85°F (25·5–29·5°C). The relative humidity should be 55–65 per cent; at lower humidities the animals may develop chest troubles. It is important that these temperatures and degrees of humidity are maintaned in the monkey rooms.

Newly imported monkeys are often infested with lice; the insects usually congregate on the chest of the monkey and can be seen as grey creatures about 1 mm in length. Gammexene powder dusted on and rubbed into the animal will soon clear this pest away, and the use of aerosols will prevent re-infestation.

The technician should inspect the animals each day and report any suspicious signs immediately to the veterinary officer. For further reading about monkeys:

The *UFAW Handbook*, 2nd Edition (1957).

VAN WAGENEN, C., 'The Monkey' in FARRIS, E. J., *Care and Breeding of Laboratory Animals*. Chapman and Hall Ltd. (1950).

RUCH, T. C., *Disease of Laboratory Primates*. W. B. Saunders Co. (1959).

'Care and Disease of the Research Monkey', *Annals of the New York Academy of Science*, 85 (May 1960).

REFERENCES

BLOUNT, W. P., *Rabbit Ailments*. Published by Fur & Feather, Bradford (1957).

CHILDS, R. T. and REECE, O., *Nature*, **181**, 1213 (1958).

LA TOUCHE, C. T., *UFAW Handbook*, p. 526. Baillière, Tindall & Cox (1957).

PATERSON, J. S., *L.A.C. Collected Papers*, **5**, 58 (1957).

SABIN, A. F. and WRIGHT, A. M., *Journal of Experimental Medicine*, **59**, 115–16 (1934).

SCOTT, P., *Journal of Physiology*, No. 118, 35–36 (1952).

SCOTT, P., Unpublished Observations (1953).

SHORT, D. J. and PARKES, A. S., *Journal of Hygiene*, **47**, No. 2, 209–12 (1949).

TUFFERY, A., *Journal of Animal Technicians Association*, **11**, 3 (1960).

CHARLES, R. T., POPPLETON, W. R. A., and STEVENSON, D. E., *Journal of Animal Technicians Association*, **13**, 1 (1962).

SHORT, D. J. and LAMOTTE, J., *Journal of Animal Technicians Association*, **9**, 1 (1958).

CHAPTER NINE

Sterilization and Disinfection

It is most important to understand clearly the special terms used in connexion with this subject. Much confusion has arisen through incorrect, loose usage of terms because they are of old derivation. For example, the terms 'antiseptic' and 'disinfectant' were first introduced some two hundred years ago, and, as is not uncommon, their meanings have been modified with the passage of time.

Sterilization

In its proper sense, sterilization is an absolute term, meaning the complete destruction or removal of all forms of life. The number of agents capable of achieving this is limited, and is confined to high temperatures (including fine saturated steam under pressure) and certain types of filters. A few of the many chemicals employed, and possibly one or two of the radiation treatments, suitably applied, are true sterilizing agents.

The term sterilization is often erroneously applied when disinfection is really meant. This should be noted in the medical field, where 'sterilization' is used to mean the destruction of micro-organisms undesirable under a particular set of circumstances. This use of the term has often led to confusion in the past, and illustrates the need for taking care always to use the correct terminology.

Disinfection

This may be defined as the process of eliminating or destroying infection; it is accomplished by the use of a *Disinfectant*. The term was introduced before the establishment of the germ theory of infection, and so because disease was always associated with foul odours, it tended to imply, primarily, the destruction or masking of these odours, although often the killing of bacteria attended. On this account the term *Disinfectant* is still frequently confined to the strong-smelling coal tar fluids, whereas, in fact, it has a much wider application and meaning. Several authorities, with some justification, prefer to confine the use of the word to the treatment of inanimate (lifeless) objects, and this is the generally accepted meaning.

Sanitization

This is an awkward word of recent vogue in the United States, and means the process of rendering sanitary or of promoting health. It is akin to disinfection,

but carries with it the inference of cleansing as well as the removal of infection. It is not a term used to any extent in Great Britain.

Antiseptic

Another much misunderstood word, antiseptic, literally interpreted from its Greek origin, means 'against putrefaction', but it has now been extended to include activity against bacterial sepsis or infection. By inference, the word conveys a meaning similar to that of 'disinfection', and there is a tendency to use the term specifically of preparations for application to living tissues, especially in surgery and hygiene. It can also be used to denote a property of inhibiting or preventing the growth of micro-organisms under prescribed conditions of usage.

Bactericide

A bactericide kills bacteria, but not necessarily bacterial spores, while a *Bacteriostat*, or *Bacteriostatic Agent* prevents the growth of bacteria and so gives rise to a state of *Bacteriostatis*. Similarly, a *Fungicide* kills fungi and a *Virucide* kills viruses. A *Germicide* kills *all* micro-organisms. The suffixes -stat and -stasis are not used in conjunction with this term.

By way of general explanation it may be said that the suffix -cide always applies to any agent producing a killing effect on the micro-organisms concerned, whereas -stat means that the agent simply prevents or inhibits growth. *Stasis* is the state of suspended animation or inhibition produced by the latter type of agent.

METHODS OF STERILIZATION AND DISINFECTION

Physical

Heat: (*a*) Wet
(*b*) Dry
(*c*) Irradiation.

Chemical

(*a*) Liquid
(*b*) Gas.

Heat

An important agent in the artificial destruction of micro-organisms, the effect of heat is to coagulate and denature cell proteins. In general, among bacteria which are parasites of mammalian animals the non-sporing forms in a moist state cannot withstand temperatures above 113°F (45°C) for any length of time. Bacteria are more susceptible to moist heat (e.g. in a steam sterilizer) than to dry heat (e.g. in a hot-air oven).

Bacterial spores

Some species of bacteria, those of the genus Bacillus and Clostridium, develop *Spores*—a highly resistant resting stage. The spore is not a reproductive structure, but it can survive unfavourable external conditions.

(A) HEAT (WET)

(i) Autoclave: sterilization by steam under pressure.
(ii) Free steaming: sterilization by steam not under pressure.
(iii) Boiling.
(iv) Washing machines.

(i) Autoclave

Autoclave is the use of steam under pressure in a specially constructed apparatus. The principle on which the autoclave depends is that water boils when the vapour pressure is equal to the pressure of the surrounding atmosphere. If therefore the pressure is increased inside a closed vessel the temperature at which water boils will rise above 212°F (100°C); the exact temperature depending on the pressure employed. The pressure generally employed is 15 lb per square inch. At this pressure water boils at 249·8°F (121°C), and 30 minutes exposure at this temperature kills all forms of organisms, *including spores*.

The point of using pressure is to raise the temperature, and it is the heat which does the sterilizing.

TEMPERATURES OF SATURATED STEAM UNDER PRESSURE

lb pressure on autoclave	°C	°F
0	100·0	212·0
5	108·4	227·1
10	115·2	239·4
15	121·0	249·8
20	126·0	258·0

It has been recognized for many years that rapid penetration of *Steam* to all parts of material in the autoclave is essential if complete sterilization is to be achieved. One of the traditional methods of removing air from steam sterilizers has been to use a steam ejector to draw out part of the air contained in the autoclave before steam was admitted. This method has proved inefficient; often it removed only about one-third of the air. Residual air prevents the penetration of steam and, thus, the attainment of the required sterilizing temperature. Recently attention has been drawn to the advantage of a preliminary evacuation to a pressure of 20 mm Hg before admitting the steam to ensure a rapid and reliable penetration. (This pressure is registered on the autoclave gauge as a *negative* 20 lb pressure.) Glick, Gremillion, and Bodmer[1] (1961) have shown that a nesting-type of cage (designed to save space during storage and sterilizing) when stacked six high in an upright position and autoclaved for four hours at 15 lb per square inch pressure still contained active test organisms. When the cages were autoclaved lying on their sides 30 minutes autoclaving at 20 lb per square inch was sufficient to kill the test organism and its spores. The authors concluded that with the cages in an

upright position pockets of air were trapped at the bottom of each cage, which excluded the steam necessary to raise the temperature.

These workers also studied the sterilization of infected carcases. Twenty dead guinea pigs were packed in fibre-board containers and autoclaved for various periods up to 16 hours. It was found that a period in excess of 8 hours was necessary for the centre of the load to reach sterilizing temperatures. For practical purposes the load was autoclaved for sufficient time to ensure sterility of the outside surfaces of the containers, and the contents were considered infectious during transport to the incinerator.

(ii) Sterilization by steam not under pressure

This method is the use of steam at atmospheric pressure in steamers or tanks. The maximum temperature which can be reached by this method is 212°F (100°C).

The Cage Sterilization Sub-Committee of the Public Health Laboratory Service[2] investigated the rate at which vegetative organisms in contaminated cage litter were destroyed by exposure to steam at atmospheric pressure applied in cabinets made of galvanized sheet iron. The committee concluded that pathogenic organisms such as *Salm. typhimurium* and tubercule bacilli were invariably killed in litter by steaming for 10 minutes. The exposure of contaminated litter, even when 4 in. deep, to steam at atmospheric pressure for 10 minutes is sufficient to destroy vegetative forms of pathogenic organisms. This committee recommended that, to ensure a wide margin of safety, steaming should be continued for 30 minutes.

It is important to note that the times given for sterilizing are AFTER the maximum temperature of 212°F (100°C) has been reached on all parts of the cages and litter. This method will not kill all organisms, and is not to be recommended if spore-forming bacteria are present.

(iii) Boiling

This method may be used for sterilizing animal cages in a large tank of water, heated by an immersion heater, or gas or steam pipes. Great care is necessary to ensure that the maximum temperatures and time of sterilizing (the same as for the previous method) are obtained and maintained. The cages are cleaned as they are sterilized. This method is not to be recommended in a modern animal house.

(iv) Washing machines

These machines can be used for *washing* and *sanitizing* cages, water bottles, and other items of equipment which can stand the rigours of this treatment. If used correctly, such machines are labour saving as large numbers of cages can be cleaned in a short time; but it must be remembered that few machines can exceed a temperature of 180°F (82°C). There are some expensive, specially designed machines which attain a temperature of 212°F (100°C) and can therefore be used to *sterilize* equipment.

(B) DRY HEAT

It should be emphasized that sterilization by dry heat depends upon the penetration of adequate heat to all parts of the article. It is therefore possible

to sterilize apparatus already assembled and pre-sealed in a container, whereas steam and gases (such as ethylene oxide) can be relied upon only to kill organisms with which the steam or gas comes into direct contact. Another advantage of this method is that objects which are damaged by water or steam (e.g. food, bedding) can be sterilized, provided the heat penetrates to all parts of the substance.

The disadvantages of the method are, first, some equipment, such as hot air ovens, take a considerable time to reach sterilizing temperature; dry sterilizing requires a higher temperature and a longer exposure time than wet sterilizing; objects (e.g. metal ones) may become oxidized at high temperatures or may not withstand the temperatures.

Wentworth Cumming[3] (1962) reported that all materials, except food, taken into a Specific Pathogen-Free Unit were sterilized in a large electric oven at 250°F (121°C), for 2 hours *after* a 30-minute warm-up period. Darmady and Brock[4] (1954) suggested that it was important to check each hot air oven carefully, as a number tested did not reach a uniform sterilizable temperature, and they showed that there might be a variation of 86–104°F (30–40°C) between different parts of the oven. They also showed that ovens fitted with a fan required a shorter time to reach sterilizing temperature than still-air ovens. The forced circulation of air reduced temperature variations to a minimum. It was also found that unless the objects in the oven were loosely packed, there would be a delay in heat penetration to the centre of the load, even in ovens fitted with a fan.

(C) RADIATION (*vide* Sykes,[5] 1958)

The range of radiations which have been used for killing micro-organisms fall into two groups: (*a*) the ionizing radiations comprising X-rays, gamma rays, cathode rays, beta rays, and the heavy particles, neutrons, protons, etc.; and (*b*) the longer electro-magnetic radiations comprising ultra-violet rays, infra-red rays, and radio-frequency radiations; and ultrasonic waves of high frequency. The terms 'radiation sterilization' and 'electronic sterilization' (terms which have become quite commonplace in present-day parlance) apply exclusively to the various forms of ionizing radiations; they do not include the radiations of group (*b*) above.

Darmady *et al.*[6] (1961) investigated sterilization by radiation of disposable medical items and found that 2·5M rad (Mega) can be recommended to give a high degree of sterility. This was best achieved, in practice, by using either high-energy electrons from a machine such as a linear accelerator or gamma radiation from an isotope source such as Cobalt-60. Both types of radiation have been used commercially for sterilization, and they have similar bactericidal properties; but they differ in dose, rate, and degree of penetration.

Radiation sterilizing is not, however, the answer to all sterilizing problems, because certain products (e.g. rubber, plastic) could be damaged, and the capital cost of installation is high. Their powers of penetration, and the absence of any thermal effect, marks the high-energy ionizing radiations of particular potential value in the sterilization of materials which are difficult to treat by the more orthodox heating methods. Thus, any material which has low heat conductivity or is adversely affected by heat is specially suited to treatment by radiation, provided there are no adverse side effects. For this reason,

investigations have been largely centred on the preservation of food and the sterilization of pharmaceutical products.

The outstanding advantage of the treatment is that it is virtually devoid of any thermal action, the maximum rise in temperature being only 3° or 4°. The treatment could be useful in the sterilization of animal bedding.

CHEMICALS

Phenol and phenol derivatives

Although phenol has occupied a prominent place in the field of disinfection since its discovery as a potential germicide by Lister in 1867, interest is now centred on the derivatives of phenol as practical disinfectants. Probably more is known about the anti-microbial properties of phenol than any other substance, and it is often used as a model for examining certain aspects of the theory of disinfection. Today its applications are virtually limited to that of the standard against which the germicides are compared and to that of a bacteriostatic for use in preparations administered by injection.

All the phenols can act either bactericidally or bacteriostatically, depending upon their concentration. Generally speaking, the phenols are not sporicidal, but they are effective against tubercle bacilli, and they exhibit anti-fungal activities, some being more active against fungi than against bacteria. They are not particularly viricidal. Small changes in concentration give rise to relatively large differences in killing rates, but the phenols remain bacteriostatic over fairly wide ranges of concentration.

The phenols become more effective with increase in temperature. The warmer the surfaces to be disinfected and the warmer the phenol solution, the better the result. Alkaline solutions are always less active than acid ones.

Mode of action

The phenols owe their anti-bacterial properties to their ability to combine with and denature proteins. This phenomenon has long been understood, but more recently other aspects of the mode of action of phenols have been studied; particularly the absorption of phenols by bacterial cells.

Effect of organic matter

The bactericidal power of phenols is always reduced in the presence of other organic matter (e.g. faeces). The organic matter may cover the organism and prevent the penetration of the disinfectant; it may neutralize the disinfecting action by combining with the disinfectant; or it may serve as an absorbing surface to reduce the amount of active disinfectant present.

Liquid synthetic phenolic disinfectants

A group of synthetic phenols is of recent advent, although the soap-based pine oil disinfectants were known forty years ago. The pine oil disinfectants are not phenolic in constitution, but are composed mainly of terpenes; however, it is useful to consider them in the present context, because many synthetic phenol disinfectants have pine oil fractions added to them. Pine oil disinfectants, if properly constituted with the right terpene fractions and a suitable soap base, are reasonably active against many bacterial types.

A correctly formulated pine oil disinfectant contains at least 60 per cent of pine oil, but many preparations on the market contain lower proportions than this of superior oils, and consequently merit being classed as little more than deodorants.

Coal tar disinfectants

The coal tar disinfectants are also phenolic in nature. They are much cruder than the pure synthetic phenols and are more toxic and irritating to the skin. Lysol, for instance, cannot be comfortably tolerated by normal skins at a concentration greater than 1 per cent.* Coal tar disinfectants should be used only for disinfecting inanimate objects.

The active constituents of this type of disinfectant are derived from coal tar distillation fractions and have only low solubility in water. Hence they have to be emulsified in order to obtain an adequate concentration for disinfectant purposes.

The germicidal activities of these disinfectants are determined by the quality of the phenol fraction used, the ratio of phenol to emulzent used and the nature of the emulzent. The mixed cresols which are the main constituents of the fraction boiling in the range 383–401°F (195–205°C) are used for making Lysol or solutions of Cresol in Soap B.P.

Lysol of the B.P. formulation contains about 50 per cent of cresol and 22 per cent of linseed oil soap. The higher the molecular weight of the compound, and the higher its boiling point, the more divergent are its activities against the gram-positive and gram-negative organisms. Thus, the various Lysols made with the boiling cresols have equal or slightly lower phenol coefficients against *Staph. aureus* than against *Salm. typhimurium.*

Too much reliance should not be placed on the phenol coefficient of a disinfectant (see Rideal Walker and Chick Martin tests). The response to these fluids differs with different organisms, and, in practice. there is also the problem of the presence of other organic matter. Generally, disinfectants of this type are used when organic matter of one sort or another is present, and the Chick Martin test was devised to meet these conditions.

Broadly speaking, the low Rideal Walker coefficient fluids are less affected by the presence of organic matter than the high ones. Thus, Lysol with a Rideal Walker coefficient of 2·5 has a Chick Martin coefficient of about 2·0, but a fluid with a Rideal Walker value of 20 may only have a Chick Martin value of 3 or 4.

Phenol coefficient test—Rideal Walker

The primary purpose of a phenol coefficient test is to compare the efficiency of phenolic coal tar disinfectants against a standard phenol solution. Rideal and Walker (1903) were the first people to standardize the conditions of testing; it included a reference germicide, phenol, and it used a culture of vegetative organisms grown in broth.

The Rideal Walker test

The Rideal Walker test is not only of historical importance, it is used extensively today, and therefore justifies consideration in some detail. In its original

* A solution of 3–5 per cent is recommended for most uses. Lysol is toxic for most animals, especially for cats, dogs, monkeys, and mice.

form, as published by Rideal and Walker (*ibid.*), the test represented the first real attempt at putting the assay of disinfectants on a quantitative basis; in its present form it is a valuable tool in the routine assessment of standard disinfectants. The test is over fifty years old and is today the same in principle as it was originally, although a number of modifications have been made, all with the object of improving the precision of the test. When first produced, it represented an entirely new departure in testing techniques in that it specified standardized cultural and other conditions and, most important, it used a standard substance, pure phenol, against which the disinfectant under examination was compared.

The test specified the species and age of the culture, the culture medium employed, the amount of inoculum used, the temperature and time of medication, the period of incubation of the subcultures, and the resistance of the test organism in terms of the phenol control. The organism used in the original test was the Rawlings strain of *Salm. typhimurium*, chosen because it is a typical representative of the entire group of the intestinal organisms and also because it was believed to possess a degree of constancy in growth characteristics superior to other organisms. For the same reason, Liebig's meat extract and Witte's peptone were specified, on the assumption that they would ensure reproducibility in nutrient properties of the culture medium, and therefore, in the growth characteristics of the organism.

In subsequent years, the authors made various modifications which they later published as an 'approved technique' (Rideal and Walker,[8] 1921). The preface contained the admonition that to avoid discrepancies 'strict observance of the conditions laid down by the authors cannot be too strongly emphasized'. The new technique included modifications to the volumes of disinfectant dilutions and of culture medium employed, the time of medication, the test temperature, and it substituted Witte's peptone by Allen and Hanbury's Eupeptone.

Calculation of the coefficient

The Rideal Walker coefficient is calculated by dividing the dilution of disinfectant which shows life after $2\frac{1}{2}$ and 5 minutes, but not after $7\frac{1}{2}$ and 10 minutes, by the dilution of phenol which shows the same end-point.

A typical test result is as follows:

DISINFECTANT	DILUTION	TIME (MIN) CULTURES EXPOSED TO ACTION OF DISINFECTANT			
		$2\frac{1}{2}$	5	$7\frac{1}{2}$	10
A	1 in 1,000	−	−	−	−
A	1 in 1,100	+	−	−	−
A	1 in 1,200	+	+	−	−
A	1 in 1,300	+	+	+	−
Control phenol	1 in 105	+	+	−	−

(+ = growth; − = no growth)

Rideal Walker coefficient = $\frac{1200}{105}$ = 11·4 (approximately).

A correctly formulated pine oil disinfectant contains at least 60 per cent of pine oil, but many preparations on the market contain lower proportions than this of superior oils, and consequently merit being classed as little more than deodorants.

Coal tar disinfectants

The coal tar disinfectants are also phenolic in nature. They are much cruder than the pure synthetic phenols and are more toxic and irritating to the skin. Lysol, for instance, cannot be comfortably tolerated by normal skins at a concentration greater than 1 per cent.* Coal tar disinfectants should be used only for disinfecting inanimate objects.

The active constituents of this type of disinfectant are derived from coal tar distillation fractions and have only low solubility in water. Hence they have to be emulsified in order to obtain an adequate concentration for disinfectant purposes.

The germicidal activities of these disinfectants are determined by the quality of the phenol fraction used, the ratio of phenol to emulzent used and the nature of the emulzent. The mixed cresols which are the main constituents of the fraction boiling in the range 383–401°F (195–205°C) are used for making Lysol or solutions of Cresol in Soap B.P.

Lysol of the B.P. formulation contains about 50 per cent of cresol and 22 per cent of linseed oil soap. The higher the molecular weight of the compound, and the higher its boiling point, the more divergent are its activities against the gram-positive and gram-negative organisms. Thus, the various Lysols made with the boiling cresols have equal or slightly lower phenol coefficients against *Staph. aureus* than against *Salm. typhimurium.*

Too much reliance should not be placed on the phenol coefficient of a disinfectant (see Rideal Walker and Chick Martin tests). The response to these fluids differs with different organisms, and, in practice. there is also the problem of the presence of other organic matter. Generally, disinfectants of this type are used when organic matter of one sort or another is present, and the Chick Martin test was devised to meet these conditions.

Broadly speaking, the low Rideal Walker coefficient fluids are less affected by the presence of organic matter than the high ones. Thus, Lysol with a Rideal Walker coefficient of 2·5 has a Chick Martin coefficient of about 2·0, but a fluid with a Rideal Walker value of 20 may only have a Chick Martin value of 3 or 4.

Phenol coefficient test—Rideal Walker

The primary purpose of a phenol coefficient test is to compare the efficiency of phenolic coal tar disinfectants against a standard phenol solution. Rideal and Walker (1903) were the first people to standardize the conditions of testing; it included a reference germicide, phenol, and it used a culture of vegetative organisms grown in broth.

The Rideal Walker test

The Rideal Walker test is not only of historical importance, it is used extensively today, and therefore justifies consideration in some detail. In its original

* A solution of 3–5 per cent is recommended for most uses. Lysol is toxic for most animals, especially for cats, dogs, monkeys, and mice.

One other advantage claimed for the quaternaries is that their solutions do not become exhausted as rapidly as do solutions of hypochlorites.

Ampholytic surface-active agents, e.g. Tego

Whereas surface-active compounds of the anionic, cationic, and non-ionic types have been rapidly developed in appropriate fields of use, the characteristics of the *ampholytic* surface-active compounds were relatively late to be recognized. *Ampholytic* surface-active compounds, as their name implies, combine the detergent properties of anionic compounds with the bactericidal properties of cationic compounds, and they remain highly active in the presence of protein.

The ampholytic surface-active agents

$$\underset{\textit{Anion}}{H_2N{\cdot}R{\cdot}COO^-} \quad \underset{\textit{Cation}}{H_3N{\cdot}{}^+R{\cdot}COOH} \quad \underset{\textit{Amphoteric ion}}{H_3N{\cdot}{}^+R{\cdot}COO^-}$$

The Tego series of compounds are nitrogenous compounds of high molecular weight. Perkins and Short[9] (1957) investigated the bactericidal property of *Tego MHG* by two *in vitro* methods. The results of their investigations are shown in Table 1, from which it was clear that 1 per cent *Tego MHG* was an effective bactericide.

TABLE 1. KILLING TIME IN MINUTES OF 1 PER CENT *TEGO MHG* AT 71·6°F (22°C)

ORGANISM	KILLING TIME MINUTES
Staphylococcus pyogenes (penicillin resistant)	<1
Staphylococcus aureus	<1
Staphylococcus albus	<1
Staphylococcus citreus	<1
Streptococcus pyogenes	<1
Streptococcus faecalis	<1
Streptococcus viridans	<1
Streptococcus haemolyticus, Group D	<1
Escherichia coli	>1 <2
Salmonella enteritidis	>1 <2
Salmonella typhi	>1 <2
Shigella flexneri Type 4b	>1 <2
Corynebacterium diphtheria	>1 <2
Pseudomonas aeruginosa	>2 <5
Pseudomonas pyocyanea	>2 <5
Proteus vulgaris	>2 <5
Clostridium welchii	>2 <5
Epidermophyton rubrum	>5 <10
Epidermophyton interdigitale	>5 <10
Mycobacterium tuberculosis H 37RV	>20 <30

The second method was a modification of the 'Use Dilution Confirmation Test' in use in the United States (Stuart *et al.*,[10] 1953). The results of the tests are given in Table 2, which shows the bactericidal nature of the surface-active agent.

With this 'Use Dilution Confirmation Test' technique it was found that a

Surface active compounds

Some substances when dissolved in water lower the surface tension and thus increase the 'wetting' capacity. The synthetic, surface-active substances which have this effect may be classified in three groups according to the electric charge (negative, positive, or both negative and positive) of the lipophilic portion of the molecule. They are referred to as anionic, cationic, or ampholytic surface-active compounds. The *anionic* surface-acting compounds possess high detergent, but only very limited bactericidal, properties. Modern synthetic soap-substitutes fall into this class.

The *cationic* surface-active compounds, as represented by the quaternary ammonium compounds, possess limited detergent, but high bactericidal and bacteriostatic properties.

Quaternary ammonium compounds ('quats'), e.g. Cetrimide, Benzalkonium Chloride, Vantoc B., and C. L. Cetavlon

The quaternaries are more active against the gram-positive organism than the gram-negative. Bacteria may readily adapt themselves to become resistant to the quaternaries in the same way as they do to the antibiotics and sulphonamides.

Sporicidal activity

Sykes (1958) reports that in spite of claims to the contrary, the quaternaries cannot be considered to be effectively sporicidal.

Antiviral activity

Sykes (*ibid.*) says that the viruses, in general, are rather more resistant than the bacteria and fungi. Because of this, quaternary ammonium compounds have been used in the preparation of vaccines and suspensions of organisms free from viable bacteria. The concentration of some compounds necessary to inactivate the influenza virus in a few minutes is of the order of 1 in 500 to 1 in 8,000, but not all quaternary compounds show such activity.

Toxicity

At the concentrations used for the purposes of disinfection the quaternaries are virtually non-toxic. At high concentrations they can cause severe skin inflammation and oedema, and when administered in large doses in the diet of animals they have proved fatal.

The uses of quaternaries

The quaternaries have been found most useful in the field of surgery (where they are used principally for pre-operative skin disinfection) and in the food industry (where they are used to disinfect food utensils, drinking glasses, and dairy equipment). The quaternaries are not recommended for sterilization of surgical instruments, because they are not active against bacterial spores.

In the food industry the particular virtues of these compounds are that they are colourless, non-staining, practically tasteless, and rapid in action.

The concentrations generally used range between 1 in 5,000 and 1 in 2,000.

One other advantage claimed for the quaternaries is that their solutions do not become exhausted as rapidly as do solutions of hypochlorites.

Ampholytic surface-active agents, e.g. Tego

Whereas surface-active compounds of the anionic, cationic, and non-ionic types have been rapidly developed in appropriate fields of use, the characteristics of the *ampholytic* surface-active compounds were relatively late to be recognized. *Ampholytic* surface-active compounds, as their name implies, combine the detergent properties of anionic compounds with the bactericidal properties of cationic compounds, and they remain highly active in the presence of protein.

The ampholytic surface-active agents

$$\underset{\textit{Anion}}{H_2N{\cdot}R{\cdot}COO^-} \quad \underset{\textit{Cation}}{H_3N{\cdot}{}^+R{\cdot}COOH} \quad \underset{\textit{Amphoteric ion}}{H_3N{\cdot}{}^+R{\cdot}COO^-}$$

The Tego series of compounds are nitrogenous compounds of high molecular weight. Perkins and Short[9] (1957) investigated the bactericidal property of *Tego MHG* by two *in vitro* methods. The results of their investigations are shown in Table 1, from which it was clear that 1 per cent *Tego MHG* was an effective bactericide.

TABLE 1. KILLING TIME IN MINUTES OF 1 PER CENT *TEGO MHG* AT 71·6°F (22°C)

ORGANISM	KILLING TIME MINUTES
Staphylococcus pyogenes (penicillin resistant)	<1
Staphylococcus aureus	<1
Staphylococcus albus	<1
Staphylococcus citreus	<1
Streptococcus pyogenes	<1
Streptococcus faecalis	<1
Streptococcus viridans	<1
Streptococcus haemolyticus, Group D	<1
Escherichia coli	>1 <2
Salmonella enteritidis	>1 <2
Salmonella typhi	>1 <2
Shigella flexneri Type 4b	>1 <2
Corynebacterium diphtheria	>1 <2
Pseudomonas aeruginosa	>2 <5
Pseudomonas pyocyanea	>2 <5
Proteus vulgaris	>2 <5
Clostridium welchii	>2 <5
Epidermophyton rubrum	>5 <10
Epidermophyton interdigitale	>5 <10
Mycobacterium tuberculosis H 37RV	>20 <30

The second method was a modification of the 'Use Dilution Confirmation Test' in use in the United States (Stuart *et al.*,[10] 1953). The results of the tests are given in Table 2, which shows the bactericidal nature of the surface-active agent.

With this 'Use Dilution Confirmation Test' technique it was found that a

spore suspension of *Bacillus cereus* NCTC 9689. which withstood boiling water for 90 minutes was killed by being brought to the boil in 1 per cent *Tego MHG*, a procedure which took, in all, 45 seconds.

Observations on the bactericidal property of *Tego MHG* thus described indicated that the use of the technique should prove effective in the sterilization of animal rooms, racks and cages.

TABLE 2. TIME TAKEN TO STERILIZE INFECTED GLASS CYLINDERS WITH 1 PER CENT *TEGO MHG* AT 96·8°F (37°C)

ORGANISM	TIME TO STERILIZE, MINUTES
Staphylococcus pyogenes (penicillin resistant) NCTC 8178	<1
**Staphylococcus aureus (penicillin resistant)*	<1
**Staphylococcus aureus (penicillin resistant)*	<1
**Staphylococcus aureus (penicillin resistant)*	<1
Staphylococcus aureus NCTC 7447	<1
Streptococcus pyogenes NCTC 8312	<1
Bacillus cereus (Vegetative cells) NCTC 9689	>1 <2
Bacterium coli NCTC 8009	>1 <2
Bacterium coli NCTC 86	>1 <2
Salmonella enteritidis NCTC 5694	>1 <2
Shigella flexneri, Type 4*b NCTC* 8336	>1 <2

* These three strains of penicillin-resistant staphylococcus aureus were recently isolated from human infections.

Toxicity

In comparisons with quaternary ammonium compounds the ampholytic compounds are ten times less toxic. For the quats, a fatal dose administered orally to rats is around 0·2–0·5 gm per kilogram of body weight. The corresponding value for the *Tego* compounds is between 3 and 4 gm per kilogram of body weight.

The ampholytic compounds approximate to the pH of the skin, but in practice they are singularly non-irritant, even when in constant use for hand washing by persons with sensitive skins. Frisby[11] (1959) reported that the *Tego* series of compounds were inactivated by soap and synthetic detergents.

HALOGENS

This group consists of the four elements fluorine, chlorine, bromine, and iodine, which have closely related and graded chemical and physical properties.

Chlorine

During the early part of the last century chlorine was found useful for disinfecting water and treating sewage, purposes for which it is still used today. Chlorine is much used for the disinfection of dairy equipment, it has application in medicine for the treatment of wounds, in the domestic sphere for personal hygiene, and in the animal house it has many uses.

Hypochlorites

The hypochlorites were the first compounds of active chlorine to be made. Calcium hypochlorite is made by allowing chlorine gas to react with slaked lime in either the solid state or in an aqueous suspension. The trade name for this is lime bleach liquor. When freshly prepared chlorinated lime (bleaching powder) may contain up to 39 per cent of available chlorine, but it is unstable under normal storage conditions, and is most sensitive to moisture and heat. Chlorinated Lime B.P. contains not less than 30 per cent of available chlorine. Sodium hypochlorite is made for a variety of industrial purposes in solutions containing up to 20 per cent of available chlorine, but other preparations are sold under numerous trade names for general disinfection purposes and personal hygiene.

The disinfecting action of chlorine

The intense reactiveness of chlorine with organic compounds generally is undoubtedly the reason for its being a rapid and effective germicide, even at quite high dilutions. The hypochlorites are the most reactive, therefore they are the most rapid in their germicidal action. Chlorine is a bactericidal agent and possesses little, if any, bacteriostatic activity. The lethal action is the result of the direct action of the chlorine on some vital part of the cell, such as its protoplasm or an enzyme system. Chlorine and its compounds are much more effective in acid solution than alkaline solution; this effect is illustrated in the following table by Charlton and Levine[12] (1935).

TABLE 3. THE EFFECT OF pH ON LETHAL ACTION OF HYPOCHLORITE AGAINST *B. METIENS* SPORES

Available chlorine, p.p.m.	pH	Time to kill 99 per cent of the spores
1,000	11·3	70 minutes
100	10·4	64 minutes
20	8·2	5 minutes
1,000	7·3	Less than 20 seconds

Effect of temperature

Studies on the effect of temperature on the bactericidal activities of chlorine compounds show that there is always an increase in activity with an increase in temperature. It has been found that about twice the concentration of hypochlorite was required to kill *Salm. typhimurium* at 35·6°F (2°C) than at 104°F (40°C).

Effect of organic matter

The bactericidal efficiency of chlorine falls in the presence of organic matter, and even quite small amounts of such material can exert a significant effect. When treating animal cages and cage trays it is important to see that all organic matter is removed before using chlorine or its compounds.

Iodine

Iodine is an element with a high chemical reactivity, and it is this reactivity which makes it an effective germicide. Among its outstanding characteristics are: (i) its lack of selectivity against different bacteria, all types being killed at about the same level of concentration; (ii) its exclusive bactericidal rather than bacteriostatic action.

The reactivity of iodine is similar to that of chlorine, but unlike chlorine, its disinfecting action is the result of the direct intervention of free iodine molecules which combine with the protein substances of the cell.

Iodine is effective against bacterial spores, but organic matter has a depressant effect on the activity of iodine.

Iodophors

The iodophors are a comparatively new development in the application of iodine for disinfection purposes, and have proved particularly useful in dairy hygiene. Strictly speaking, they are not compounds of iodine, but simply mixtures of iodine with surface-acting agents of all types which act as vehicles for the iodine.

The solutions have all the characteristics of iodine as a germicide, but display a complete lack of odour and of staining power and low irritant properties. Cationic, anionic, and non-ionic detergents can be used with equal success, but generally the more stable preparations are found with the non-ionic group.

Some of the latest iodine compounds have 'built-in' colour molecules which show whether or not the disinfectant is still active.

GASES AND VAPOURS

The sterilization of equipment, bedding, and food is usually thought of in terms of heat treatment, but at least one other method of sterilizing is available, i.e. by bactericidal gases or vapours. This treatment can, of course, effect only surface sterilization, but in dealing with solids this is practically all that is necessary. When the material to be sterilized is porous, or when it is in fine crystalline or powder form, most gases can be used for sterilizing.

The particular advantages of this form of treatment are that it can be carried out at normal or only slightly elevated temperatures, and the gas can be removed completely from the treated material after sterilizing. One important thing to be considered apart from the bactericidal efficiency of such agents is the reactivity of the chosen gas or vapour. No gaseous disinfectant is chemically inert—it would not be germicidal if it were so—but certain of them, such as sulphur dioxide and chlorine, are much too reactive for practical consideration.

Of the substances which are more acceptable, formaldehyde, ethylene oxide, and beta-propiolactone (B.P.L.) have received most attention. But even these have their limitations; they are all toxic to humans above certain concentration and they exhibit other unpleasant or undesirable side effects.

Formaldehyde

Under optimum conditions formaldehyde is lethal to bacteria, bacterial spores, viruses, fungi, and yeasts, and has been employed as a fumigant with varying degrees of success for the past fifty years.

Formaldehyde has the advantage of being cheap, easy to handle, not injurious to fabrics, paints, or metals, and of being rapidly neutralized by ammonia. Its pungent and irritating odour gives adequate warning of its presence at concentrations (5 p.p.m.) too low to be toxic.

Formaldehyde gas can be generated by heating a solution of Formaldehyde B.P. (popularly known as formalin), which is a 37 per cent solution of formaldehyde in water stabilized by the addition of a small amount of methyl alcohol.

Another method employed is to mix about two parts of formalin with one part of potassium permanganate and allow the heat generated by the oxidative reaction to volatilize the remainder of the formaldehyde gas. The bactericidal efficiency of formaldehyde vapour is a direct function of the concentration, relative humidity, and temperature.

Concentration

Sykes (1958) found that bacterial cultures dried on cotton threads required several hours in a gaseous concentration of 1 or even 2 mg of formaldehyde per litre of air before a complete kill was obtained. Nordgren[13] (1939), using bacteria dried on metal and glass, found that organisms died in an atmosphere containing 1 mg of formaldehyde per litre of air within 20–50 minutes. Glick, Gremillion, and Bodmer[14] (1959) advise the vaporization of 1 ml of formalin or formalin–methanol solution for each cubic foot of space in treating rooms or buildings.

Temperature

An increase in the temperature influences disinfection in three ways: by increasing the amount of vapour, by reducing the loss of formaldehyde due to polymerization (the union of two or more molecules of the same compound to form larger molecules), and by reducing adsorption on to fabrics if they are present. A temperature of not less than 64°F (18°C) is recommended.

Relative humidity

At low humidities disinfection is slow, the rate of killing increases with rising humidity, reaching a maximum at 80–90 per cent. Above this level efficiency falls sharply, particularly if the objects are grossly wet.

Ethylene oxide

Kelsey[15] (1959) reports that experience has shown that some of the early claims made for ethylene oxide as the perfect sterilizing agent were not justified. Attempts had been made to define a safe process in terms of concentration, time, temperature, and humidity analogous to accepted standards for steam sterilization, but none could be accepted with complete confidence. Glick, Gremillion, and Bodmer report the extensive use of ethylene oxide in the form of a low pressure mixture with two of the chloro-fluorohydrocarbons

TABLE 4. COMMON

CLASS OR TYPE	INDUSTRIAL NAME (U.K.)	MANUFACTURED BY	CONCENTRATION NORMALLY USED
Halogens			
Chlorine	—	—	—
Iodine	—	—	—
Hypochlorites	Chloros	ICI	1–5%
	Sod. Hypochlorite (10% ww Chlorine)	Hopkin and Williams	3–5 (liquid)
Phenols and related compounds			
Cresol	Lysol B.P.	Prince Regent Tar Co. Ltd	Not exceeding 5%
Cresol and chlorxylenol	Jeypine and other brands	Jeyes	Not exceeding 5%
Quaternary ammonium compounds			
	Nonidet 32G	Shell	0·5%
	Cetavlon	ICI	1·0%
	Cetrimide B.B.	ICI	1·0%
Ampholytic compounds			
	Tego MHG	House, Hoseason & Co. Ltd., Manchester	1%

(Freons) in a disposable can. Any steam autoclave can be inexpensively converted for applying the ethylene oxide–freons mixture without interfering with the conventional use of the autoclave. This conversion enables complete mechanical equipment and heat-labile substances (substances prone to undergo changes with heat) to be sterilized using the same piece of equipment.

Chemically, ethylene oxide is a powerful alkylating agent, and its bactericidal effect is probably due to this action. It is lethal to bacteria and viruses. It forms an explosive mixture when more than 3 per cent of it is present in air. Its toxicity may be compared with that of ammonia.

If mixed with carbon dioxide or freons in proportions of not more than 12 per cent by weight the mixture is not inflammable (Kelsey, *ibid.*).

A definite drawback to the use of ethylene oxide is the required exposure

CHEMICAL DISINFECTANTS

EFFECTIVE AGAINST	NOTES
Most bacteria	Quick acting
Most bacteria	All bacteria killed at same level of concentration
Most bacteria and viruses	Cheap; effective, somewhat corrosive to metal. Neutralized by soil, faeces, etc.
Most bacteria and viruses	Cheap, effective, somewhat corrosive to metal. Neutralized by soil, faeces, etc.
Most bacteria, including *Myco tuberculosis*, not spores or *Ps. pyocyaneus*	50 per cent cresol in soap solution. Good general disinfectant
Most bacteria, including *Myco tuberculosis*, not spores or *Ps. pyocyaneus*	General disinfectant. Compatible with soap
Most bacteria, including *Myco tuberculosis*, not spores or *Ps. pyocyaneus*	A bactericidal detergent for general use. Not to be mixed with other materials, e.g. soap
Most bacteria and viruses, including *Myco tuberculosis*, not spores or *Ps. pyocyaneus*	A bactericidal detergent for general use. Not to be mixed with other materials, e.g. soap. Low toxicity; useful for water bottles
Most bacteria and viruses, including *Myco tuberculosis*, not spores or *Ps. pyocyaneus*	A bactericidal detergent for general use. Not to be mixed with other materials, e.g. soap. Low toxicity; useful for water bottles
Most bacteria and some viruses	One of the most useful of the modern disinfectants. Low toxicity to animals

time. In concentrations practical for use (11 gm per cubic foot of air) a minimum of 6 hours is required to sterilize materials contaminated with bacterial spores.

Ethylene oxide does not readily penetrate thick layers of dirt, grease, or oil. It is active over a wide range of humidities.

Beta-propiolactone

The use of beta-propiolactone (B.P.L.) as a vapour disinfectant is relatively new. However, it has been found to be effective in the decontamination of rooms, buildings, and closed chambers in which airflow can be held to a minimum. The temperature and humidity requirement are the same as for formaldehyde.

Kelsey (personal communication, 1962) writes that beta-propiolactone is a carcinogen and, as such, this substance should be treated with the utmost respect.

Beta-propiolactone has the following advantages over formaldehyde as a vapour disinfectant:

1. It does not polymerize as readily on surfaces, so there is little or no residue.
2. It acts more rapidly. However, in the liquid state B.P.L. is more toxic than formaldehyde, and in handling care must be taken to see that it does not contact the skin.

Glick, Gremillion, and Bodner (*ibid.*) report that a vaporizer suitable for the dissemination of B.P.L. and also formaldehyde has been used successfully, 1 gallon of the lactone being vaporized for each 12,000 cu ft of space.

As with formaldehyde, the room or building need not be hermetically sealed and, after a waiting period of 2–3 hours, doors and windows can be opened and forced ventilation resumed. At this point entry into treated areas should be made with protective clothing and respiratory protection. After good airing for a further 3 hours normal entry can be made.

REFERENCES

1. GLICK, C. A., GREMILLION, C. G., and BODMER, G. A., *Proceedings of Animal Care Panel* (February 1961).
2. MONTHLY BULLETIN M.O.H. and P.H.L.S., *The Sterilization of Animal Cages by Steam*, **47**, 16, 64.
3. WENTWORTH CUMMING, C. N., 'The Commercial Production of Rats under S.P.F. Conditions', *Journal of Animal Technicians Association*, **12**, No. 4 (March 1962).
4. DARMADY, E. M. and BROCK, R. P., *Journal of Clinical Pathology*, **7**, 29 (1954).
5. SYKES, G., *Disinfection and Sterilization*. Spon (1958).
6. DARMADY, E. M., HUGHES, K. E. A., BURT, M. M., FREEMAN, B. M., and POWELL, D. B., 'Radiation Sterilization', *Journal of Clinical Pathology*, **14**, 55 (1961).
7. RIDEAL, S. and WALKER, J. T. A., *Journal of the Royal Sanitary Institute*, **24**, 424 (1903).
8. RIDEAL, S. and WALKER, J. T. A., *An Approved Technique of the Rideal Walker Test*. H. K. Lewis, London.
9. PERKINS, F. T. and SHORT, D. J., 'A New Technique in the Sterilization of Animal Houses, Racking, and Cages', *Journal of Animal Technicians Association*, **8**, No. 1 (1957).
10. STUART, ORTENZIS, and FRIEDL, *Journal of the Association of Agricultural Chemists*, Washington, **36**, 466 (1953).
11. FRISBY, B. R., 'Tego Compounds in Hospital Practice', *Lancet*, pp. 57–58 (July 1959).
12. CHARLTON, D. B. and LEVINE, M., *Journal of Bacteriology*, **30**, 163 (1935).
13. NORDGREN, G., *Acta Pathologica Microbiologica Scandinavica*, Suppl. 40 (1959).
14. GLICK, C. A., GREMILLION, C. G., and BODMER, G. A., *Proceedings of Animal Care Panel* (February 1961).
15. KELSEY, J. C., 'Sterilization by Ethylene Oxide', *Journal of Clinical Pathology*, **14**, 59 (1961).