ventilation by means of wire mesh windows (see Fig. 1). Spacing bars should be fixed to the top and sides of the box to prevent the ventilation areas being blocked during stowage.
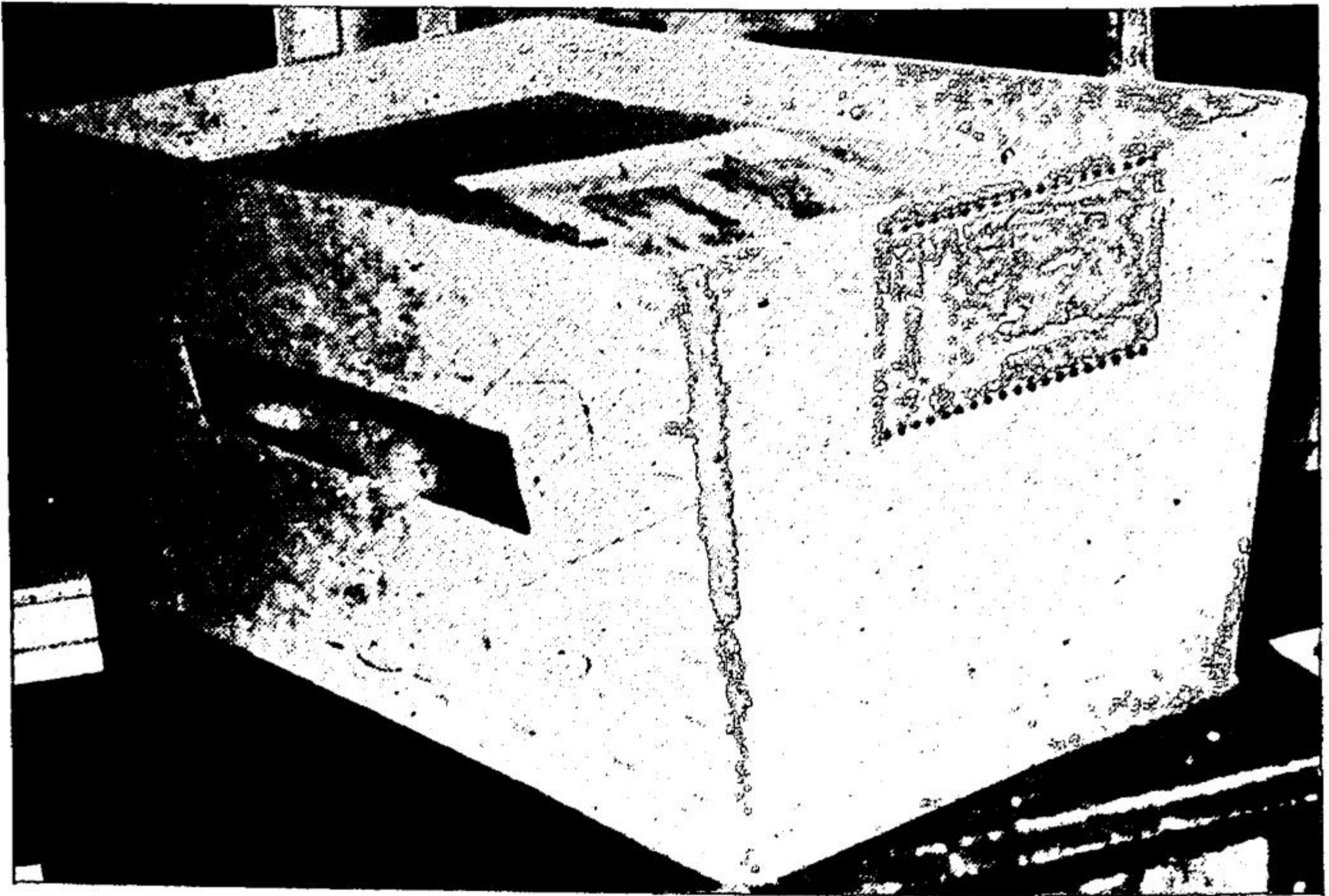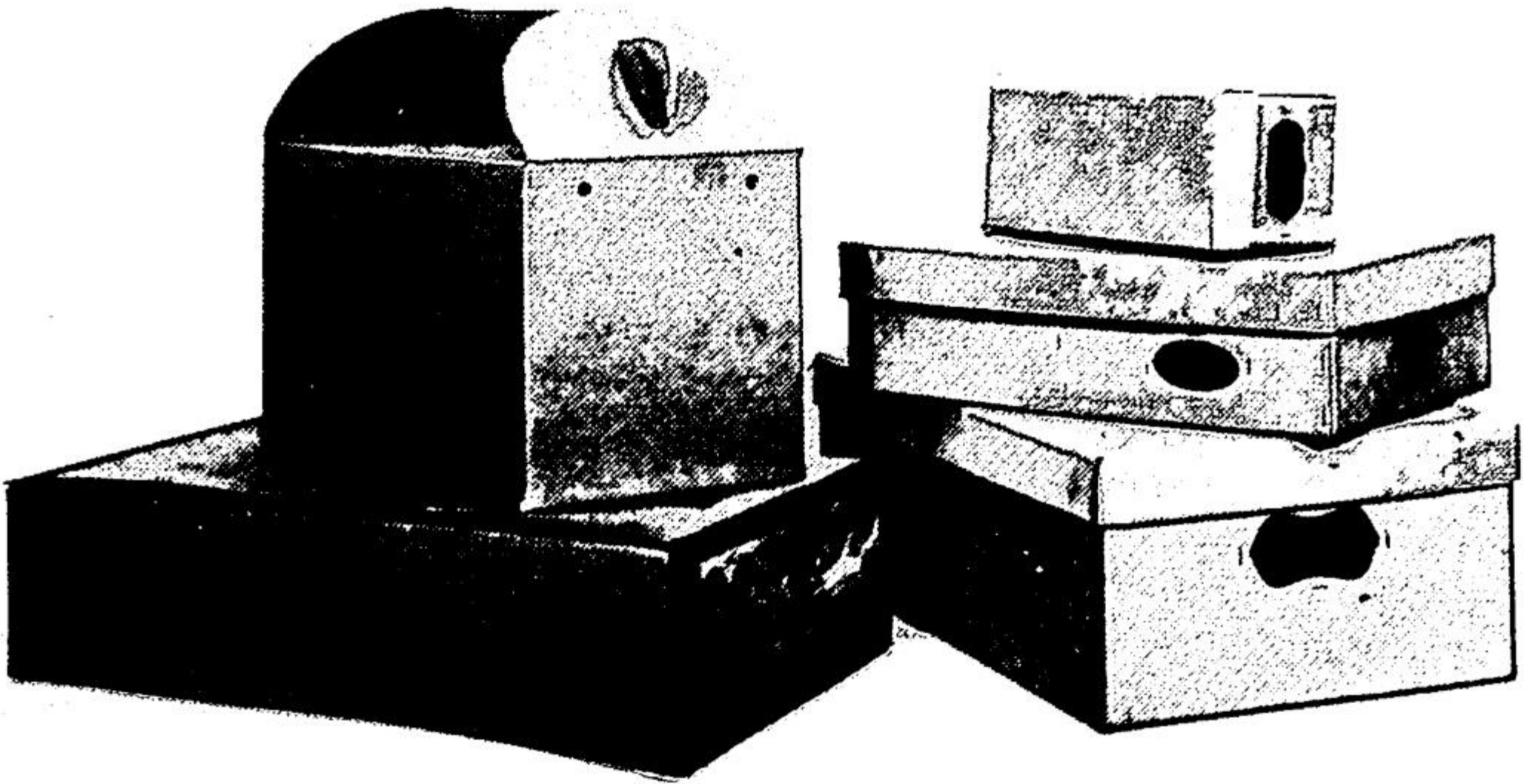

FIG. 1

For recommendations about travelling containers and the requirements of some transport authorities before packing animals, reference should be made to the density chart to choose the appropriate size of cage for the weight and number of animals.

Birds

LIVE POULTRY. The British Transport Commission rigidly enforces the following conditions for the packing of live poultry:

1. Crates must be substantially constructed and of a height and size reasonably sufficient for the type and number of birds. Maximum floor area of the crates must not exceed 24 sq ft, and any one compartment must not exceed 10 sq ft. The crates must be constructed in a manner which will prevent head, legs, and wings protruding through the top, bottom, or sides. Baskets covered with wire netting are permitted under the above conditions. Flimsy cardboard boxes are not acceptable, but the recognized pullet boxes are. If more than one species of bird is being dispatched in one crate the species must be in separate compartments.

2. If sent by passenger train delivery must be ensured within 30 hours.

3. *Feeding and watering.* Birds will suffer no hardship if they are deprived of food for up to 48 hours. Water must be given at intervals not exceeding 12 hours. Instructions to this effect should be printed on the crate. *Birds will not eat or drink in the dark.*

4. *Day-old chicks* should be packed in recognized day-old-chick containers. Chicks generate much heat, therefore adequate ventilation is necessary. However, they must not be left in draughts. They are liable to suffocate if the packages are stacked too high or too tightly. They can survive for 48 hours without food or water.

During cold weather transport should be in heated and well-ventilated vans.

Cats

Wicker hampers and wooden crates serve well as containers for cats. Crates should have solid floors, and the sides and top should be of 2-in. slats, $\frac{1}{2}$ in. thick and set $\frac{1}{2}$ in. apart. These crates may be divided into three separate compartments by using two movable wooden partitions. Drinking-vessels should be securely fixed in each compartment. Dimensions of crate or hamper 3 ft × 16 in. × 1 ft 3 in. high, as recommended by the RSPCA.

Dogs

Dogs may travel loose if they are provided with stout collars and chains and are securely muzzled. The labels must be attached to the collars beyond the reach of the animals.

Dogs may also be sent in well-ventilated crates with solid floors of the type described for cats. Drinking-vessels must be provided, because dogs must be watered every few hours.

Ferrets

Ferrets must travel in strong boxes or hampers from which they cannot escape. The lids must be firmly secured to prevent opening *en route*. Adequate ventilation must be provided. Ferrets will not be accepted for transport in Britain if the journey is to exceed 12 hours.

Frogs and Toads

A cage intended for the carriage of frogs and toads should be constructed of wood, hardboard, or plywood. The cage or box should be well ventilated and all ventilation holes covered by wire gauze or perforated metal sheet. The number per box or compartment should be kept to a minimum, because these animals pile on top of each other. Bedding: damp, rough, leaf-mould, in which the animals can bury themselves.

Goats

Goats may travel loose, under the same conditions as dogs. Kids should be sent in crates.

Guinea pigs

For short journeys stiff cardboard boxes (reinforced, if necessary) are suitable. These should have solid floors and tops and ventilation holes, 1 in. in diameter, on all sides.

For long journeys boxes should be constructed of hardboard, on a framework of 1-in. square timber, with solid floors and lids. The lids should be hinged and secured by a catch or a spring-loaded hook. Ventilation should be by 1-in. diameter holes on all sides, and a bar should be fixed around the box to prevent blocking of the ventilation holes; light metal boxes may also be used, but not in hot climates.

Hamsters

These may be packed in strong wooden boxes or metal tins with adequate ventilation (as for guinea pigs). The floors and lids should be solid.

Mice

For short journeys, stiff cardboard boxes with perforated metal windows inserted on all sides for ventilation are satisfactory.

For long journeys (over 6 hours) light timber boxes are preferable. The whole of one side of the box should be of perforated metal or wire scrim. Light metal boxes (e.g. half-size biscuit tins) with adequate ventilation holes on all sides are ideal for sending small numbers of mice by air.

Rabbits

Boxes should be constructed on a framework of 1-in. square wood. The floor, lid, and lower third of every side should be of hardboard or plywood, the remainder being covered with wire netting. The lid must be hinged and secured by a catch or lock-nut.

Rats

Pack as mice, but consult density chart.

Reptiles

All reptiles should, however, be kept at a temperature not lower than 60°F (15°C), and they should not be exposed to extremes of temperature. Boxes should be constructed of wood or plywood, and the ventilation holes should be covered with strong gauze or perforated metal sheet.
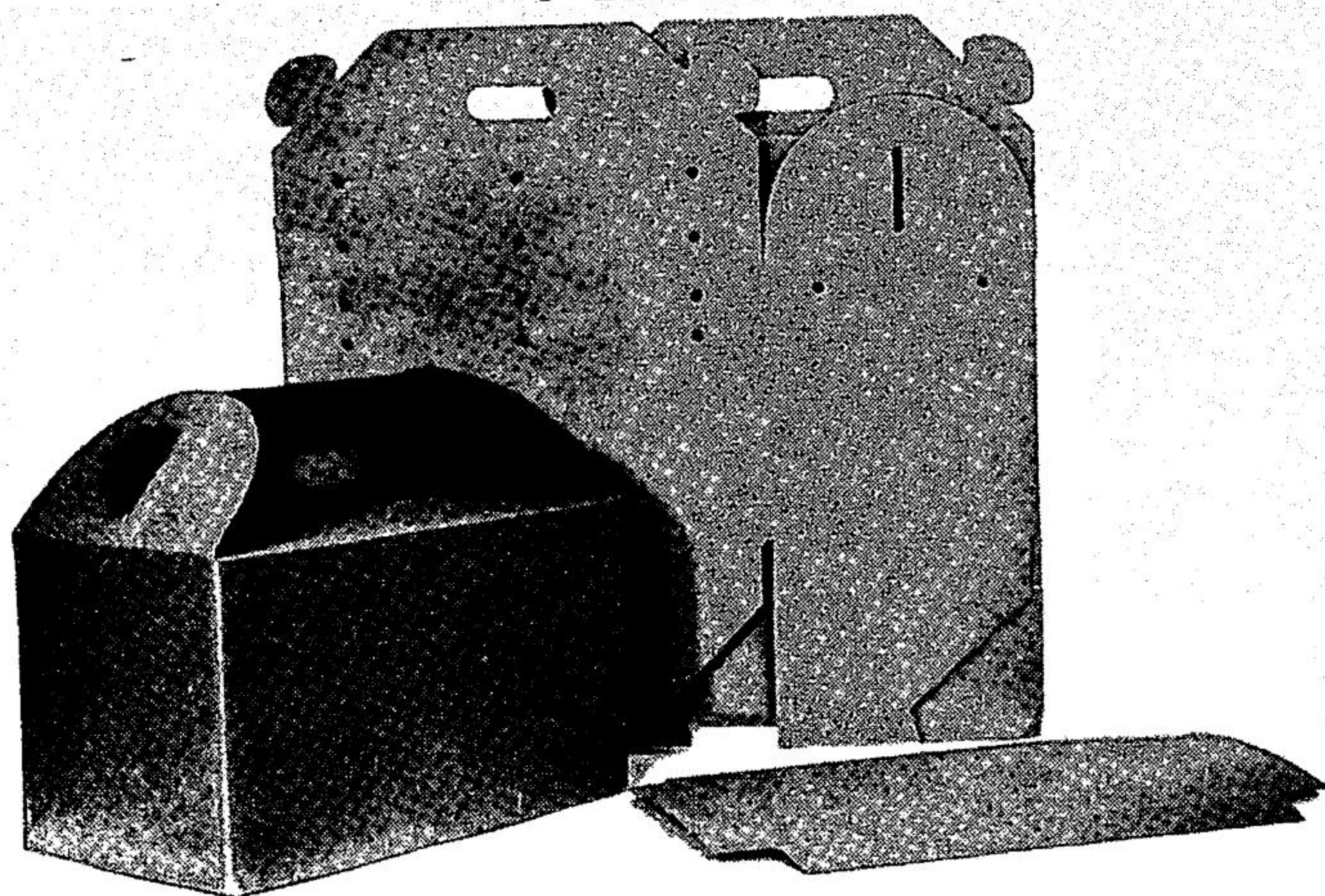
FIG. 2. An efficient cardboard travelling box for rabbits for journeys up to 12 hours.

Snakes should be packed singly in strong jute cloth bags. These bags should be slung inside a well-constructed box in such a way that the bags cannot touch each other or the sides of the box.

Each bag should be clearly labelled, stating the species it contains and whether or not the snake is venomous.

DENSITY CHART

Species	Weight of animal	Maximum number per compartment	Sq in. per animal (sq cm)	Height of box, in. (cm)
Guinea pigs	170–280 gm	12	14 (90)	6 (15)
	280–420 gm	12	25 (160)	6 (15)
	over 420 gm	12	36 (230)	6 (15)
Hamsters	Young	12	5 (32)	5 (13)
Mice	15–20 gm	25	3 (20)	5 (13)
	20–35 gm	25	4 (26)	5 (13)
Rabbits	Under 2·5 kg	4	120 (770)	8 (20)
	2·5 kg	2	150–180 (970–1160)	10 (25)
	Over 5·0 kg	1	220 (1400)	12 (30)
Rats	35–50 gm	25	6 (40)	5 (13)
	50–150 gm	25	8 (52)	5 (13)
	Adult	12	16 (100)	5 (13)

H

For further information on the transport of reptiles see British Standards *Recommendations for the Carriage of Live Animals by Air*, Part 6.

Food and water

All animals should have access to food and water as late as possible before dispatch.

Food in the form of grains, pellets, or cubes, sufficient for the journey, should be placed in the travelling box.

Moisture must be provided, but preferably not in the form of a wet mash, which quickly becomes soiled and unpalatable. Rats, mice, hamsters, and guinea pigs will travel well if moisture is supplied by succulents, e.g. apples, beetroot, raw potatoes, raw carrots, or grapes; in this case water bottles or other containers are necessary.

Water bottles invariably leak because of jolting in transit. Water bottles are suitable for use only during resting stages in the journey, and they should be provided by the sender, together with full instructions for their use.

However, for journeys of not more than five days the majority of rodents will obtain all their water requirements from succulents packed with them, and the need for watering will not arise. If the consignor wishes his animals to be watered on the journey he must provide a suitable utensil—bottle, dish, etc.— *and full instructions on the container*. Otherwise the label should state specifically that no food/water is required and should not be given.

Bedding

The floors of all types of containers for rodents must be covered with fine softwood sawdust, granulated peat moss, or fine silver sand to a depth of 1 in.

Rabbits, guinea pigs, and hamsters should be provided with a liberal supply of hay or shredded paper. Travelling boxes containing rodents should be almost filled with bedding through which the animals can burrow and remain dispersed throughout the box.

Adequate bedding must be provided for the following reasons:

1. For the comfort of the animal.
2. To absorb moisture.
3. To protect against shock, which is unavoidable even with the best handling.
4. To insulate against temperature fluctuations.
5. To keep the animals dispersed throughout the container.

Labels

All boxes must be well and clearly marked with the following information:

(a) Name, address, and telephone number of the consignor.
(b) Name, address, and telephone number of the consignee.
(c) The word LIVESTOCK in bold red letters.
(d) Label—'Box contains rodents: *Please do not open*'.
(e) Instructions for feeding and watering, if necessary, or instructions 'Do *not* feed or water'.
(f) Instructions on the care of the animals in the event of delays *en route*.
(g) Label marked 'To be called for'.

DOCUMENTATION FOR EXPORT

(i) Clearance certificates must be obtained from the recipients of the animals, stating that the livestock will be admitted subject to local regulations.

(ii) Transport arrangements should be made with one of the recognized shipping agents or with the commercial attaché of the receiving country. Livestock should be delivered to the airport or to the agent's depot. The recipient must be informed of the flight number, time of departure, and time of arrival at destination, so that arrangements can be made for collection from the receiving airport.

(iii) Veterinary certificates must be provided in triplicate, signed by a veterinary surgeon whose signature is acceptable by the recipient's Government.

One copy of the veterinary certificate (clearly marked) must be fixed in a prominent position on the travelling box. Two copies must be handed to the shipping agent or airport official.

GENERAL CARE DURING TRAVEL, AT LOADING, AT INTERMEDIATE STOPS AND AT UNLOADING

1. Animals should be kept under cover at all times and should be protected from sudden and drastic changes of temperature, strong draughts, rain, strong sunlight, noise, and rough handling.

2. Animals should not be left on railway platforms, airport aprons, or in general merchandise goods sheds, but should be removed to a warm room (or a cool room, in the tropics). Animals should not be placed against hot pipes, open fires, or radiators.

3. Loading and unloading should be quick and efficient, and be done immediately the animals arrive.

Customs authorities and transport personnel are usually very helpful about livestock and will, if asked, give clearance priority to consignments of animals and permit the consignee's vehicle on to the airport tarmac to collect animals.

4. Livestock which suffers unexpected delay in the course of a journey (e.g. through the grounding of aircraft) should be handed over to a person competent to feed, water, and repack, if necessary, according to instructions on the box. Instructions relating to the consignment must be strictly adhered to, because some animals may bite or be dangerous to handle, and others will endeavour to escape.

The consignee should be fully informed of any delay and the new time of arrival.

Animals should not be reshipped before notifying a qualified person, who should either inspect them or give instructions for their care.

Summary

The successful transport of livestock is dependent on the following:

    The consignor packing the animals well.

    The consignee collecting the animals immediately on their arrival.

    The transport personnel treating all livestock as urgent and valuable cargo.

PROHIBITED SPECIES AND ITEMS

Certain countries prohibit the entry of wood and wood by-products. There are other countries who prohibit the entry of cereals, seeds, straws, dried grass, and herbage. A number of species of rodent are on the prohibited list in some countries.

Dogs and cats imported into England require a licence, and are subject to six months quarantine.

Only two mammals are at present barred entry into Great Britain: the grey squirrel and the musk rat.

When in doubt regarding import/export regulations write to Ministry of Agriculture & Fisheries, Hook Rise, Tolworth, Surbiton, Surrey.

Full information regarding these species and items may be obtained on application to the Natural Science Division of UNESCO, 9 Place de Fontency, Paris 7e, France.

The following table is appended as a guide, and is reproduced by permission of the Editor of the *Journal of Animal Technicians Association*.

The Table refers to exports from the United Kingdom. The Import permit and Quarantine on arrival columns refer to the receiving country only.

Country	Livestock	Export Licence required	Import permit required	Quarantine on arrival	Remarks
Australia	Dogs Cats, Rats and mice Birds*	No No No No	No No Yes Yes	Yes Yes	Special permit required for Alsatian dogs
Canada	Dogs Cats Rats and mice Birds*	No No No No	No No No	No No No No	
India	Dogs Cats Rats and mice Birds*	No No No No	No No No No	No No No No	
Kenya	Dogs Cats Rats and mice Birds*	No No No No	Yes Yes No	No No No	Larger birds must have had blood test for Bacillary White Diarrhoea and show negative T.B.
New Zealand	Dogs Cats Rats and mice Birds*	No No No No	No Yes Yes Yes	Yes Yes	Cats must be free from ticks. Special permit required for rabbits

* Birds—excluding table poultry, game, and parrots.

Country	Livestock	Export Licence required	Import permit required	Quarantine on arrival	Remarks
South Africa	Dogs Cats Rats and mice Birds*	No No No No	Yes Yes Yes Yes	No No	Birds not permitted entry if sent from districts in the United Kingdom where proved cases of equine encephalomyelitis have been found
United States	Dogs Cats Rats and mice Birds*	No No No No	No No Yes No	No No No	Shepherd dogs quarantined and inspected for tapeworm. Alexandrian rat prohibited
Denmark	Dogs Cats Rats and mice Birds*	No No No No	No No Yes	No No	
Sweden	Dogs Cats Rats and mice Birds*	No No No No	Yes Yes Yes Yes	Yes	Dogs subjected to blood test against leptospirae. Musk rat prohibited

* Birds—excluding table poultry, game, and parrots.

4th March 1957

CERTIFICATE OF HEALTH

I hereby certify that I have today examined 400 mice and have found them to be free from any visible signs of disease.

(*signed*) A. N. Other,

M.R.C.V.S.

APPENDIX A

Medical Research Council recommendations on the humane shipment of monkeys by air

I GENERAL REQUIREMENTS

1. The duration of time in transit should be as short as possible.
2. Factors causing stress to monkeys should be reduced as much as possible.
3. Monkeys under six months of age should not be transported by air (a guide is given in Appendix B on estimating the age of monkeys by weight).
4. Monkeys in the same cage should be approximately the same weight.
5. Pregnant monkeys should not be shipped except when specifically requested by the importer.
6. Pregnant monkeys and monkeys over 10 lb (4·5 kg) in weight should be shipped in cages specially approved for this purpose.
7. When possible, only monkeys of the same species should be shipped in the same

aircraft. It is most desirable that no other species of animal or bird are carried at the same time.

8. At no time during transit should monkeys be left unattended. At least one animal handler should be present at all times when the plane is on the ground.

II TRAVELLING CAGES

1. The type of travelling cage recommended and the containers for food and water are shown in the diagram in Appendix A.

2. A cage of the type shown in the Appendix must not contain more than twelve monkeys.

3. The total weight of monkeys in one cage must not exceed 50 lb (23 kg).

4. No individual monkey in this cage shall weigh more than 10 lb (4·5 kg).

III CERTIFICATE OF FITNESS

A certificate of fitness, on the prescribed form (see Appendix C), must accompany each consignment of monkeys. In the absence of such a certificate the captain of the aircraft will not accept the consignment for shipment.

The certificate must be signed by a person whose qualifications and experience are acceptable to the Government of the exporting country, to the shippers, and to the consignee.

IV CARE IN FLIGHT

1. Ventilation, temperature, and light

(*a*) A minimum of twelve changes of air per hour is required.

(*b*) Draughts are to be avoided.

(*c*) There should be no 'dead' pockets of air.

(*d*) The temperature considered optimum is 75°F (23·9°C) (sixteen air changes per hour). Maximum 80°F (26·7°C) (twenty air changes per hour). Minimum 65°F (18·3°C) (twelve air changes per hour). The variation in temperature should be no greater than 1°F (0·6°C) every 5 minutes, and extremes of temperature should be avoided.

(*e*) Humidity should be kept as low as possible during shipment.

(*f*) Except when monkeys are being fed and watered, it is better that they should travel in semi-darkness. This will make the monkeys quieter and less inclined to fight and give them better opportunities for resting.

2. Food (Food container—see Appendix A)

A monkey can comfortably go without food for 24 hours if it has been fed regularly during the quarantine period before shipment.

If the period from the scheduled time of departure is longer than 24 hours the animals must be fed at the expiration of this period and thereafter at intervals of 12 hours. A sufficient stock of food must be available on the plane and at likely stopping-places for this purpose. Three ounces (85 gm) of food per monkey is required daily. The food should consist of dry cereal grain or gram. Fruit and vegetables should not be offered immediately before or during shipment, as experience has shown that this is liable to upset the monkeys.

3. Water (Water container—see Appendix A)

Monkeys must be watered not less than every 6 hours. The water supplied must be fit for human consumption and piped from tanks within the aircraft. Manifold taps from a pipe running along the roof of the aircraft must be suitably placed (e.g. at 10-ft intervals) so that a movable tap can be used to fill the water containers in the cages.

A minimum of ¼ pint (142 ml) of water must be allowed for each monkey daily, i.e. 50 gallons (approximately 227 litres) for 1,600 monkeys.

4. Sickness and injury of monkeys

Wherever feasible, injured and dead monkeys should be removed. Injured monkeys should be put into a spare cage kept for this purpose and dead ones into impervious disposable bags. Tongs should be provided for handling dead monkeys. Sick animals should not be removed from their travelling cages.

Experience has shown that the application of dressings and administration of medicines to individual monkeys during flight is of little value.

V HYGIENE

It is desirable that any aircraft carrying monkeys be kept as tidy as possible during the flight. Cage trays must be cleaned not less than once every 24 hours and the refuse put into the impervious disposable bags which must be provided.

VI LOADING, INTERMEDIATE STOPS, AND UNLOADING

The conditions required during the flight are required also after the aircraft has landed at intermediate stops and at destination. In addition, it is considered necessary that:

1. The monkeys should be under cover from the time of leaving the collecting unit.
2. Loading and unloading should be carried out quickly and efficiently. A roller conveyor with a canopy could be used for this purpose.
3. The stowing and lashing of cages should be supervised by the senior animal handler.
4. Every effort should be made to avoid subjecting the monkeys to extremes of temperature, draughts, etc.
5. On arrival at destination the Customs authorities should be asked to give clearance priority to shipments of monkeys.
6. Airport authorities should allow the consignee's vehicles on to the tarmac to collect monkeys on arrival.

VII ANIMAL HANDLERS

There should be an approved list of animal handlers. Facilities can be offered by the importing organization for training handlers in the proper care of monkeys. Every approved handler will sign a statement certifying that he has received a copy of the M.R.C. recommendations and has read them.
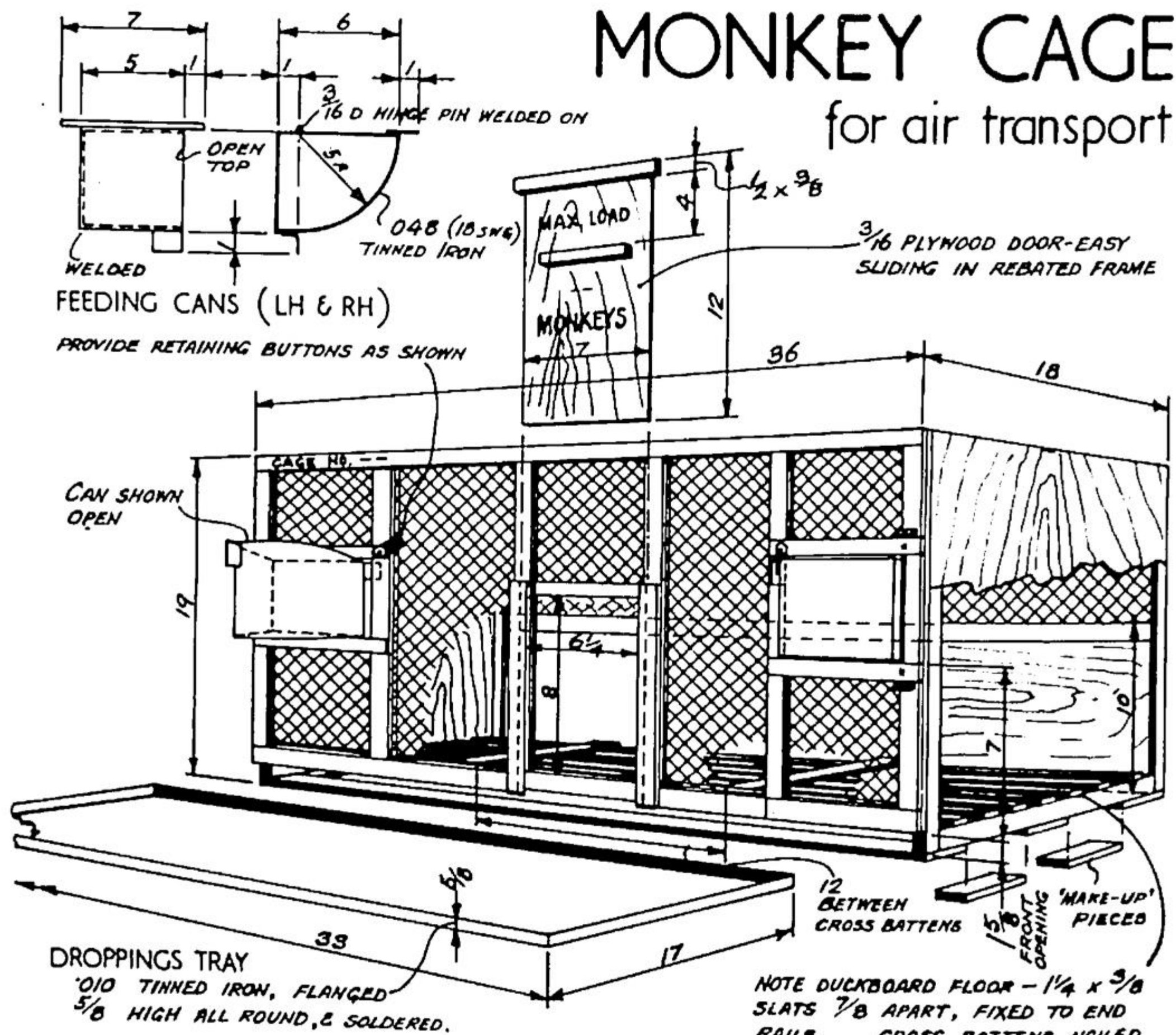
Medical attention

Prior to approval, animal handlers will be required to produce evidence of physical fitness and good health, including freedom from active tuberculosis. All animal handlers should receive six-monthly chest X-ray examination, and tuberculin-negative personnel should be retested for tuberculin conversion at six-monthly intervals. In the absence of anti-tuberculosis vaccination, tuberculin convertees will be laid off duty until further evidence of freedom from active tuberculosis has been produced. It is advisable that protection against tetanus and the enteric group of fevers should be offered to all animal handlers as a routine.

Note: 'Carriage of Live Animals by Air', 'Monkeys for Laboratory Use' **B.S. 3149:** Part 1, 1959 (Available from British Standards Institution, 2 Park Street, London, W.1. Price 4*s*.).

MONKEY CAGE
for air transport

FEEDING CANS (LH & RH)

7
5
6
3/16 D HINGE PIN WELDED ON
OPEN TOP
5/8
·048 (18 SWG) TINNED IRON
WELDED

PROVIDE RETAINING BUTTONS AS SHOWN

MAX LOAD
MONKEYS
7
1/2 × 9/8
4
12
36
18

3/16 PLYWOOD DOOR-EASY SLIDING IN REBATED FRAME

CAN SHOWN OPEN

CAGE NO.
19
6 1/4
8
7
10

DROPPINGS TRAY
·010 TINNED IRON, FLANGED
5/8 HIGH ALL ROUND, & SOLDERED.

33
6/8
17

12 BETWEEN CROSS BATTENS
5/8 FRONT OPENING
'MAKE-UP' PIECES

NOTE DUCKBOARD FLOOR — 1 1/4 × 3/8 SLATS 7/8 APART, FIXED TO END RAILS. CROSS BATTENS NAILED TO SLATS AND FIXED AT FRONT & REAR. NAILS MUST NOT PROJECT THROUGH, OR MUST BE TURNED OVER.

EXPLODED VIEW – ONE END PARTLY CUT AWAY

36 WIDE × 1/2 × ·028 GALVANISED IRON WIRE MESH (IN ONE LENGTH) TO BE TRAPPED UNDER 1 × 3/16 WOOD SLATS NAILED ONTO RAILS. NAILS OR WIRE ENDS MUST NOT PROJECT INSIDE CAGE.
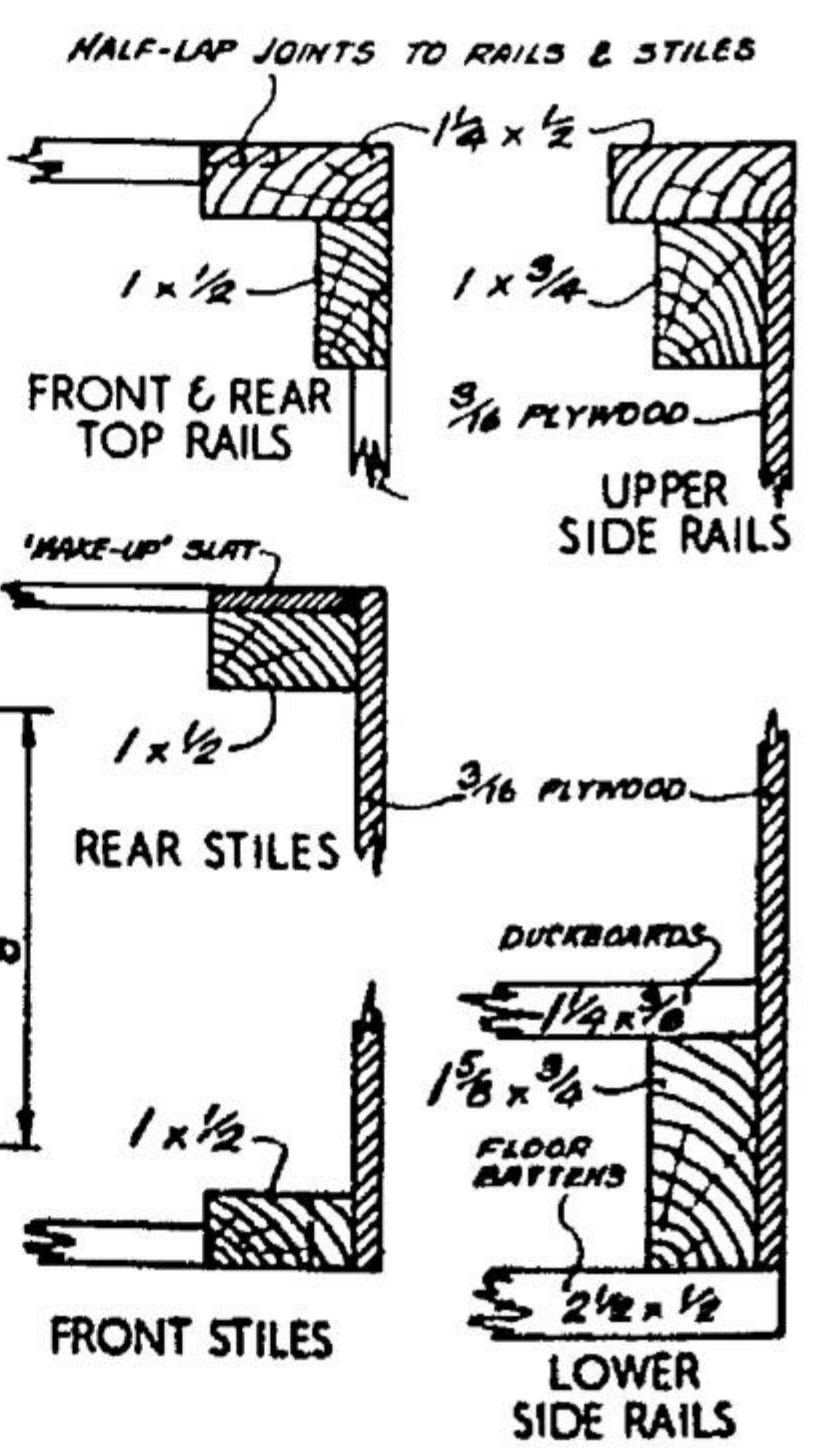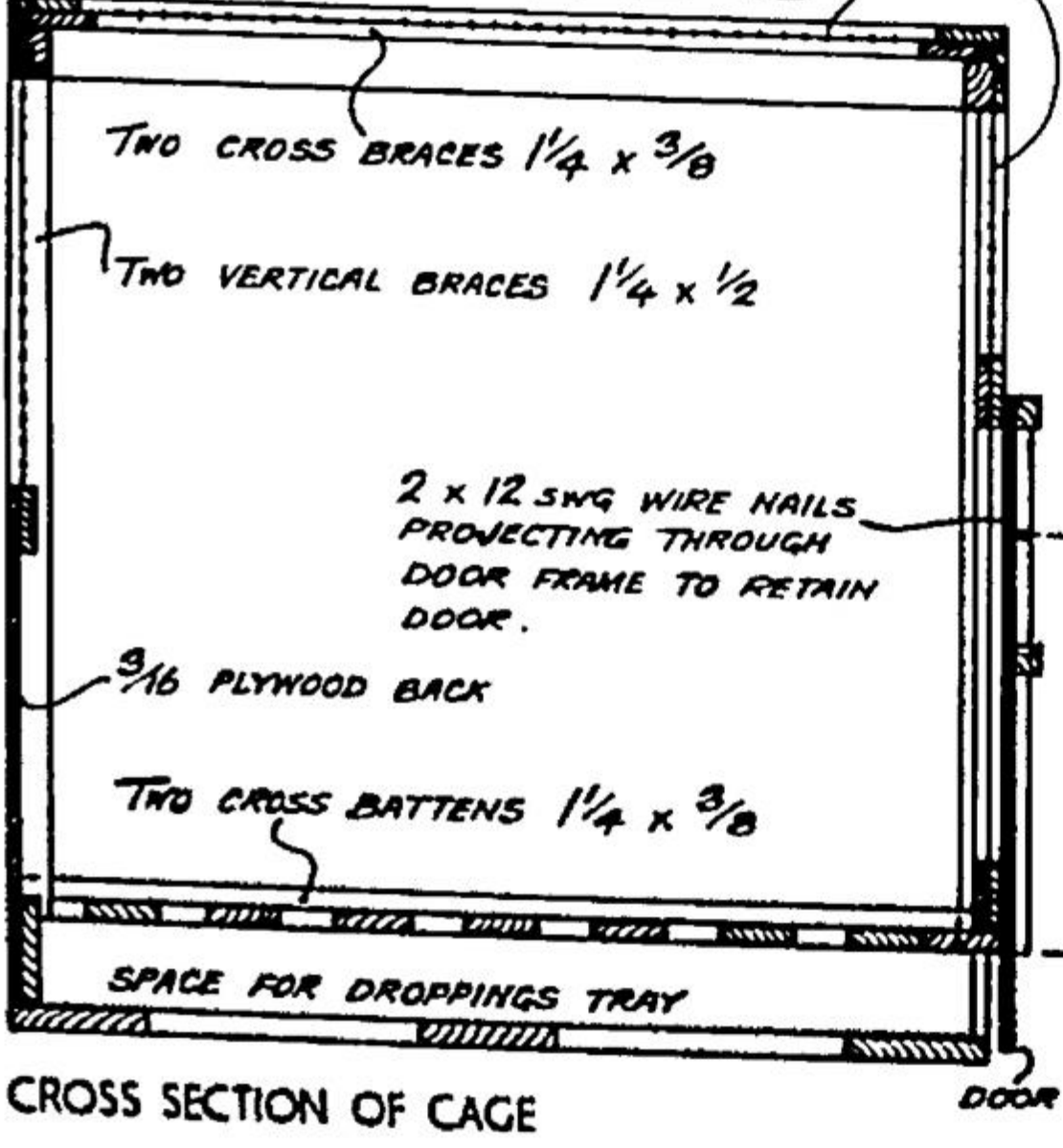
TWO CROSS BRACES 1 1/4 × 3/8

TWO VERTICAL BRACES 1 1/4 × 1/2

2 × 12 SWG WIRE NAILS PROJECTING THROUGH DOOR FRAME TO RETAIN DOOR.

3/16 PLYWOOD BACK

TWO CROSS BATTENS 1 1/4 × 3/8

SPACE FOR DROPPINGS TRAY

CROSS SECTION OF CAGE

DOOR

HALF-LAP JOINTS TO RAILS & STILES

1 1/4 × 1/2
1 × 1/2
1 × 3/4
3/16 PLYWOOD

FRONT & REAR TOP RAILS

UPPER SIDE RAILS

'MAKE-UP' SLAT
1 × 1/2
3/16 PLYWOOD

REAR STILES

8

DUCKBOARDS
1 1/4 × 3/8
1 5/8 × 3/4
FLOOR BATTENS

1 × 1/2

FRONT STILES

2 1/2 × 1/2

LOWER SIDE RAILS

Fig. 3

APPENDIX B

Veterinary Certificate of Fitness (Monkeys)

This certificate is to be completed and signed by a duly authorized person in the country of export in respect of all shipments of monkeys consigned to or transported via the United Kingdom. Attention is drawn to the Recommendations of the Medical Research Council on the Humane Shipment of Monkeys by Air (revised 1959).

Date and time of examination ....

Number of cages ....   Species ....

Number of animals ....   Species ....

Shipped from ....   To ....Via ....

    I hereby certify that I have read the M.R.C. recommendation *Humane Shipment of Monkeys by Air* (revised 1959).

(1) That, at the request of (consignor) ....

....

    I examined the above-mentioned animals in their travelling cages not more than 12 hours before their departure.

(2) That they appeared to be in good health and were free from signs of contagious and infectious disease.

(3) That no animal was under 6 months of age, and that no animal appeared to be pregnant.*

(4) That they were adequately fed and watered within 2 hours of scheduled time of departure.

(5) That the cages were packed and loaded in accordance with the regulations and recommendations of the country of export and of the carrier.

        Signed ....

        Address ....

        Qualifications ....

        Authorized by the Government of ....

* Pregnant monkeys may be shipped provided:

    1. That the importer has made a special request for pregnant monkeys.

    2. That such monkeys are individually caged.

APPENDIX C

Livestock Progress Report

Service No. ....   Captain ....
                                                (on arrival in London)

Aircraft Registration ....   Animal handlers ....

Date of Departure: London...........   Arrival: Singapore ....

              •   Singapore ....   London ....

Consignment No. (most important)	Cage No.	Number of animals or birds dead	Notes

Were the M.R.C. recommendations observed? If not, in what respects were they not observed? ....

Signature of Senior Handler...................................

NOTES TO ANIMAL HANDLERS:
   (*to be completed by BOAC*)

PART TWO

THE RECEPTION OF ANIMALS FROM OUTSIDE SOURCES

The most important point to remember when receiving animals from outside sources is that they are NOT NORMAL ANIMALS. This problem, often discussed, but about which little is published, is the condition of animals when reaching their destination. All animals sent on a journey of any length from 100 yards to 500 miles, are exposed to elements which effect them:

> Change of surroundings
> Change of temperature
> Change of food
> Overcrowding
> Draughts
> Change of personnel
> Noise
> Exposure to infection

It is important that all newly received animals should be inspected immediately on arrival, by a competent person who could recognize anything out of the normal and deal with it.

For economy, animals are somewhat crowded when packed for travel, and on arrival want special attention, and the very best of care.

(i) ALL ANIMALS RECEIVED from outside sources should be put into quarantine for at least 14 days. Isolated quarantine areas for holding animals from outside sources should be clearly designated as quarantine space, with transfer of personnel, animals, or equipment from this area to the disease-free animal building strictly prohibited. Distance alone does not provide sufficient isolation to prevent the spread of infection from the quarantined newly received animal to the normal unit. All such rooms should be fitted with its own set of equipment, its own hot- and cold-water supply, and controlled heating 72–75°F (22·2–23·9°C) for rats and mice, 60–65°F (15·6–18·3°C) for cavies.

(ii) ROUTINE HYGIENE METHODS. All personnel must change their coat and wash their hands before handling the animals, and wash their hands after they have finished.

(iii) OVERCROWDING usually proves disastrous. A drop in room temperature or fright will cause them to huddle in one corner. Those at the bottom will invariably smother. Unless adequate drinking facilities are provided, some will be without water, with a resulting high rate of dehydration; this is particularly true of mice, rats, and cavies. This factor may cause animals to crowd round watering devices; the remedy for this is to provide more water sources during

113

the first few hours. The following table will give some idea of the number of animals that should be put together with safety:

Mice	15–20
Rats	6–10
Cavies	4–6
Rabbits	Singly
Cats	Singly
Monkeys	Singly
Dogs	Singly

DIET

Too much emphasis cannot be placed on the subject of diet, where an institute is exposed to animals coming from various sources. It is most important to know what diet the animals have been fed on. The growth curve of immature mice, rats, and rabbits, when exposed to a sudden change of diet, will be found to drop and gradually level off before reaching a normal climb. This is particularly serious where immature mice are involved which are purchased regularly for routine use. Their bodyweight will not correspond with their age in comparison with normal growth. A percentage seldom respond and will eventually die. It is advisable in the case of rats, mice, guinea pigs, and hamsters to restrict the diet to one-third of the daily ration in the first day of arrival, two-thirds on the second, and full rations on the third day.

All boxes, crates, and baskets in which the animals are received should be suspected of carrying infection and infested with pests of the animal house. As soon as the animals are removed, the containers should be burnt or sterilized in a way best suited to the establishment, but on no account should it be delayed; if this is impossible at the time, then the container should be put in the open and not left in the quarantine.

Animals from breeders are, generally speaking, an advertisement for the breeder, who will take steps to ensure that his animals are in good condition when he sends them to the purchaser.

Animals from a dealer are always suspect; they may have changed hands a number of times, and consequently their condition and resistance to disease is lowered.

Dealers are in the game from a purely profit motive, and the care and conditions under which they house the animals leaves much to be desired.

Rabbits

On arrival rabbits should be examined for symptoms of diarrhoea, nasal discharge, ear canker, sore hocks, and injury. Coccidiosis is found in the majority of domestic rabbit colonies, and rabbits so infected frequently develop severe diarrhoea when exposed to changes in diet and the stress of travelling. Rapid diagnosis and quick treatment are very necessary in these cases.

Cats

Upon arrival cats should receive a prophylactic injection of infective feline-enteritis antiserum. For cats that are to remain for long periods the appro-

priate doses of feline-enteritis vaccine should be given. Cats procured from outside sources have usually been exposed to numerous infections, and since they are highly susceptible to respiratory diseases, prompt diagnosis, treatment, and isolation is important. Routine faecal examination should be carried out, and should parasite ova be found, the infested animal must be treated with the appropriate vermifuge (see chapter on Diseases), and if necessary, action should be taken against the intermediate host. All newly arrived cats should be inspected for lice, fleas, and mites.

Dogs

When procured from breeders or dealers dogs will have been exposed to stress, changes in diet, and numerous infections; they should be quarantined as infectious animals and carefully observed. Prophylactic treatment with antisera and vaccines as well as appropriate vermifuges are recommended for all long-term experimental dogs (see chapter on Diseases). A dog should be quarantined for at least a fortnight, preferably in solitude for the first few days until it settles down. Make friends with the dog and encourage it to take its meals; if necessary, tempting it to eat by offering a variety of foodstuffs. During the quarantine period the dog should be examined for parasites. Fleas, lice, and mites may be treated by the application of Lorexane. Dogs may be bathed at any age, but only white Windsor soap (or a proprietary dog shampoo) should be used, and the animals should be dried thoroughly so that they do not get chilled.

Dogs may be infected with roundworms or tapeworms, both of which can be seen with the naked eye. Roundworms look like anything from shiny pieces of watch spring to lengths of spaghetti, and are white or dirty pink in colour. They usually cause vomiting and worms may be found in the vomit, and, occasionally, in the faeces. Tapeworm segments look like semi-transparent pieces of ribbon. Both types of worm may be treated with proprietary tablets.

To prevent reinfection with worms, wild rodents must be kept out of the kennels and fleas must be eliminated from the animals and rooms. All dogs and puppies on long-term studies will have to have vaccines to protect them from distemper, hardpad, and canine hepatitis. A combined vaccine is available.

Monkeys

Before newly arrived monkeys may be handled it is vital that the handler be fully protected with gloves, face mask, and protective clothing. These precautions are necessary because of the B. Virus infection of monkeys which can be transmitted to man, to whom it is fatal. The B. virus was first isolated by Sabin and Wright[1] in 1932 from the central nervous system of a laboratory worker who had died after being bitten by a monkey.

This infection is the greatest hazard to monkey-house personnel, since there have been at least twelve authenticated fatal human infections, most of them in the last three or four years. The disease is carried in the mouth of the monkey, and the most easily recognized gross lesions are formed on the surface of the tongue and on the lips.

It is well to remember that monkeys arriving at the laboratory are in relatively poor condition after having been subjected to extensive environmental

Common Diseases of Laboratory Animals

MICE

BACTERIAL

Salmonellosis (mouse typhoid)

CAUSAL AGENTS. Bacteria of the Salmonella genus: *Salmonella typhi-murium*, *Salmonella enteritidis*, and more rarely other members of this genus.

SYMPTOMS. The acute form is characterized by rapid loss of condition, sometimes followed by purulent conjunctivitis and/or diarrhoea. Death occurs within seven to fourteen days of exposure.

The chronic form probably follows a sub-acute stage which passes unnoticed. The animal progressively loses condition as infection spreads. Clinical recovery may occur without treatment, though the animal can continue to excrete Salmonella organisms in the faeces for a long period.

POST-MORTEM. In the acute stage the animal may only show a generalized inflammation of the internal tissues. The more chronic the infection, the greater is the degree of involvement of the body tissues. The following lesions occur—peritonitis with excess peritoneal fluid; enlargement of the mesenteric and colonic lymph glands; hepatic enlargement, congestion, and necrosis; liver surface studded with small microscopic foci; splenic enlargement with thickening of the capsule. Sometimes the lungs are consolidated and there is excessive pleural exudate. Inflammation of the gut and hypertrophy of Peyer's patches.

DIAGNOSIS. During life the causal organism can be cultured from the faeces by the methods described for Salmonellosis in the guinea pig. These methods are also used for the examination of post-mortem materials.

ROUTE OF INFECTION. Probably by the oral route, though it may be that the conjunctiva is also a portal of entry.

SOURCE OF INFECTION. Investigations over the past few years have revealed the ubiquitous nature of the Salmonella, and many sources of the infection are possible.

Food and bedding may be contaminated by the excreta of domestic and wild-animal carriers or actual cases of Salmonellosis. Outbreaks of Salmonellosis have also often followed fresh importations from infected carrier stocks.

CONTROL. The following points may be made:

(*a*) Mice for breeding purposes obtained from an outside source should never be introduced into the existing colonies without first undergoing a period of quarantine of at least fourteen days. During this time it is worthwhile, should the facilities exist, to test faecal pellets from each animal for the presence of Salmonella. If these organisms are isolated, then the whole consignment should be destroyed and the equipment and quarantine room efficiently sterilized.

(*b*) If the disease is detected in an established colony, then eradication measures will depend on the system of management. For example, if, of a number of well-isolated rooms, only one is affected, then it may be sufficient to sacrifice the animals in that room and carry out sterilization procedures. However, should overt cases and carriers disperse throughout the stocks, eradication of the disease may involve destruction of all animals. After slaughter, equipment, rooms, etc., must be sterilized. Salmonella eradication is possible provided the methods used are sufficient. No lasting benefit is achieved by vaccines, drugs, or antibiotics.

Tyzzer's disease

CAUSAL AGENT. This disease is usually attributed to a gram negative bacillus designated *Bacillus piliformis*. The taxonomic position of this organism and its exact role in the aetiology of Tyzzer's disease is obscure because the organisms cannot be cultivated on laboratory media, nor will tissue suspensions containing it readily produce the disease.

SYMPTOMS. There is a loss of weight and condition and an associated diarrhoea; death is common.

POST-MORTEM. The liver is studded with 'target-like' white and yellow necrotic foci. These tend to remain discrete. The gut may be inflamed, particularly at the ileo-caecal junction.

DIAGNOSIS. The typical appearance of the hepatic foci is usually sufficient for diagnosis. The slender gram negative bacilli can readily be seen in stained sections of the liver and the affected areas of the gut. The macroscopic lesions can be confused with those caused by Salmonella or Corynebacterium, but can be differentiated by successful cultivation of causative bacteria in these cases.

ROUTE OF INFECTION. Probably oral; mice have also been artificially infected by the intravenous injection of infected material.

SOURCE OF INFECTION. This organism is apparently host-specific. Infection, when it occurs, is usually endemic, with occasional outbreaks of epidemic disease. Once introduced, faulty management, such as overcrowding, favours epidemics, while with good management few cases of overt disease appear. Possibly wild mice are sometimes responsible for fresh introductions of the disease.

CONTROL. Mice with diarrhoea should be killed and the liver and gut carefully examined. In-contacts should be sacrificed, equipment sterilized, and the bed-

ding destroyed. The author has experience of a colony in which endemic infection disappeared after the introduction of sterilized bedding in place of bedding known to be liable to contamination with the faeces of wild mice.

Arthritis (mouse rheumatism).

CAUSAL AGENT. *Streptobacillus moniliformis* (*Actinomyces muris*): a gram negative bacillus.

SYMPTOMS. Acute: the animal loses weight and condition, there is an accompanying semi-purulent conjunctivitis and occlusion of the palpebral fissures. Death frequently ensues within a few days of infection.

Chronic: a more characteristic syndrome is observed. Weight and condition are progressively lost. There is conjunctivitis and keratitis. The legs and feet become oedematous, ankylosed, and arthritic, and they may ulcerate; the tail may be similarly affected. Subcutaneous nodules may be found, and there is sometimes enlargement of the inguinal and maxillary glands.

POST-MORTEM. The liver and spleen are enlarged and show areas of necrosis, which may become confluent. There is necrosis affecting the splenic pulp. Infection of the heart is not uncommon, e.g. pericarditis and endocarditis. The lymph glands are enlarged, and examination of the joints reveals disintegration with caseous exudation.

DIAGNOSIS. This is based on clinical symptoms, post-mortem findings, and the isolation of the causal organism by cultivation on a medium containing a high proportion of serum.

ROUTE OF INFECTION. Not definitely known; could possibly be by bites or through the conjunctiva. Feeding the organisms orally does not result in active disease.

SOURCE OF INFECTION. Other affected animals: primary introduction can be by the introduction of infected stock or even wild rodents, since the maintaining of rats in rooms with mice is a likely source of infection for the latter.

CONTROL. Mice injected with a heat-killed suspension of the causal organisms develop an immunity, but it is doubtful if this is a practical method of control. In a colony where an epidemic occurs it is usually necessary to sacrifice all the animals, sterilize equipment and rooms, and start afresh with non-infected animals.

Mouse septicaemia

CAUSAL AGENTS. Various bacterial agents may be responsible for sporadic cases of murine septicaemia and the following merit some consideration.

AGENT (1). *Corynebacterium kutscheri*. A gram-positive bacillus of the corynebacterium genus.

SYMPTOMS. Loss of weight and condition.

POST-MORTEM. Tubercular-like septic foci may be found in the lungs, the spleen, the kidneys, the cervical gland, and, less frequently, in the liver. The foci contain free or caseated pus, and may become so numerous as to coalesce over considerable areas.

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DIAGNOSIS. The causal organisms are demonstrable in gram-stained smears of affected tissue and are recoverable by culture of the tissues on blood-agar plates.

ROUTE OF INFECTION. Probably by oral ingestion.

SOURCE OF INFECTION. Not known.

CONTROL. Sacrifice of immediate contacts.

AGENT (2). *Klebsiella pneumonia*: a gram-negative bacillus.

SYMPTOMS. Dyspnoea, loss of condition, and finally death.

POST-MORTEM. Congestion and consolidation of the lungs and enlargement of the spleen are the chief lesions.

DIAGNOSIS. The organisms are demonstrable on gram-stained smears of affected tissue. They are heavily capsulated, best shown by preparations stained with a special capsule stain. The organism can be cultivated on routine laboratory media.

ROUTE OF INFECTION. Probably the respiratory route.

SOURCE OF INFECTION. Not established.

CONTROL. Sacrifice of in-contacts and sterilizing of cages, etc.

AGENT (3). *Erysipelothrix muriseptica*: a gram-positive bacillus.

SYMPTOMS. Loss of weight and condition, and there may be a conjunctivitis.

POST-MORTEM. Septic foci may be present in the liver, spleen, and lungs.

DIAGNOSIS. The causal organism can be cultured from infected material by inoculating blood-agar plates.

ROUTE OF INFECTION. Unknown.

SOURCE OF INFECTION. Unknown.

CONTROL. It is usually sufficient to kill in-contacts.

In addition to the above causal agents, there are numerous others which may be responsible for sporadic cases of murine septicaemia, e.g. *Streptococcus haemolyticus*, *Diplococcus pneumoniae*, *Bordetella bronchiseptica*, etc.

VIRAL

Ectromelia (mouse pox)

CAUSAL AGENT. The Ectromelia virus—a member of the pox group of viruses.

SYMPTOMS. Acute: the animal may show no obvious signs of illness before being found dead. The first sign of the disease is usually an inconspicuous oedematous skin lesion which develops a scab, but this often goes unnoticed.

Chronic: animals surviving the acute stage develop a secondary generalized skin eruption followed by scab formation about two weeks after infection. In chronic cases the tail, one or both hind feet, or even forefeet become oedematous and later gangrenous, with sloughing of the affected extremity.

POST-MORTEM. Acute: sometimes few characteristic lesions are seen. The spleen is usually somewhat enlarged and presents white areas of fibrosis. The liver may be yellow and show minute necrotic foci or more diffuse necrosis. Fluid is often present in the peritoneal cavity. In the author's experience, the disease may result in sporadic deaths in a colony and the only post-mortem finding a mild hepatitis or splenitis.

Chronic: the superficial skin lesions have been described. There is extensive hepatic necrosis and numerous small foci on the surface of the liver and the spleen; necrosis of the pancreas and mesenteric fat is characteristic.

DIAGNOSIS. Ectromelia should always be suspected when any lesions suggestive of the disease are seen and unexplained losses follow the pattern of an epidemic. Confirmation may be sought in the following way. The spleen should be ground in horse-serum saline to give a final 10 per cent W/V suspension. 0·1 ml of this is injected intraperitoneally into two mice and 0·05 ml into the sole of the hind feet of two other mice. These mice must be from ectromelia-free stock. If the virus is present the mice injected intraperitoneally die within a few days, with hepatitis and splenitis as prominent lesions at autopsy. Those injected in the foot develop localized oedema and gangrene of the foot, and may die if the infection becomes generalized. If sections are prepared from the oedematous skin the presence of large cytoplasmic inclusions in the epithelial cells is diagnostic. Diagnosis can also be confirmed in other ways. For example, mice recovered from infection with vaccinia are immune to ectromelia; the sera of mice recovered from ectromelia neutralizes the vaccinia virus and prevents the formation of pocks on the chorio-allantoic membrane of fertile eggs. The haemagglutinative inhibition test may also be used to identify the virus or to detect antibodies against the haemagglutinin.

ROUTE OF INFECTION. Virus excreted by infected animals gains entry to a new host chiefly through skin abrasions of the tail and feet.

SOURCES OF INFECTION. Usually by direct contact with infected mice, both overt cases and carriers, or contact with material soiled by such mice.

CONTROL. Should ectromelia be present in a colony, the usual procedure is to sacrifice the colony and take the usual measures to destroy residual virus by adequate disinfection before a new stock of mice is introduced. Although mice develop solid immunity to ectromelia after inoculation with vaccinia virus, it is likely that a colony infected with ectromelia virus will continue to harbour this virus even after immunization and thus remain a source of infection for healthy mice. Vaccination, however, is of value under conditions where the introduction of ectromelia is a permanent unavoidable hazard. Furthermore, by making use of the fact that mice which have had ectromelia do not react to vaccination, the inoculation of vaccinia offers a test to ascertain that fresh intakes from outside sources are not infected. Thus, in the author's establishment it is the practice to test fresh intakes of foundation stock by the injection of 0·05 ml of dilute vaccinia intradermally into the tail. Non-immune mice show a local positive inflammatory response after seven days, and a mouse reacting in this way can be regarded as being free from infection and previous exposure to ectromelia virus. Ninety per cent positive 'takes' on any group of mice are regarded as evidence of freedom from infection.

Infantile diarrhoea

Two virus diseases of infant mice have been described by Kraft:

(a) Epidemic diarrhoea of infant mice (EDIM).
(b) Lethal intestinal virus of infant mice (LIVIM).

SYMPTOMS. This is a disease occurring in mice of less than fourteen days old. Whole litters are usually affected and show a yellow fluid diarrhoea which bespatters the litter. The disease is usually first noticed about ten days after birth. Mortality may be high, and survivors are often retarded in development. Infection may involve from 1 to 80 per cent of all litters and have a disastrous effect on output.

POST-MORTEM. The gut contents are semi-fluid and the animal dehydrated. There are no other microscopically demonstrable changes.

DIAGNOSIS. By the clinical symptoms.

ROUTE OF INFECTION. The route of the infection is probably oral, but the virus is disseminated in droplets and spreads rapidly over considerable distances. Presumably nursing mothers carrying the virus infect their offspring, and this is followed by widespread dissemination to neighbouring litters.

CONTROL. Owing to the great capacity of the infection to spread, eradication has only so far been possible under special conditions, known virus-free parents rearing their progeny in virus-proof isolators. This method has, as yet, been used only to study the disease, but could be applied to the foundation of 'infantile-diarrhoea-virus free' colonies. It seems probable that the toll exacted by the disease depends very much upon the management and genetic constitution of the mice, apart altogether from eradication of infection. With conventional methods of management success in control of the disease has been claimed by the provision of special diets and types of cages.

FUNGAL

Ringworm

CAUSAL AGENT. Ringworm fungus. *Trichophyton mentagrophytes.*

SYMPTOMS. Loss of hair; scaling and encrusting of lesions. The tail may be affected.

DIAGNOSIS. Skin scrapings and hair from infected areas are mounted in 10 per cent potassium hydroxide and examined microscopically for the presence of spore sheaths and mycelia invading infected hair roots. Cultures may be made on a suitable medium, such as Sabouraud's, to provide final identification of the causal fungus.

CONTROL. Infected mice and their in-contacts should be sacrificed and equipment, etc., sterilized. Treatment is not worthwhile; topical applications are of doubtful efficacy, and systemic fungicides, at present, are too expensive for routine use. *T. mentagrophytes* is pathogenic for man and an infected mouse is a hazard to the handler.

Note. Another Trichophyton—*T. Quickeanum* causes favus in mice. It is identified by the methods described above.

PROTOZOAL

Though the mouse gut is inhabited by several types of protozoa, e.g. coccidia, flagellata, and entamoeba, they are seldom of pathogenic importance.

ECTO-PARASITES

Body mange

CAUSAL AGENT. A mite—*Myocoptes musculinus.*

SYMPTOMS. Infestation is characterized by a marked loss of hair and sometimes generalized inflammation of the underlying skin. Frequently the mites produce these symptoms only in the breeding females and youngsters; the adult male, though infested, shows no hair loss or skin inflammation.

DIAGNOSIS. Hair and skin scrapings are mounted in 10 per cent potassium hydroxide and examined microscopically. Mites in all stages of development from ova to adults are usually present in large numbers.

CONTROL. It is usually sufficient to dust with benzene hexachloride powder (Lorexane) and put the treated animals in sterilized quarters. The treatment requires repeating twice at weekly intervals.

Head and neck mange

CAUSAL AGENT. A mite—*Myobia musculi.*

SYMPTOMS. Inflammation of the above areas is followed by serous exudation and scab formation. Males are more frequently affected than females.

DIAGNOSIS. Skin and hair scrapings are made as described for *Mycoptes musculinus*. *Myobia musculi*, however, burrows deeply, and relatively deep scrapings may be necessary to demonstrate its presence.

CONTROL. Simple dusting with an acaricidal powder is not usually satisfactory. The affected mouse and its contacts require to be totally immersed in a diluted solution of tetraethylthiuram monosulphide (Tetmosol, I.C.I.) one volume in nine volumes of warm water. Alternatively, a 0·2 per cent solution of D.M.C. (de-*p*-chlorophenyl methyl carbinol) in 50 per cent alcohol has been recommended as a dipping agent. The animals require to be transferred to sterile quarters after dipping. The treatment and transfer are repeated twice at weekly intervals.

Ear and body mange

CAUSAL AGENT. A mite—*Psorergates simplex.*

SYMPTOMS. This mite is concerned in two forms of mange. In the first the ears of the infected animal are found almost completely occluded by a firmly adherent, white, waxy mass which consists mainly of adult mites and early developmental stages. The second form, body mange, is characterized by the mites burrowing under the skin and forming skin pouches—nidification.

DIAGNOSIS. Pieces of ear wax or material dissected from skin pouches are mounted in 10 per cent potassium hydroxide and examined microscopically.

CONTROL. For the treatment of ear mange, a large drop of undiluted dibutyl phthalate is instilled into the ear and the treatment repeated after a period of seven days. It is unnecessary and inadvisable to attempt to remove the adherent wax. Care should be taken to prevent the chemical from entering the eyes of either the mouse or the operator, where it can cause serious irritation. For the treatment of body mange, a 2 per cent aqueous solution of 15 per cent Aramide [(2-(*p*-tert-butyl phenoxy)isopropyl-2-chlorethyl sulphite)] is used twice as a topical application at intervals of a week.

Notes. (*a*) A technique has been described for the detection of ecto-parasites in mouse stocks. A percentage of the mice are killed and immediately pinned out on black paper covered with transparent sticky tape. As the mouse slowly 'cools' the mites leave the body and are trapped on the paper, where they are detected, examined, and identified.

(*b*) A new method of dipping mice, which is claimed to kill the three mange-causing ecto-parasites and also lice, has been recently described. A dip is prepared by dissolving 2 gm DMC [di(*p*-chlorophenyl)methylcarbinol] in 3 gm ethanol and 67 gm Tetmosol (25 per cent solution tetraethylthiuram monosulphide, I.C.I.) in 1 litre of warm water. These two solutions are mixed and kept at 98·6°F (37°C) during use. Mice are given two dippings 1 hour apart and transferred to clean cages after the second dipping. Treatment is repeated after an interval of one to three weeks.

HELMINTHS

Roundworm infestations of the caecum and large colon

CAUSAL AGENTS. Two species of oxyuridae—*Aspicularis tetraptera* and *Syphacia obvelata*.

SYMPTOMS. Neither of these two worms cause recognized syndromes. Nevertheless, such heavy infestations as commonly occur, especially in the caecum, must be deleterious to well-being.

DIAGNOSIS. At post-mortem adult worms are found mainly in the caecum. The two species are distinguishable by the microscopic examination of gravid females.

CONTROL. Complete eradication is difficult in a large colony. However, reasonable control is achieved by the addition of piperazine acid citrate to the drinking-water (12 gm to 8 litres) for a period of seven days. This should be repeated on two occasions, with a rest period of seven days between each treatment. Individual mice can be treated by dosing orally with 50 mg of the drug contained in 0·2 ml of water. Eradication is difficult to achieve as

(*a*) piperazine, while highly active against the adult worms, is less efficient in the eradication of immature forms;

(*b*) the gravid females of *S. obvelata* can deposit their ova around the anal exterior, where they hatch and migrate back into the colon, and before this migration takes place may escape contact with the drug being used; and

(*c*) foodstuffs contaminated by wild-mouse faeces will almost certainly contain ova of these helminths. Re-introduction of the parasite quickly results from ingestion of foodstuffs contaminated with faeces from wild mice.

Cysticerosis

CAUSAL AGENT. The cystic stage, *Cysticercus fasciolaris*, of the cat tapeworm, *Taenia crassicollis*.

SYMPTOMS. Seldom symptomatic, and infestation is usually revealed only at autopsy.

POST-MORTEM. One or more cysts are found in the liver, and these contain the strobilocereus. Occasionally they may become infected by micro-organisms.

ROUTE OF INFECTION. Oral.

SOURCE OF INFECTION. Materials contaminated by the faeces of cats infested with *Taenia crassicollis*. Bedding, such as sawdust and wood shavings obtained from sawmills, is a common source of infestation.

CONTROL. Avoidance of possibly contaminated materials or the adequate sterilization of these before use in the colony.

CHEMICAL AGENTS

The volatile vapours of chloroform and carbon tetrachloride are unusually toxic to mice. When chloroform is used in animal houses to kill unwanted animals it sometimes happens that adult male mice housed nearby die and show diffuse tubular nephritis at autopsy.

RATS

The rat is less liable to serious diseases than most of the other laboratory animals, but it is prone to chronic respiratory infection.

BACTERIAL

Labyrinthitis (Middle ear disease—'circling')

CAUSAL AGENTS. At least two agents appear to be implicated, viz. Mycoplasma (pleuro-pneumonia organisms—P.P.L.O.) and a bacterium, *Streptobacillus moniliformis*.

SYMPTOMS. Middle ear disease is characterized by the affected animal tilting its head to one side and moving in a curve rather than a straight line. There may be difficulty in regaining balance should the animal fall over.

POST-MORTEM. There is infection of the middle ear. This may be due to an extension of infection from the outer ear or, alternatively, from the upper respiratory passages.

DIAGNOSIS. This is usually made by observing the clinical symptoms. *Streptobacillus moniliformis* can be isolated by inoculation of infected material on rich laboratory medium such as 30 per cent serum-agar plates. Mycoplasma can only be cultivated with regular success on the special media used for these organisms.

ROUTE OF INFECTION. Probably by either the aural route or the upper respiratory tract.

SOURCE OF INFECTION. Other rats.

CONTROL. Virtual elimination of the disease can be accomplished by frequent examination and destruction of animals with symptoms of labyrinthitis or upper respiratory tract distress.

Salmonellosis

CAUSAL AGENTS. *Salmonella typhi-murium* and *Salmonella enteritidis.*

SYMPTOMS. As described under mouse salmonellosis. In this country salmonellosis in rat colonies is not common. Post-mortem appearance, etc., is as described in mice.

VIRAL

Epidemic murine pneumonia (E.M.P.)

CAUSAL AGENT. Probably a virus.

SYMPTOMS. This disease, though probably the most common of all rat diseases, frequently causes little distress in spite of the presence, in individuals, of considerable lung damage. Close examination, particularly of old breeders, will often reveal dyspnoea, sometimes accompanied by a 'chattering noise'.

POST-MORTEM. Varying degrees of pulmonary involvement are noted, from discrete foci to widespread consolidation and hepatization.

DIAGNOSIS. In the past a large number of different bacteria have been thought responsible for rat pneumonia, but are now regarded as secondary invaders, and the true agent, a virus. Diagnosis is dependent on the examination of histological sections of lung tissue and the inoculation of suspensions of suspect lung into mice by the nasal route. The mouse inoculation test is carried out by preparing a 10 per cent W/V suspension of lung and dropping 0·05 ml on to the noses of anaesthetized mice; an infected suspension causes pneumonia within twenty-eight days.

ROUTE OF INFECTION. Respiratory.

SOURCE OF INFECTION. Adult females infect their offspring soon after birth.

CONTROL. Has not proved successful by any of the conventional methods used to combat disease. Every rat is at least a carrier of the virus, and a high proportion of adults will have demonstrable lung lesions. The disease impairs the development of rats and can seriously interfere with some kinds of research. A method has recently been introduced to establish rat colonies free of the disease. Caesarean-delivered animals are removed under aseptic conditions and hand reared. They then become the progenitors of a colony maintained under a system of management which effectively excludes reintroduction of the causal agent.

PROTOZOAL

Coccidiosis

CAUSAL AGENTS. Three species of coccidia have been described: *Eimeria miyairii*, *Eimeria separata*, *Eimeria nieschulze*.

SYMPTOMS. Acute diarrhoea is a common symptom only in weaning rats.

POST-MORTEM. Haemorrhagic enteritis of the small intestine is the common lesion.

DIAGNOSIS. Large numbers of oöcysts are demonstrable microscopically in the gut contents and the faeces.

ROUTE OF INFECTION. Oral: by the ingestion of sporulated oöcysts present in faecal material from infected rats.

SOURCE OF INFECTION. Other rats.

CONTROL. It is probable that all rats are at some time sub-clinically infected with Eimeria spp. and that the infection only becomes evident when massive infection takes place. Provided the system of management ensures adequate nutrition and cleaning, signs of coccidiosis are seen rather rarely and only in young rats.

ECTO-PARASITES

Body mange

CAUSAL AGENT. The following species of mites can be responsible for body mange: *Myobia ratti*, *Myobia musculi*, *Bdellonyssus bacoti*, and *Notoedres spp.*

SYMPTOMS. Irritation of the skin and occasionally skin and tail lesions.

DIAGNOSIS. The particular agent responsible for the occurrence of mange can be demonstrated by microscopic examination of hair and skin scrapings after treatment with 10 per cent potassium hydroxide.

SOURCE OF INFECTION. Other infested rats.

CONTROL. Several agents provide treatment: benzene hexachloride powder, diluted tetraethylthiuram monosulphide, DDT powder, etc. Treatment should be repeated at least once, seven days after the first treatment.

Note. Rats have been noted to harbour a variety of fleas; Cenatophyllus, Nopposyllus, Centocephalides, and Leptosylla. Treatment is the same as that described for mite infestations.

HELMINTHS

Cysticercosis

CAUSAL AGENT. The cystic stage, *Cysticercus fasciolaris* of the cat tapeworm, *Taenia crassicollis*. Both the rat and mouse act as intermediate hosts to the tapeworm, and the information about this parasite given in the section on mice applies also to rats.

OTHER PARASITES

Though the rat may carry in its intestine, roundworms, tapeworms, protozoa, etc., these do not usually, apart from coccidia, give rise to recognizable disease.

PHYSIOLOGICAL

Ringtail

CAUSE. Attributed to low humidity in rat houses, viz. less than 50 per cent.

SYMPTOMS. Newborn rats develop swollen tails with distinctive corrugated ringing, and the tails may eventually fall off. The other extremities sometimes become affected.

NUTRITIONAL

Vitamin E deficiency

CAUSE. Diet containing insufficient vitamin E.

SYMPTOMS. Infertility or lowered fertility, resorption of foetuses, abortion, and testicular inactivity.

CONTROL. This deficiency is less common than formerly, possibly because synthetic vitamin E is frequently added to compounded diets.

GUINEA PIGS

BACTERIAL

Pseudotuberculosis

CAUSAL AGENT. *Pasteurella pseudotuberculosis*: a gram-negative bacillus.

SYMPTOMS. Usually chronic: the animal becoming emaciated and often dying, though recovery may take place.

POST-MORTEM FINDING. The primary lesion is usually found in either the mesenteric or colonic lymph nodes. The infection spreads to the gut wall, the liver, and the spleen. Infection may also be present in the genito-urinary tract and mammary tissues; there is seldom pulmonary involvement. The lesion is a white or yellow nodular necrotic focus containing free or caseated pus.

DIAGNOSIS. The causal organism is recoverable by culturing suspect material on routine laboratory media such as 5 per cent blood agar.

ROUTE OF INFECTION. Oral: by the ingestion of materials contaminated with the organism. Infection may occur rarely by other routes, e.g. through the peridontal tissues surrounding the incisors.

SOURCE OF INFECTION. Primary infections are introduced from outside the colony, and secondary infections then occur by spread within the colony. Birds can be carriers of infection or overt cases. In the author's experience, pigeons are a frequent source of infection, contaminating growing green foods such as kale with excreta containing *P. pseudotuberculosis*.

CONTROL. (*a*) By exclusion from the colony of materials liable to be contaminated. (*b*) By the elimination of diseased animals: early non-clinical cases may be detected by palpation of the primary lesions in the gut glands.

Salmonellosis

CAUSAL AGENT. Bacteria of the Salmonella genus: *Salm. typhi-murium*, *Salm. enteritidis*, *Salm. dublin*, etc.

SYMPTOMS. Acute: animals may die without showing any symptoms: occasionally there may be an associated diarrhoea.
    Chronic: wasting and emaciation.

POST-MORTEM. Acute: splenic and hepatic enlargement which may be accompanied by a haemorrhagic and necrotic invasion of the gut glands.
    Chronic: enlargement of the liver and spleen. Infected foci containing free or caseated pus may be present in the liver, the lymph glands, and the walls of the gut: there may be a peritonitis.

DIAGNOSIS. The chronic form of the disease may resemble, at post-mortem, pseudotuberculosis. A preliminary distinction may, however, be made by staining material from affected tissue by Gram's method. Salmonella infected tissue usually contains large numbers of gram-negative bacilli: *P. pseudotuberculosis* is more difficult to detect. Final diagnosis is, however, dependent on the isolation of the causal organism on laboratory media, such as McConkey's agar, or more selective media, such as selenite F and desoxycholate citrate agar. Suspect isolates are identified by biochemical and serological techniques.

ROUTE OF INFECTION. Infection is believed to occur by the oral route, though some evidence suggests that infection of the eye may occur and spread by the local lymph glands to the bloodstream and other tissues.

SOURCE OF INFECTION. Several sources of infection have been incriminated. The contamination of feeding stuffs and bedding by wild rodents: flies can also spread contamination. It is now also recognized that certain of the materials used in compounded animal pellets can harbour Salmonellae, though it is probable that these are largely destroyed during processing. It is perhaps significant that the species of Salmonella usually responsible for the disease in laboratory animals are those commonly associated with Salmonellosis in cattle, though a direct association may be difficult to demonstrate.

CONTROL. (*a*) The removal and sacrifice of active cases and their contacts. (*b*) The sterilization of infected equipment and the destruction of bedding, etc. (*c*) It may be necessary in severe outbreaks to sterilize the animal house by physical and/or chemical methods. (*d*) If possible, the source of infection should be discovered and eliminated.

Streptococcal pneumonia

CAUSAL AGENT. *Streptococcus pyogenes*: a gram-positive coccus producing a soluble haemolysin and usually of Lancefield's Group C.

SYMPTOMS. Emaciation, respiratory distress, and nasal discharge: the urine may contain haemoglobin.

POST-MORTEM. The lung consolidates in varying extents. Infection may be found in the pericardium and myocardium, and the kidneys may show congestion.

DIAGNOSIS. The causal organism can be seen in gram-stained films made from infected tissue. It may be isolated on 5 per cent blood agar, which will also show the haemolytic nature of the organism.

ROUTE OF INFECTION. Probably by the respiratory route.

SOURCE OF INFECTION. Unknown.

CONTROL. (*a*) The elimination of infected animals and their contacts. (*b*) The sterilization of equipment and the destruction of bedding, etc.

Streptococcal lymphadenitis

CAUSAL AGENT. *Streptococcus pyogenes*: (Lancefield Group C—see streptococcal pneumonia).

SYMPTOMS. Acute: there is a fulminating septicaemia, the animal dying within three to four days of infection.

Chronic: the animal frequently remains in normal health. The cervical glands and other neck glands are obviously enlarged. Occasionally the infection may involve the axillary and inguinal glands, and there may be an associated arthritis and cellulitis. The disease in pregnant females sometimes results in abortion.

POST-MORTEM. Acute: some degree of lung hepatization is usually found. There may be a diffuse haemorrhagic peritonitis and congestion of the spleen and liver.

Chronic: free pus is found in the affected glands. There may be small abscesses present in the liver, lungs, peritoneum, and the gut wall.

DIAGNOSIS. Streptococci are seen in gram-stained smears made from infected pus or tissue. Cultures are made as in streptococcal pneumonia.

ROUTE OF INFECTION. The organism is considered to enter the body through abrasions in the buccal mucosa caused by ingestion of materials such as the thistles sometimes found in hay. In the acute form the lymph glands fail to localize the organisms.

SOURCE OF INFECTION. There is little definitely known, haemolytic streptococci are a common parasite of humans and animals, and either may be responsible for primary introduction.

CONTROL. Cases and their contacts should be sacrificed, equipment sterilized and bedding, etc., should be destroyed. Hay containing abrasive ingredients should not be used. Should infection become widespread, it may even be necessary to destroy the colony and restock from a clean nucleus.

Bronchiseptica pneumonia

CAUSAL AGENT. *Bordetella bronchiseptica*: a small gram-negative bacillus formerly known as either *Brucella bronchiseptica* or *Haemophilus bronchisepticus*.

SYMPTOMS. Affected animals may show signs of dyspnoea, though early cases may go undetected.

POST-MORTEM. The lungs show areas of consolidation which may contain a purulent exudate; other tissues are not involved.

DIAGNOSIS. Recognition of the organism in gram-stained films of infected material is difficult. Cultures of infected tissue on blood-agar plates or bile-sale agar plates usually results in profuse pure growths of the organism. It is identifiable by its failure to ferment carbohydrates and its ability to produce ammonia from urea.

SOURCE OF INFECTION. Probably many normal guinea pigs harbour *B. bronchiseptica* in the respiratory tract, and some disturbance of the host–parasite relationship allows the organism to become pathogenic. Rabbits, rats, cats, and dogs also harbour this organism, and may occasionally be the source of infection for guinea pigs.

CONTROL. Usually only isolated cases of this disease occur, and no control measures required other than removal of the affected animal and close observation of in-contact animals. However, widespread epidemics can take place, and it may be advisable to sacrifice in-contacts and sterilize contaminated materials. An autogenous vaccine has been used with success in eliminating pneumonia in rats caused by *B. bronchiseptica* and guinea pigs can also develop a good immunity following vaccination. However, carriers persist and may infect non-vaccinated stock.

Cervical adenitis

CAUSAL AGENT. *Streptobacillus moniliformis*: a gram-negative bacillus.

SYMPTOMS. There is a lymphadenitis of the cervical glands, these become obviously swollen and may rupture through the skin. The general health of infected animals is not usually seriously impaired.

POST-MORTEM. The affected glands contain pus: infection sometimes extends to the trachea, the bronchi, and the lungs, where it manifests itself as pustular nodules. Infection of the axillary and inguinal glands has been observed but is uncommon.

DIAGNOSIS. The causal organism is not easily found in stained smears of pus: it is isolated by cultures of pus on media containing a high proportion of serum.

ROUTE OF INFECTION. Through abrasions in the buccal mucosa.

SOURCE OF INFECTION. The respiratory tract of most rats is infected with this organism and, in mice, it produces chronic arthritis. While little is definitely

known about the source of infection for guinea pigs, rats constitute a dangerous reservoir of infection. Minor injuries to the mouth tissues may be the portal of entry.

VIRAL

So far, no well-defined virus diseases of major importance have been recognized in guinea pigs.

FUNGAL

Mucormycosis

CAUSAL AGENT. *Absidia ramosa*: a mucor mould.

SYMPTOMS. The infected animal is usually asymptomatic, though, as described below, rare cases are found where the infection becomes generalized and the animal dies. In the usual asymptomatic cases the primary lesion is a nodular lymphadenitis of the mesenteric lymph gland. This swelling is sufficiently large to be palpable, and may be confused with glandular enlargement caused by *Pasteurella pseudotuberculosis* or the Salmonella spp.

POST-MORTEM. Benign: the usual lesion is chronic enlargement of the mesenteric lymph node which contains free or caseated pus.

Generalized: infection is found spreading from the lymph gland to the kidneys, spleen, liver, and lungs. These organs are enlarged, congested, and necrotic. There is sometimes a marked excess of peritoneal fluid and a pleural effusion.

DIAGNOSIS. Typical non-septate hyphae are demonstrable microscopically in wet preparations of pus and infected tissue treated with 10 per cent potassium hydroxide. Cultures on blood-agar plates develop fine cotton-wool-like growth typical of mucor spp.

ROUTE OF INFECTION. Oral.

SOURCE OF INFECTION. Absidia sp. are widely distributed in nature. Heavy growths of *Absidia ramosa* may be found in hay, particularly when made during wet weather, and probably this represents the principal source of infection for guinea pigs.

CONTROL. The avoidance of poor-quality, mouldy hay.

Note. The importance of this disease rests in the likelihood of its being confused with the lymphadenitis caused by infections with Salmonella or *P. pseudotuberculosis.*

PROTOZOAL

Coccidiosis

CAUSAL AGENT. *Eimeria caviae.* A protozoan.

SYMPTOMS. Diarrhoea and emaciation.

POST-MORTEM. The disease is characterized by a gelatinous oedema of the large gut just beyond the caecal junction. The contents of the large gut are semi-fluid without the normal pellet formation.

DIAGNOSIS. This condition may be diagnosed during life by the microscopic detection in faecal material of large numbers of oöcysts.

ROUTE OF INFECTION. Oral: by the ingestion of materials contaminated with the faeces of guinea pigs, containing sporulated oöcysts.

SOURCE OF INFECTION. *Eimeria caviae*, like other coccidia, is host specific. As guinea pigs are not found in the wild state, infection must arise within the colony. Most guinea pigs harbour coccidia, and active disease is usually only found in animals from four to eight weeks of age, probably the period when maternal passive immunity has declined and active immunity not yet developed.

CONTROL. Oöcysts, after being shed in the faeces, take five to eight days to sporulate to the infective stage. Hence, regular cleaning and sterilization of equipment in shorter periods of time than five days will greatly minimize spread of infection. Similarly, any system of management which prevents gross faecal contamination will tend to lessen the liability to overt disease. Overt cases should be removed and sacrificed, and to prevent further spread it may be necessary to treat in-contacts with the coccidiostats used for poultry, such as sulphamezathine or sulphaquinoxaline. Bactericides are usually ineffective against oöcysts, which will, for example, sporulate in solutions of formalin and hypochlorites. Oöcysts are, however, killed by heat (steam or flame) and by 10 per cent ammonia solution.

Lice

CAUSAL AGENT. Three biting lice (Mallophaga) are commonly associated with guinea pigs. These are *Gyropus ovalis*, *Glirocola porcelli*, and *Trimenopon jenningsi*. A fourth type, *Menopon extraneum*, is only rarely found.

SYMPTOMS. Guinea pigs can be heavily infested without showing symptoms. However, in individual cases the animal may scratch and cause skin irritation.

DIAGNOSIS. Naked-eye examination will usually detect infestation, though a hand lens is of value. Identification of the type of louse present requires microscopic examination.

SOURCE OF INFECTION. Other guinea pigs.

CONTROL. Where only a few animals are infested they can be treated by dipping in diluted tetraethylthiuram monosulphide, or dusting with DDT or benzene hexachloride. These treatments need repeating twice at intervals of a week. The animals should be transferred to sterilized equipment after each treatment. The above methods are not practicable for a large number of animals, and a measure of control can be achieved by the use of some form of continuously flowing insecticidal vapour.

HELMINTHS

Three worms have been described as causing pathological conditions in guinea pigs. They are: the fluke, *Fasciola hepatica*, the roundworm, *Paraspidodera uncinata*, and the larvae of *Trichinella spiralis*. It is not proposed to describe these in detail, as their occurrence in guinea pigs is rare. Briefly, *F. hepatica* has been found in the liver and musculature; cysts containing *Tr. spiralis* larvae have been found in the limbs, the tongue, and the palate. *P. uncinata* is found in the caecum and colon of the guinea pig, and heavy infestations have been thought to cause loss of condition, emaciation, and diarrhoea.

PHYSIOLOGICAL

Stillbirth and inanimation

High losses may be experienced in guinea pigs from stillbirths and deaths shortly after birth. Certain colonies have experienced up to 30 per cent losses. These losses are a problem of management rather than disease control, and have been much reduced by replacement of breeding stock by the progeny of mothers with a satisfactory breeding record. Unsuitable diet and cages may also play some part in this problem.

Pregnancy complications

Dystocia is not uncommon and results from a number of causes. Common ones are dual presentation at full term; torsion of the uterus; the mating of females when too young; delaying mating until they are too old.

Pregnancy toxaemia also occurs, presumably due to faulty metabolism in the later stages of pregnancy.

Anal and vaginal fissures are sometimes found in heavily pregnant females. These are probably initiated in part by excessive urination, with consequent continuous wetting of the affected areas. Chronic inflammation may follow and produce oedema in the adjacent tissues.

Vitamin C deficiency

Guinea pigs require a regular intake of ascorbic acid. A growing animal needs about 2 mg daily and the breeding female about ten times this amount. Sufficient of the vitamin is usually available in the quantities of fresh greens normally fed. Should the feeding of greens be, for some reason, stopped, then the diet should be supplemented by crystalline ascorbic acid. Deficiency results in scurvy and finally death. At autopsy the animal appears emaciated, the ribs showing enlargement at the costochondral junctions, and there are widespread petichial haemorrhages.

Hay deficiency

Guinea pigs eat large quantities of hay, and if deprived of it they show adverse effects, particularly in young stock, such as high mortality and bizarre dentition, which interferes with nutrition. The exact nature of the deficiency has not been determined.

Soft tissue calcification

This is a condition found occasionally in breeding boars, and more rarely in breeding sows. The affected animals are in poor condition and lose weight. At autopsy calcium deposits are visible on the external wall of the colon flexure, the heart, the kidneys, the external wall of the colon flexure, the heart, the kidneys, the external wall of the stomach, and the genito-urinary systems, particularly the seminal vesicles of the male. The condition has been produced experimentally by the feeding of diets deficient in magnesium.

Nephritis

Chronic nephritis is not uncommon in old breeding stock. It is thought to be due to the absorption, over long periods, of toxic substances which may be present in materials used for guinea pigs, such as the toxins present in wood shavings often used for bedding. Guinea pigs will, for example, eat large quantities of shavings if deprived of sufficient hay.

Exudative hepatitis and oedema (Paget's disease)

A number of deaths have been reported as occurring among young weaner guinea pigs, following the feeding of certain batches of pellets prepared according to the formula of the Bruce–Parkes Diet 18. The affected animals have staring coats and a characteristic dropsy of the abdomen. At autopsy the most marked feature is a subcutaneous oedema due to the collection of clear, watery fluid in the connective tissues of the abdomen, chest, and neck. There is sometimes pleural effusion, free ascitic fluid in the peritoneal cavity, and enlargement of the mesenteric lymph gland. The liver may be pale, but otherwise appears normal, though histological examination shows marked hepatic changes. There is dilatation of the liver-cell columns, cellular infiltration of the portal tracts and dilatation of the portal lymphatics. The mesenteric gland and spleen may show considerable hyperplasia of the sinusoidal reticulum cells, and there may be an increase in the number and size of the islets of the pancreas. Biochemical tests have shown serum protein values lower than those found in comparable normal guinea pigs.

Investigations so far have not established the cause of this disease. It does not appear to be due to an infective agent, and it has been suggested that it is either a nutritional deficiency or caused by some toxic substance present in certain batches of diet.*

RABBITS

BACTERIAL

Snuffles (infectious nasal catarrh)

CAUSAL AGENTS. Believed to be bacteria: at least three gram-negative bacilli are regarded as associated with outbreaks of snuffles—*Pasteurella septica*,

* Recent investigations have demonstrated that a condition indistinguishable from that described above can be produced by feeding guinea pigs food containing GROUND NUT MEAL which has become contaminated with a toxin (aflatoxin) produced by *Aspergillus flavus*. It is probable that the outbreak of the above guinea pig disease was caused by feeding Diet 18 which contained ground nut meal.

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Bordetella bronchiseptica, and members of the Haemophilus spp. That so many agents have been considered to cause a disease syndrome raises the suspicion that the true cause may not yet have been discovered.

SYMPTOMS. Sero-purulent discharge from the nostrils, though symptoms may vary from occasional sneezing to marked dyspnoea. Rabbits with dyspnoea make snuffling noises, from which the disease has received its popular name. Some cases are mild and chronic, but others develop a severe pneumonia which often terminates fatally.

POST-MORTEM. Lesions may vary from mild inflammation of the nasal passages to severe rhinitis, sinusitis, and pleuro-pneumonia.

DIAGNOSIS. This is usually dependent on the clinical symptoms. Bacteriological examination of exudates and infected tissues results in the isolation of either one or a combination of the bacteria mentioned above.

ROUTE OF INFECTION. Probably by the respiratory route.

SOURCE OF INFECTION. Other infected rabbits.

CONTROL. When an isolated case occurs in a rabbitry, sacrifice of the animal and sterilization of the cages, bedding, etc., may prevent further spread. However, the disease is more often endemic in a colony, and then successful control is difficult. There is not yet any fully satisfactory curative treatment, although some success has been claimed by parenterally administered sulphonamides, water-fed antibiotics, and bactericidal inhalants. At least one bacterial species associated with snuffles may be found in the respiratory tract of normal rabbits, and their complete eradication would be difficult. An adequate diet, sufficiently ventilated quarters, and the regular provision of cleaned and sterilized cages, etc., usually control the disease sufficiently to prevent serious outbreaks.

Pneumonia

CAUSAL AGENTS. The bacteria associated with snuffles.

SYMPTOMS. There is usually marked dyspnoea, but often this is overlooked, and the disease is only recognized after death.

POST-MORTEM. There is a pneumonia, though the amount of lung involved varies widely. Sometimes almost all the lung substance is consolidated and large cavities are filled with creamy pus.

DIAGNOSIS. Associated bacteria are recoverable by culture of infected material on blood plates, though richer medium, such as chocolate-agar plates, may be necessary for the isolation of organisms of the Haemophilus spp.

ROUTE OF INFECTION. Probably by the respiratory tract.

SOURCE OF INFECTION. Presumably other rabbits, but most of the organisms associated with the disease can be recovered from other species of laboratory animals.

CONTROL. Pneumonia may follow as a sequel to snuffles, or it may occur in a rabbit without involvement of the upper respiratory tract. Methods of treatment and control described under snuffles apply to pneumonia.

Pseudotuberculosis

CAUSAL AGENT. *Pasteurella pseudotuberculosis.*

Pseudotuberculosis in the rabbit is essentially the same as that described in guinea pigs. The disease is much less common in rabbits.

Rabbit syphilis (venereal disease).

CAUSAL AGENT. A bacterium, *Treponema cuniculi.*

SYMPTOMS. Ulcerative lesions are found on the penis and prepuce of the male, and the vulva and vagina of the female. There may be involvement of the anus, the eyelids, the lips, and the nose.

POST-MORTEM. Death from rabbit syphilis is not common, though it has been recorded after generalization of infection.

DIAGNOSIS. The causal organism is a spirochaete and is difficult to demonstrate by routine staining methods. Serous exudates require to be examined as wet preparations under the dark-ground illuminated microscope or in the dry state to be stained by a silver impregnation method.

ROUTE OF INFECTION AND SOURCE. By sexual intercourse with infected rabbits.

CONTROL. The disease is curable by a single injection of neosalvarsan or penicillin. Either of these agents should eradicate the disease from a closed colony. Before introduction of rabbits for breeding, they should be examined to ensure their freedom from this disease.

Listeriosis

CAUSAL AGENTS. *Listeria monocytogenes*: a gram-positive bacillus of the genus Listeria.

SYMPTOMS. The disease appears to be confined to young stock and pregnant does.

Young stock. Rapid loss of flesh, with death occurring suddenly.
Breeding does. Abortion of dead foetuses near term or delayed parturition with putrid foetuses.

POST-MORTEM. Young stock. Focal necrosis of liver, heart, and spleen, enlarged mesenteric glands.
Breeding does. Focal necrosis of liver, metritis, and associated peritonitis.

DIAGNOSIS. Isolation of *Listeria monocytogenes.*

ROUTE OF INFECTION. Not known.

SOURCE OF INFECTION. Not known.

CONTROL. The disease does not spread within a colony to any extent if management is good, but slaughter of all in-contact animals is recommended.

VIRAL

Myxomatosis

CAUSAL AGENT. Myxomatosis virus.

SYMPTOMS. The primary lesion of myxomatosis occurs on the eyelids, which are at first congested and swollen. Later a severe blepharo-conjunctivitis accompanied by a purulent exudate occurs. The nose and lips are swollen, and the genitalia may be similarly affected.

POST-MORTEM. Little change may be present in the internal organs. The spleen may be slightly enlarged, and subcutaneous tumours are sometimes found.

DIAGNOSIS. Domestic rabbits are highly susceptible to the myxomatosis virus. The clinical symptoms and very high mortality are sufficient to make a probable diagnosis. Confirmation can be obtained, by the histological examination of sections made from lesions, and by inoculation of suspected material into normal rabbits.

ROUTE OF INFECTION. The virus is introduced into the bloodstream by the bite of either mosquitoes or rabbit fleas which have previously fed on an infected rabbit.

SOURCE OF INFECTION. Fortunately, so far in this country, the mosquito has not been the vector responsible for many outbreaks of myxomatosis among wild rabbits, the rabbit flea has been the usual vector. The control measure necessary in Britain has been the exclusion from rabbitries of wild rabbits. However, where there is any likelihood that the virus may be spread by mosquitoes, steps must be taken to exclude them from buildings housing rabbits. A vaccine prepared from the Shope fibroma virus confers immunity against myxomatosis for about six months. This would be useful for immunization of contact rabbits if a case happened to appear in a colony of domestic rabbits.

Rabbit pox

CAUSAL AGENT. Rabbit pox.

SYMPTOMS. The most common and obvious features are blepharitis and keratitis, accompanied by a blood-stained mucopurulent discharge: there is a similar discharge from the nose. Close examination reveals glandular enlargement, particularly of the head and neck glands. Macular and papular rashes occur over the whole body, being most evident on the ears, exposed areas of skin, and the genitalia. The tissues of the mouth and tongue are swollen and necrosed. The disease is very contagious, and mortality rates are high.

POST-MORTEM. There is hepatic enlargement, and grey nodules are distributed throughout the liver substance. The spleen is enlarged, and areas of focal necrosis may be seen. The testicles of the male and the uterus and ovaries of the female usually show many small lesions. A few lesions may be present in the lungs. The lymph glands, particularly of the head and neck, are enlarged,

haemorrhagic, and oedematous, and may show focal lesions. The nose and associated sinuses are haemorrhagic and contain a mucopurulent exudate.

DIAGNOSIS. Suspensions of infected material inoculated on to the chorio-allantoic membrane of suitable fertile eggs give rise to pocks, but such pocks are absent if the infectious material is incubated with immune vaccinia serum prior to inoculation of the eggs.

CONTROL. This is not a common disease in this country, though several outbreaks have been recorded from the United States. It is mentioned because this virus is sometimes used in laboratories and may easily spread to normal rabbits housed close by. Such an outbreak has been recorded in Britain. A solid immunity is conferred by vaccination with vaccinia virus.

FUNGAL

Ringworm

CAUSAL AGENT. Trichophyton and Microsporon spp.

SYMPTOMS. There is destruction of the hair follicles, resulting in areas of baldness which may exude serous fluid and become encrusted.

DIAGNOSIS. Scrapings made from infected areas and mounted in 10 per cent potassium hydroxide show fungal hyphae and spores when examined microscopically. Cultures are made by inoculating hair and skin scrapings on Sabouraud's medium.

ROUTE AND SOURCE OF INFECTION. Probably by contact with infected rabbits or other rodents.

CONTROL. It is doubtful if ringworm in rabbits is worth attempting to cure. Topical applications are of doubtful value, and oral treatments are expensive. Infected animals mostly recover without treatment, but since they are likely to infect others before recovering, it is probably more satisfactory to sacrifice the animal and effectively sterilize the cages, bedding, etc.

PROTOZOAL

Intestinal coccidiosis

CAUSAL AGENTS. Two species of Eimeria: *Eimeria perforans* and *Eimeria magna*.

SYMPTOMS. Diarrhoea accompanied by loss of condition, often terminating fatally.

POST-MORTEM. Small infected foci can be seen in the internal wall of the small gut. Often these lesions are accompanied by a more diffuse enteritis.

DIAGNOSIS. The oöcysts can be demonstrated microscopically during life by examination of faecal suspensions or at autopsy in scrapings from the gut mucosa.

ROUTE OF INFECTION. Oral: by the ingestion of material contaminated by the faeces of rabbits excreting oöcysts which have sporulated since being voided.

SOURCE OF INFECTION. Other rabbits: Eimeria spp. are host species specific.

CONTROL. Reasonable control depends upon removal of faeces before the contained oöcysts have sporulated. This requires a high standard of management to ensure regular cleaning and sterilizing of cages, etc. In addition, it has become a common practice to include sulphadimidine or sulphaquinoxaline in the drinking-water of breeding does immediately prior to littering and of growing stock for some days following weaning. Certain manufacturers now include the above-mentioned drugs and other coccidiostats in compounded feeding pellets, though the efficiency of such treatments has not yet been fully tested.

Hepatic coccidiosis

CAUSAL AGENT. *Eimeria stiedae.*

SYMPTOMS. Mild cases may show no clinical signs of infection, but gross involvement of the liver results in faulty metabolism, loss of weight, and death.

POST-MORTEM. In early and mild cases the only finding may be of the presence of oöcysts, demonstrable microscopically in the bile of the gall bladder. As the diseases develop there is increasing invasion of the hepatic bile ducts, which thicken and show themselves as multiple white lesions throughout the liver substance. The lesions, when opened, are filled with greenish creamy fluid packed with oöcysts.

DIAGNOSIS. Microscopic examination of the bile or material from infected foci reveals the oöcysts. They are also voided in the faeces and require to be distinguished from intestinal coccidia.

ROUTE OF INFECTION. Oral: as in intestinal coccidiosis. It is said that the sporozoites liberated from sporulated oöcysts find their way to the liver via the portal vein.

CONTROL. As in intestinal coccidiosis; the so-called coccidiostats used in either the drinking-water or the feed appear to give a much higher degree of control of hepatic coccidiosis than they do of the intestinal disease.

ECTO-PARASITES

Ear canker

CAUSAL AGENT. Either of two species of mite may be involved. *Chorioptes cuniculi* or *Psoroptes communis.*

SYMPTOMS. Animals with severe ear canker usually show signs of discomfort, such as excessive scratching of the ears; mild cases often go undetected.

DIAGNOSIS. The ears contain yellow-white crust: a piece of the crust crushed in 10 per cent potassium hydroxide and examined microscopically will show the causal mites, nymphs, and eggs.

SOURCE OF INFECTION. Other infested rabbits.

CONTROL. The canker can be cured by a single installation into the ears of a diluted solution of tetraethyl-thurium monosulphide (Tetmosol, I.C.I.) one volume in nine volumes of warm water; it is unnecessary to remove the cankerous crust. Regular aural examination of rabbits should be practised. This can conveniently be done when the rabbits are set up for mating, at weaning, and when issued for experimental use. Such a routine eradicates ear canker or reduces the number of cases to a minimum.

Body canker

CAUSAL AGENT. *Notoedres spp.* and *Sarcoptes spp.* of mites.

SYMPTOMS. Irritation of the skin, followed by loss of hair and serious lesions.

DIAGNOSIS. The causal mites are demonstrable in hair and skin scrapings mounted in 10 per cent potassium hydroxide. Body mange is not common in domestic rabbits in this country. However, should it occur, it can be treated with Tetmosol, DDT, benzyl benzoate, benzene hexachloride, etc.

HELMINTHS

Cysticercosis (bladder worms)

CAUSAL AGENT. *Cysticercus pisiformis* and *Cysticercus serialis*, the cystic stages of the dog tapeworms, *Taenia pisiformis* and *Taenia serialis*.

SYMPTOMS. Neither of these worms cause serious ill effects. The cysts of *C. pisiformis* are usually situated internally. The cysts of *C. serialis* develop in the subcutaneous tissue, causing large round swellings under the skin. The flank of the animal is a common site.

POST-MORTEM. *C. pisiformis* is found in the peritoneal cavity. It frequently occurs as clusters of multiple small cysts adherent to the pancreas, the liver, the stomach, and the mesentery.

C. serialis is found subcutaneously.

DIAGNOSIS. The presence of these typical cysts is diagnostic. When opened, immature tapeworms can be expelled from them.

ROUTE OF INFECTION. Oral.

SOURCE OF INFECTION. Foodstuffs, bedding, etc., which have been contaminated by the faeces of a dog harbouring the tapeworms. The larvae hatch from the ingested eggs and migrate to their final site by passing through the liver, their passage leaving fibrous tracts in that organ.

CONTROL. By avoiding the use of materials contaminated by dog faeces or sterilizing materials which may have been exposed to infection. The subcutaneous cysts of *C. serialis* can easily be removed, provided care is taken not to rupture them.

INJURIES

Fractures

Rabbits are easily frightened by noises, inexperienced handling, etc. Fracture of the spine may occur as a result of the animal jumping in fright and catching its back on some obstruction. Rabbits hind-legs are easily trapped in weld mesh, broken wooden boxes, etc., and a fracture results from the animals attempting to free the trapped limb. Injuries to the spine often pass unnoticed at the time of occurrence, and are detected later when signs of hind-quarter paralysis develop, resultant upon damage to nerves.

THE NON-SPECIFIC ENTERIC COMPLEX

Growing rabbits are prone to several ill-defined enteric conditions which are not yet fully understood. These conditions are mainly found in animals of from six to twelve weeks of age, the critical period of post-weaning adjustment. Many rabbits are ill and often die, showing at post-mortem examination either one or a combination of the following lesions.

Mucoid enteritis and/or mucoid typhlitis

The affected area of the gut contains large quantities of jelly-like mucus, which is sometimes excreted with the faeces.

Enteritis and/or typhlitis

There is inflammation of the gut, which may vary from a mild irritation to a severe haemorrhagic reaction.

Diarrhoea

A simple diarrhoea with fluid intestinal contents is seen. Evidence of pellet formation in the large gut is absent.

Impaction

The contents of the large gut present a solid impaction. There is gross distension of the gut wall, which is often without tone and is 'parchment-like' in appearance.

Fluid distention

This condition often follows the onset of impaction, the animal drinking excess water, causing distension of the stomach and intestines.

Although poorly understood, these conditions account for a large part of the losses in rabbitries. A mortality of 20–25 per cent of all litters born is not uncommon. Various causes have been suggested: faulty nutrition, inadequate housing, pathogenic bacteria, viruses, protozoa, etc. However, the true aetiology is still obscure and presents a challenge to all those responsible for the investigation of rabbit diseases.

MONKEYS

The diseases described are those commonly affecting the *Macaca mulatta* species. These are still the most widely used monkeys in this country. However, should other species become used in large numbers, then other diseases may require to be described at some later date. For example, naturally occurring malaria is absent in the *M. mulatta* monkey, but is widespread in other species found in Africa, Asia, and South America.

BACTERIAL

Bacillary dysentery

CAUSAL AGENTS. Gram-negative bacilli of the Shigella genus.

SYMPTOMS. There is a diarrhoea which varies from a stool of semi-fluid consistency to a frank exudate of blood and mucus. The animal loses weight and condition, and in untreated cases the condition may terminate in death.

POST-MORTEM. The carcase is emaciated and dehydrated: the whole of the gut may show inflammatory and haemorrhagic changes and necrosis.

DIAGNOSIS. The presence of blood and mucus in the faeces is strongly diagnostic (microscopic examination of the exudate shows large numbers of pus cells). The causal organisms are recovered by inoculating the faecal exudate or gut contents on a selective medium such as desoxycholate citrate agar. Suspect isolates are examined biochemically and serologically. Commonly incriminated Shigella are *Shigella flexner* types 1, 2, 3, 4, 5, X, and Y; *Sh. sonnei* and *Sh. schmitzei*.

ROUTE OF INFECTION. Oral.

SOURCE OF INFECTION. Other infected monkeys and man.

CONTROL. Many monkeys imported into this country are either clinical cases or carriers of Shigella. Infection rates of between 30 and 40 per cent have been recorded in batches of these animals. Should the monkeys be penned together in large numbers after their arrival, outbreaks of dysentery may be severe. It is now common practice to cage monkeys in single units, and to treat them as a curative and prophylactic measure with broad spectrum antibiotics such as chloramphenical injected parenterally or terramycin given in the drinking-water. Resistant cases may be treated orally with a chemotherapeutic agent such as sulphaguanidine. Strict hygiene is also necessary; trays containing faeces should be removed, cleaned, and sterilized daily, and freshly sterilized cages regularly provided. Apart from the danger of spread among monkeys, Shigella are also human pathogens, and there are many recorded cases of handlers becoming accidentally infected. It is probable that most of these infections would have been avoided had adequate hand washing taken place after handling the animals or infected fomites.

Pneumonia

CAUSAL AGENTS. *Diplococcus pneumoniae*: a gram-positive diplococcus. *Klebsiella pneumoniae*: a gram-negative bacillus. Members of the Pasteurella group of gram-negative bacilli.

SYMPTOMS. Respiratory distress, inappetence, loss of condition and, if left untreated, the pneumonia often terminates in death.

POST-MORTEM. The lungs may be congested, consolidated, and hepatized.

DIAGNOSIS. During life there is usually apparent respiratory distress, which can be confirmed by stethoscopic examination and X-ray examination of the chest. At post-morten examination the particular causal organism involved can be demonstrated by plating infected material on laboratory medium such as blood agar. *Diplococcus pneumoniae* and *Klebsiella pneumoniae* are well-defined bacterial types and are easily identified. Members of the Pasteurella group may present more difficulties in classification.

ROUTE OF INFECTION. Respiratory tract.

SOURCE OF INFECTION. Other monkeys or humans.

CONTROL. The building containing monkeys should be warm, 70–75°F (21–24°C), dry, and adequately ventilated, the latter reducing opportunities for the occurrence of cross-infection. Diagnosed cases can usually be successfully treated with parenteral antibiotics.

Tuberculosis

CAUSAL AGENT. *Mycobacterium tuberculosis* var. *hominis* and var. *bovis*; more rarely *Mycobacterium avium*. (These are 'acid-fast' bacilli which require, for microscopic demonstration, to be stained by a technique such as the Ziehl Neelsen's method. After staining, the bacilli resist decolourisation by strong acid solutions and the application of a blue counterstain shows the organisms as red bacilli on a blue background.)

SYMPTOMS. The monkey becomes progressively emaciated and may, when the lungs are involved, show respiratory distress. Death is a common sequel to infection.

POST-MORTEM. Two main types of infection are usually encountered. The first is a generalized infection; whitish necrotic foci being distributed in all organs. Principal sites are the lymph nodes, the alimentary tract, the liver, and the spleen: infection may spread to the lungs. The second type of infection is primarily pulmonary, though there may be secondary spread. Lesions in the lungs can be very large, giving rise to large cavitations.

DIAGNOSIS. During life this is mainly by the use of the tuberculin test, which will be described below. At post-mortem material from infected areas can be stained to demonstrate the organisms. These may not be always easy to find, and a careful microscopic search is required. Cultures can be made on a selective medium, such as Lowenstein and Jensen's medium. Visible growth on culture medium is not present until after three to four weeks' incubation at 98·6°F (37°C).

ROUTE OF INFECTION. Probably orally or by the respiratory tract.

SOURCE OF INFECTION. Primary infection in wild monkeys is probably contracted from man, although the majority of infections in captive monkeys arise within the colony.

CONTROL. Freshly imported monkeys should be screened for the presence of reactors. Diluted mammalian tuberculin or its protein-purified derivative (P.P.D.) is used. The injection is made into the eye-lid, which, within three days in a positive reactor, becomes reddened and oedematous, with the eye sometimes closing completely. Reactors should be killed. The test, while of value, possesses certain disadvantages. Very advanced cases of tuberculosis may not react positively. A number of positive reactors subsequently sacrificed are found to show no macroscopic evidence of infections. Very early cases may fail to react positively. This last source of error can be partially corrected by testing a colony at frequent intervals. No attempt should be made to treat tubercular monkeys, as these animals represent a real hazard to human attendants. The sick animals would require to be kept in strict isolation and managed under conditions designed to ensure the safety of the attendants. These conditions can seldom be met, and there is rarely any justification for attempting treatment. As in other infectious diseases of monkeys, the spread of infection can be minimized by the caging of the animals in small units; by the provision of warmth and adequate ventilation, and the regular changing and sterilization of equipment. The use in monkey houses of bactericidal substances (e.g. hexyl resorcinol) which vaporize on heating to give continuous clouds of bactericidal particles are said to be of value in preventing the spread of disease.

VIRAL

B virus infection

CAUSAL AGENT. Herpes virus simian.

SYMPTOMS. 'Herpes-like' lesions are found on the lips, hard and soft palate, buccal surface of cheeks, and the tongue. These sites may show considerable ulceration. The lesions start as small vesicles which ulcerate and scab. There is sometimes an accompanying nasal discharge and conjunctivitis. The animal usually shows little signs of systemic disturbance, and the lesions disappear within two weeks from the appearance of the vesicles: recovery is apparently complete.

POST-MORTEM. Pathological changes can only be demonstrated by histological examination of the central nervous system, liver, and kidneys.

DIAGNOSIS. By observing the typical lesions.

ROUTE OF INFECTION. It is thought that there are two methods of infection: by contact with food and water contaminated by the saliva of infected animals and by scratching and biting.

SOURCE OF INFECTION. Other monkeys.

CONTROL. Monkeys are often infected on arrival in this country. One series of observations disclosed that of 14,400 screened animals, more than 2 per cent had active lesions. It is doubtful, however, if the disease affects any monkeys adversely. The importance of the disease lies in that it is communicable to man by a bite or scratch from an infected animal, and the resulting B virus infection is usually fatal: of fifteen recorded cases, thirteen have terminated in death. Though many individuals are bitten by monkeys without apparently contracting this disease, the possibility that infection may occur is real, and precautions should be taken when handling these animals. They should, for example, be caged in such a way that they can be caught without undue handling. During any handling rubber gloves should be worn. Should, in spite of all precautions, a handler be bitten, then the wound should immediately be thoroughly cleansed and treated with an efficient antiseptic and the individual placed under medical supervision. Existing scratches and broken skin on the hands and arms of handlers also present a potential portal for virus entry.

HELMINTHS

Oesophagostumum infestation

CAUSAL AGENT. A nematode: *Oesophagostumum apiostumum*.

SYMPTOMS. In severe infestations there may be a diarrhoea, with loss of weight and condition; the animal may die. It is more common, however, for an animal to be only slightly infected and show no signs of illness.

POST-MORTEM. Black rounded nodules are found in the wall of the large intestine, which when opened are found to contain developing worms measuring about 10 mm in length. The gut contents may be bloodstained and contain adult worms.

DIAGNOSIS. During life the adult worms may be found in the faeces and deposited ova from gravid females detected by a suitable flotation method.

ROUTE OF INFECTION. Oral.

SOURCE OF INFECTION. By the ingestion of material contaminated with the nematode larvae from the faeces of other infected monkeys.

CONTROL. By good hygienic management preventing the occurrence of opportunities for cross-infection. In known infected cases phenothiazine has been used to eliminate the worms from the gut, though immature forms encysted in the gut may resist such treatments.

ECTO-PARASITES

Pulmonary acariasis

CAUSAL AGENT. Members of the Pneumonyssus species of acari, e.g. *Pneumonyssus simicola*.

SYMPTOMS. Though these parasites have been said to cause pulmonary distress and other lung complications, opinions are varied as to their pathogenicity, and the condition is described here because infestation is widespread, and evidence of this is frequently seen at routine autopsy.

POST-MORTEM. The lungs show discrete foci which may vary in appearance from small white spots to greenish-yellow-coloured lesions measuring a few millimetres in diameter.

DIAGNOSIS. The pulmonary foci, when opened, contain the causal mites, which can be demonstrated microscopically. The finding of the typical foci containing mites is diagnostic.

SOURCE OF INFECTION. Other monkeys.

ROUTE OF INFECTION. This has not yet been definitely established. It has been suggested that the mite is inhaled into the respiratory passage or, alternatively, that it is ingested and reaches the lungs via the lymphatic system and the blood stream.

CONTROL. As the true pathogenicity of the parasites is in doubt, no real measures have been introduced, though cross-infection has been said to be preventable by dusting animals with DDT.

NON-SPECIFIC

After arrival in this country a number of young monkeys refuse to eat any appreciable quantity of food, even when a large variety of foodstuffs is offered. The animals behaving in this way are not necessarily suffering from any recognizable disease, though the refusal to eat frequently terminates in death due to inanition. Monkeys need more personal attention than most other laboratory species, and a special effort is often necessary on their arrival in the animal house to provide the extra care and interest needed. Following the critical period of acclimatization and adaptation to food and environment, losses from disease and inanition are usually negligible, providing the system of management is good. It is becoming common practice to treat batches of monkeys freshly arrived in this country with a mixture of antibiotics such as aureomycin and terramycin, fed in either the drinking-water or the diet. It is thought that these prophylactic measures aid in the control of specific disease and so-called non-specific complaints.

DOGS

The diagnosis and treatment of the diseases of dogs and cats require the services of a qualified veterinarian. It is, however, advisable that the animal technician should possess a knowledge of the more common diseases which may be encountered during the care and management of these animals, and they are described for this reason.

Distemper/hard pad

It is now accepted that distemper and hard pad are disease manifestations caused by the same virus, vaccination against distemper giving an immunity which prevents the occurrence of both disease syndromes.

Vaccination

Composite vaccines are now available for the simultaneous immunization of dogs against distemper, contagious hepatitis, and leptospirosis; primary

vaccination normally being carried out at eight weeks of age, followed by a second injection two weeks later. Some authorities advise the giving of a further so-called booster dose twelve months after the second injection. These three diseases are described below, and when vaccination is mentioned it is assumed that the vaccine used will be one producing an immunity against all three.

Quarantine

Dogs obtained from outside sources should, irrespective of their age and condition, undergo a period of quarantine of at least fourteen days. This period should be used to free the animals of skin parasites and intestinal worms and, in the absence of well-authenticated records of satisfactory vaccinations, immunization should be carried out against distemper, contagious hepatitis, and leptospirosis.

VIRAL

Distemper

CAUSAL AGENT. A virus.

SYMPTOMS. Distemper usually occurs in animals between three and twelve months of age. There is an elevation of temperature 103–104°F (39·4–40°C) and refusal to eat. The eyes discharge and show a conjunctivitis and keratitis. A nasal discharge is usually present consisting of serous or mucopurulent material. Distressed breathing occurs as a result of bronchitis and bronchopneumonia, and there is often vomiting. Ulcers may be found on the tongue and inside the cheeks. Diarrhoea may be present, and involvement of the central nervous system leads to convulsive fits and paralysis.

DIAGNOSIS. By the clinical symptoms.

SOURCE OF INFECTION. The infected secretions from the nose and eyes of infected or carrier dogs.

CONTROL. By the method of vaccination described above. Should distemper occur in the absence of, or despite, vaccination, then an anti-serum is available, from commercial sources, which is used in treatment. There is, at present, no specific therapeutic agent active against animal viruses, though, should secondary bacterial infection occur, sulpha drugs and antibiotics are often used.

Hard pad

CAUSAL AGENT. A virus.

SYMPTOMS. The disease received its popular name from the swelling and thickening of the pads of the feet which cause a tapping sound when walking. Other symptoms are as described under distemper.

DIAGNOSIS. As described under distemper.

SOURCE OF INFECTION. As described under distemper.

CONTROL. As described under distemper.

Contagious hepatitis/infectious canine hepatitis

CAUSAL AGENT. A virus.

SYMPTOMS. Overt-disease occurs most frequently among dogs between three and twelve months old, though many dogs apparently suffer a sub-clinical infection which passes unnoticed. In obvious clinical cases the temperature is raised 103–104°F (39·4–40°C). There is refusal of food, though the animal may drink copiously. Conjunctivitis and an accompanying discharge from the eyes and the nose is common. The blood clotting time is increased, leading to the appearance of petechial haemorrhages of the skin and to severe bleeding should the dog injure itself.

DIAGNOSIS. In the acute fatal form of the disease the dog may show few symptoms before dying. Viral inclusions can then be found in histological sections of liver tissue collected at autopsy. In less-acute cases it may be difficult to differentiate the disease from distemper and, indeed, it is not uncommon for the two diseases to occur simultaneously in the same animal. The onset of canine hepatitis is, however, usually more rapid than distemper, and prolongation of the bleeding time of diagnostic value.

SOURCE OF INFECTION. Dogs recovered from the disease may continue to excrete the causal virus in the urine for many months. During active infection the virus is present in all excretions from the body.

CONTROL. By the method of vaccination described above.

Leptospirosis (Stuttgart disease, infectious jaundice)

CAUSAL AGENTS. *Leptospira canicola* and *Leptospira icterohaemorrhagiae*.

SYMPTOMS. The animal refuses food and may vomit, the temperature is raised 104°F (40°C), and there is often unwillingness to rise owing to muscular stiffness and pain. There is yellowing of the eyes and mucus membranes of the mouth, and the latter may show haemorrhagic patches. Sometimes the gums bleed, and there may be a bloodstained diarrhoea.

DIAGNOSIS. By clinical symptoms. The causal spirochaete is excreted in the urine and can be demonstrated using the dark-ground-illuminated microscope or in dried smears of urine stained by a silver impregnation method. Antibodies are present in the serum and can be demonstrated by serological tests.

SOURCE OF INFECTION. *L. canicola* is found in the urine of infected or carrier dogs. *L. icterohaemorrhagiae* is harboured by wild rats; up to 40 per cent of groups of these animals have been found to excrete *L. icterohaemorrhagiae* in the urine, and the organism infects dogs following contact with materials contaminated by the urine of carrier rats. Access to the body is probably through the membranes of the mouth, though Leptospira can penetrate unbroken skin.

CONTROL. By the method of vaccination already described. Wild rats should never be allowed to exist in or around dog kennels. It should also be recognized that *L. icterohaemorrhagiae* is a human pathogen, and an infected animal may, unless care is taken, infect handlers.

HELMINTHS

Ascariasis

CAUSAL AGENTS. *Toxocara canis* and *Toxascaris leonina*. Nematode round worms.

SYMPTOMS. The mature worms are commonly found excreted in the faeces, and as they may attain a length of 90–180 mm, they are easily seen. Many adult dogs harbour round worms without showing noticeable signs of illness, though heavy infestations, particularly in young puppies, lead to loss of weight and condition and even death. A puppy may harbour so many worms that they cause occlusion of the small intestine.

DIAGNOSIS. Adult worms are seen in the faeces, and eggs can be detected microscopically using a flotation concentration method.

SOURCE OF INFECTION. Other dogs excreting round worm ova.

CONTROL. Piperazine acid citrate is effective in eliminating both species. Treatment usually needs to be carried out twice. Puppies *in utero* can be infected with *T. canis* from their mother, and bitches should be treated during the gestation period and young puppies a few weeks after birth. Fresh entrants into the kennels can be treated during the period of quarantine.

Tapeworms

CAUSAL AGENTS. Tapeworms of the Taenia genus. *T. pisiformis*, *T. hydatigena*, *T. ovis*, *T. multiceps*, and *Echinococcus granulosus*. A single worm of the Dipylidium genus occurs, *D. caninum*; this is probably the most common tapeworm found in dogs.

SYMPTOMS. In light infestations the animal usually shows no noticeable signs of illness. Heavy infestations can lead to loss of weight and condition and even death. Fits can be caused by helminthic toxins. Segments passing the anus may cause extreme irritation.

DIAGNOSIS. The finding of segments (proglottids) in the faeces; ova can be detected by microscopic examination.

SOURCE OF INFECTION. Taenia species require an intermediate host in which the ova excreted by the host animal, in this case the dog, develop as a cystic stage (coenurus), e.g. the cysts of *T. pisiformis* (*C. pisiformis*) are found in the rabbit. The dog eating that part of the rabbit containing the cyst becomes infected. A single species may be found in several intermediate hosts, e.g. the cysts of *E. granulosus* are found in man and domesticated mammals, and may be the cause of serious illness.

CONTROL. The use of cooked and tinned foods has led to a decrease in the infestation of dogs with certain tapeworms. However, this does not apply, for example, to *D. caninum*, where the intermediate hosts are the dog flea and louse. Control is by the exclusion from the kennels of animals or materials which may harbour cysts. Two drugs commonly used to expel tapeworms from infested animals are arecoline hydrobromide and acetarsol.

Ear canker

There is little value in attempting to tabulate the causes of canine ear canker. The examination, diagnosis, and treatment require the services of a veterinarian. It is commonly thought that ear canker is caused by a mite, *Otodectes cynotis*, and while it is true that this mite may be involved, it is far from being the sole cause of canine ear canker, which is often a complex condition, requiring lengthy and sometimes even surgical treatment.

Skin diseases (mange/ringworm/pruritus/eczema/dermatitis)

As in ear canker, skin diseases of dogs may arise from a variety of causes, e.g. sarcoptic mange (a mite, *Sarcoptes scabei*), ringworm (*Microsporum canis*), allergies, nutritional deficiencies, etc. Though a veterinarian is needed to attend to the skin diseases of dogs, it is nevertheless good practice to thoroughly bath all fresh intakes with a proprietary shampoo, such as a preparation of selenium sulphide in a detergent (Seleen), which is effective in killing fleas and lice, and also has some value in the treatment of demodectic mange and the so-called non-specific dermatoses. Lice can also be killed on the animal by using benzene hexachloride or DDT, though fleas will infest bedding and buildings and return to reinfest the animal unless thoroughly eradicated.

Nephritis

This is common in old dogs, and may cause serious illness. It is common practice in certain establishments to routine test the urine of dogs for the presence of albumin, a positive test indicating the necessity for further investigation.

CATS

Quarantine

Freshly imported cats should, like dogs, undergo a period of quarantine of at least fourteen days, during which time treatment can be given for fleas, lice, and helminths, and vaccination carried out to immunize against panleucopaenia (see below).

BACTERIAL

Kittens and young cats reared in catteries may develop septicaemic conditions due to various bacterial invaders; examples of these being *Streptococcus haemolyticus*, *Pasteurella septica*, and *Haemophilus influenzae*. The author has personal experience of epidemic cat septicaemia where the causative organism was *S. haemolyticus*, and this condition is described. It is similar to septicaemias caused by other micro-organisms.

Septicaemia

CAUSAL AGENT. *Streptococcus haemolyticus* (Lancefield Group G).

SYMPTOMS. Generalized bacterial septicaemia occurs in kittens during the first few weeks of life, though older cats may be infected. When the disease is

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acute and fulminating the kitten may show no noticeable symptoms before death. In more chronic cases there is raised temperature, loss of appetite, loss of condition, and dyspnoea if the lungs become involved.

DIAGNOSIS. During life diagnosis of isolated cases is difficult. During an epidemic detailed examination of dead animals, at autopsy, usually results in the isolation and identification of the causative organism. Post-mortem examination shows infection of the liver and spleen and peritonitis; the lungs may become infected. The causal organisms can be seen in gram-stained smears of infected tissues and isolated on a laboratory medium such as blood agar.

SOURCE AND ROUTE OF INFECTION. Very young kittens can be infected via the navel cord, the mother carrying the causal bacteria in her respiratory or genito-urinary tract and infecting her offspring during post-parturition cleansing. It is also possible that infection may arise from biting injuries or possibly by the aural route (see Ear canker), or by the respiratory passages.

Humans can be carriers of *S. haemolyticus* and *H. influenzae*, and could be responsible for introducing infection.

CONTROL. The use of parenterally administered broad-spectrum antibiotics. Should breeder cats be known to harbour potential pathogens in their genito-urinary tract, it is of value to install into the vagina a topical application of a suitable antibiotic. For example, intramammary penicillin, containing 100,000 units per tube, instilled for two to three days before parturition will prevent the occurrence of streptococcal 'navel ill' in the youngster.

Pneumonia

CAUSAL AGENT. *Bordetella bronchiseptica*. (This is the same bacterium as that associated with pneumonia in the dog, rabbit, and guinea pig.)

SYMPTOMS. Dyspnoea, loss of appetite, and loss of condition.

DIAGNOSIS. During life by the clinical symptoms. At autopsy the lungs show a broncho-pneumonia with varying degrees of consolidation. *B. bronchiseptica* can be recovered by inoculating infected lung on to blood-agar and bile-salt-agar plates.

SOURCE OF INFECTION. Cats or other animals harbouring *B. bronchiseptica* in the respiratory tract.

ROUTE OF INFECTION. Respiratory.

CONTROL. Overt cases respond to parenterally administered antibiotics and injections of specific anti-serum. Vaccination can be of value as a prophylactic measure; the breeders being vaccinated annually, thus passing on a useful degree of passive immunity to their offspring. Though vaccination does not eliminate carriers, it can lead to a substantial reduction in the incidence of overt cases of *B. bronchiseptica* pneumonia.

VIRAL

Feline panleucopaenia/infectious feline enteritis

CAUSAL AGENT. A virus.

SYMPTOMS. The onset is often sudden; the animal refusing to eat: it may vomit and pass fluid stools. Panleucopaenia is highly contagious and fatal; mortality rates in young cats can be as high as 60–90 per cent.

DIAGNOSIS. The disease receives its name from the occurrence of aplasia of the bone marrow, this being reflected in a marked diminution of the leucocytes circulating in the peripheral blood. Leucocyte counts often fall as low as 2,000 per cu mm, and cases have been recorded where circulating white cells were absent. The clinical symptoms allied to a low leucocyte count and high mortality rates are highly suggestive of panleucopaenia.

POST-MORTEM. There is aplasia of the sternum bone marrow, the lymph glands are oedematous, and there is an enteritis, typhlitis, and colitis: the ileum being most affected.

SOURCE OF INFECTION. Other cats whose excretions are infected with the virus.

CONTROL. By the use of protective vaccination. An inactivated vaccine prepared from the tissues of infected cats is available from commercial sources. Kittens are inoculated at six weeks of age, followed by a second inoculation two weeks later. Adult breeders should receive a booster vaccination each year.

Feline pneumonitis

CAUSAL AGENT. A virus or viruses.

SYMPTOMS. Pneumonitis is a highly contagious disease of cats which is seldom fatal. There is loss of appetite, rise in temperature, conjunctivitis with a muco-purulent discharge, and a nasal discharge, the animal frequently sneezing.

POST-MORTEM. The anterior and diaphragmatic lobes of the lungs are consolidated; the trachea and larynx are inflamed and may contain thick mucus.

DIAGNOSIS. By the clinical symptoms. Elementary viral bodies contained in mononuclear cells can be seen in Giemsa-stained smears of mucus from the trachea and larynx and those areas of lung showing pneumonitis.

SOURCE OF INFECTION. Cats, either carriers or overt cases, who sneeze and disseminate the virus over large areas.

ROUTE OF INFECTION. Probably respiratory.

CONTROL. The respiratory tract of the cat is now known to harbour a number of viruses, at least two of these cause the disease syndrome detailed above. A vaccine has been prepared, from one of these viruses, grown in the yolk sac of the embryonic chick, but this, so far, is only available from commercial sources in the United States. Though there is no specific therapeutic anti-viral

agent, pneumonitis is frequently complicated by secondary bacterial infection, particularly of the eyes and nose, and these respond well to topical installations of antibiotics. Parenterally administered antibiotics are often of value when there is bacterial invasion of the lungs. If it is possible to persuade the sick cat to eat well recovery usually takes place more rapidly.

FUNGAL

Ringworm/favus

CAUSAL AGENTS. *Trichophyton felineum*, *Microsporom felineum*, *Microsporom canis*, and *Achorion Quinckeanum*.

SYMPTOMS. Skin lesions may be distributed over the whole of the body, common sites are the head, neck, face, and the fore-paws. The lesions may vary from small bald patches to large areas of scaling and crusting.

DIAGNOSIS. Hairs and skin scrapings from affected areas are mounted in 10 per cent potassium hydroxide and examined microscopically. Spores and/or hyphae of the causal fungus can be seen in or around hairs and scrapings. Cultures are made by inoculating suspect material on to plates of a selective medium such as Sabouraud's. A Wood's light is of value in the diagnosis of *microsporosis*. Infected hairs when illuminated by this type of ultra-violet lamp exhibit the phenomenon of fluorescing, appearing as green coloured. Diagnosis, using a Wood's light, can be confirmed by microscopical and cultural examination of fluorescing hairs.

SOURCE OF INFECTION. Usually other cats and sometimes dogs. *A. Quinckeanum* infection can be contracted from wild mice.

CONTROL. Griseofulvin, a systemic antimycotic, is now used to treat ringworm. The occurrence of a case of ringworm should result in the isolation of the animal and adequate sterilization of the vacated quarters and the eating and drinking utensils, etc.

Body mange

CAUSAL AGENTS. Two mites *Notoedres cati* and *Demodex folliculorum* var. *cati*.

SYMPTOMS. These mites burrow into the skin, lesions being commonly found on the face and around the ears.

DIAGNOSIS. The mites and developmental stages are demonstrable by the microscopic examination of hair and skin scrapings mounted in 10 per cent potassium hydroxide.

SOURCE OF INFECTION. Other cats.

CONTROL. These parasitic manges usually respond to treatment with acaricidal preparations such as benzyl benzoate emulsion and suspensions of benzene hexachloride. Care must be taken during treatment to prevent the animal licking these preparations, which may be toxic to cats when ingested. Demodectic mange may be difficult to cure, and treatment is often prolonged.

Ear canker

CAUSAL AGENT. A mite, *Otodectes cynotis*.

SYMPTOMS. The animal may shake its head and scratch its ears, which contain a dark-brown waxy mass. Occasionally there is an underlying bacterial infection shown by the presence of free pus under the wax.

DIAGNOSIS. The causal mites and other developmental stages can be seen by microscopic examination of the aural exudate mounted in 10 per cent potassium hydroxide.

SOURCE OF INFECTION. Other cats.

CONTROL. A good measure of control can be achieved even when large numbers of cats are affected by *O. cynotis*, providing the system of treatment is adequate. A number of preparations are effective against *O. cynotis*, examples being dibutyl phthalate, benzyl benzoate emulsion, and 5 per cent piperonyl butoxide. Mixed otodectic and bacterial infections are not uncommon, and certain proprietary preparations contain both acaricidal and bactericidal substances and a locally acting analgesic agent which reduces discomfort. Cats' ears should regularly be examined and treated. It is convenient to examine adult breeders before parturition and kittens when they are vaccinated against panleucopaenia. Such a routine will eradicate ear canker or reduce cases to a minimum.

HELMINTHS

Roundworm infestations

CAUSAL AGENTS. The nematode worms, *Toxascaris leonina* and *Toxocara mystax*.

SYMPTOMS. Cats may harbour these worms in the gut without showing noticeable signs of illness. Heavy infestations may, however, cause severe loss of weight and condition.

DIAGNOSIS. Adult worms are found in the faeces, and ova can be detected microscopically after concentration by a flotation method.

SOURCE OF INFECTION. Other cats.

ROUTE OF INFECTION. Oral.

CONTROL. Piperazine acid citrate is efficient administered orally; treatment may have to be repeated once or twice.

Tapeworm infestations

CAUSAL AGENTS. *Taeniaeformis* (*T. crassicollis*), *Taenia pisiformis*, and *Dipylidium caninum*.

SYMPTOMS. As in roundworm infestations, the cat may harbour tapeworms without showing noticeable signs of illness. Heavy infestations can cause loss of weight and condition. There may be vomiting and diarrhoea.

DIAGNOSIS. Tapeworm segments (proglottids) are found in the faeces, and ova may be detected by microscopic examination of faecal suspension.

SOURCE AND CONTROL OF INFECTION. The cystic stage of *T. taeniaeformis* is found in the liver of the mouse and the rat, and the similar stage of *T. pisiformis* is found in the abdomen of the rabbit. Therefore, infection can be prevented by not feeding raw rabbit meat and excluding wild mice and rats. *Ctenocephalides felis*, the cat flea, is an intermediate host for *D. caninum*. The larvae of the flea eat the tapeworm eggs and when the larvae become adult fleas the tapeworm embryos become cysticercoids; the cat being infected by eating these fleas. Therefore infection of cats with *D. caninum* does not take place in the absence of fleas. Drugs commonly used for the elimination of mature tapeworms from the cat are arecoline hydrobromide, drocarbil, arecoline acetarsol, and tetrachlorethylene. Great care needs to be taken in the estimation of dosage of these drugs and capsules of tetrachlorethylene can cause asphyxiation unless swallowed without chewing.

Flea and louse infestations

CAUSAL AGENTS. *Ctenocephalides felis*, the cat flea, and *Felicola subrostrata*, the cat louse.

SYMPTOMS. Heavy infestations may lead to discomfort, scratching, and inflammation of the skin.

DIAGNOSIS. The finding and identification of the causal parasite.

SOURCE OF INFESTATION. Other infested cats.

CONTROL. Fleas and lice can be killed on the infested animal by the use of a number of substances, examples being DDT, benzene hexachloride, and pybuthrin. These need to be rubbed into the animal's coat and then brushed out before the cat has an opportunity to lick its coat. Lice are usually eradicated by treatment of the host animal; fleas, however, lay eggs in bedding, floor cracks, wooden boxes, etc., and these may hatch and live for long periods away from the host animal, and no lasting benefit results from treatment unless the bedding is destroyed and fleas and their eggs eradicated from the living-quarters.

Urinary calculi

A common occurrence in breeding tom cats is the presence, in either the neck of the bladder or the urethra, of large calculi causing urinary obstruction. The cat is restless and irritable, and is often seen attempting to urinate and only succeeding in passing a drop or two of urine which may be bloodstained. Palpation of the abdomen shows the bladder to be full.

Urinary calculi are composed of substances, such as magnesium ammonium phosphate, urates, calcium carbonate, cystine, etc., and when a calculus is detected it is usually so large as to require surgical removal, and recurrences are not uncommon.

Various remedies have been suggested for the prevention of the formation of calculi: lowering the mineral content of the diet, increasing the vitamin A intake, altering the urinary pH by feeding substances such as sodium acid phosphate, etc. None of these remedies are of proven value, and may be difficult to apply in a large cattery.

Pests of the Animal House

PART ONE

ANIMAL PESTS

There are many pests which can infect animals and animal houses. Pests feed on the blood (lice, fleas) or skin debris (mites) of living animals, or on undigested food present in excreta (house flies), or on foodstuffs intended for consumption by animals (wild rodents). Pests breed on living animals (lice, mites), in undisturbed dirt and bedding (fleas, house flies), or in cracks in walls or furniture (cockroaches, bedbugs).

The best precaution against infestations is a well-designed, constructed, and equipped animal house which is regularly and thoroughly cleaned. In a well-designed animal house the interior surfaces are hard, smooth, impervious, and washable; there are no sharply angled joins or corners where dirt can accumulate; service pipes and ducts are easily accessible for cleaning; woodwork is kept to a minimum; windows fit tightly. The objects of such a design are to minimize both the opportunity for access by pests and the provision of breeding places for them, and to facilitate cleaning of the building. The fabric of the building must be watched for signs of deterioration or damage, and such defects must be reported. Ideal breeding places for some pests are offered by cracks in plaster work (notably between walls and window- or door-frames), where service pipes enter walls, behind light fittings, flaking paint or loose or broken tiles, or in rotting wood. Wild rodents can enter a building by gnawing through door-posts, through the gap between the bottom of a door and a worn doorstep, through broken air bricks, or secreted in sacks or bales of food and bedding. Once established in a building, wild rodents will live and breed in hollow walls and floors and in service ducts. Buildings having solid walls and floors and unenclosed service pipes offer little shelter for these pests. Flies and mosquitoes enter through any fissure. Flies are attracted by the smell of rotting vegetation and excreta, so all refuse should be incinerated as quickly as possible. If flies are particularly troublesome and their breeding-place is unknown or unassailable the entrance to the animal house should be fitted with double screen doors and the windows kept shut or be screened. House flies do not attack laboratory animals directly, but they are vectors of disease, and their presence is therefore undesirable. Food and bedding should be inspected before delivery is accepted, and if the materials are infested the whole consignment should be returned to the supplier.

While it is important that the fabric of the building should be in good repair, it is essential that the building and its contents are kept clean. The importance of the regular and thorough cleaning of an animal house cannot be over-stressed, for the control of diseases and infestations depends on the conscientious performance of this seemingly inglorious task. A brief account of the life cycles and habits of common pests of animals and animal houses will show how regular cleaning can break across the life cycles of some pests and thus prevent them from attaining sexual maturity.

It may be impossible to eradicate, without treating the animals themselves, pests which never leave the host animal. All infestations cause continuous irritation to the host animals, and treatment for infestations may cause them temporary distress. It is the duty of animal technicians to see that the animals in their care are not unnecessarily exposed to either experience. Details of suitable treatments are given in the table at the end of the chapter.

PARASITES

Pests which feed by attacking, without killing, other animals are called *parasites*. The attacked animal is called the *host*. Parasites which live on, or immediately beneath the surface of, the skin of animals are called *ectoparasites*.

Ectoparasites are harmful because they: (i) debilitate the host by sucking its blood; (ii) cause much irritation by biting or burrowing; (iii) carry diseases which can be transmitted to the host; and (iv) cause chronic diseases of the skin.

Insect pests include *flies, moths, beetles, bugs, fleas, and lice*. The bodies of insects are divided into three regions, the *head*, the *thorax*—from which arise *three pairs of legs*, and the *abdomen*; some have one or two pairs of wings. All insects are bi-sexual, and the females lay eggs. From the egg hatches either a miniature adult (nymph) or a grub (larva), which has to pass through a *pupa* stage from which the adult form emerges. In insects growth to the sexually mature form is achieved by *moulting* from stage to stage. The number of moults undergone varies from species to species.

The *Arachnida* are represented by the *mites* and *ticks*, neither of which is typical of this group. The bodies of mites and ticks are not so clearly divided into regions as are the bodies of insects. They have *four pairs of legs*, and do not have wings. They are bi-sexual and lay eggs from which *larvae* hatch. The larvae resemble the adults, but are smaller and have only *three pairs of legs*. Growth is achieved by moulting.

FLEAS

Fleas are blood suckers and can transmit disease through their bites. They are dark in colour and are about 2 mm long. Female fleas lay eggs anywhere, but often on the fur of the host, from where they fall, or are shaken, to the ground. Larvae hatch from the eggs and feed on decaying organic matter, particularly the excreta of adult fleas. The larvae spin cocoons around themselves in which they develop into pupae before finally reaching the adult form. In warm conditions the complete life cycle may take from three to four weeks, and a flea may live for three to four months. In cool conditions the flea may

remain quiescent in the pupa stage for many weeks, and the adult life span may be as long as a year.

Each variety of flea has its favourite host species on which it prefers to feed, but each can, and does, feed from other hosts. Thus the Great Plague of the Middle Ages was spread from infected rats to man by the bite of fleas.

Murine typhus and the protozoan infection, *Trypanosoma lewisi*, are spread from rat to rat by fleas. Several tapeworms (Cestodes) spend part of their life cycle in the flea. (The flea is then said to be an intermediate host.) Tapeworm eggs are eaten by flea larvae and the tapeworm begins to develop. If an infected flea is eaten by a mammal the immature tapeworm is liberated to develop to the adult state within the gut of the mammal. Dog tapeworms are transmitted by this method.

Eradication

Animals must be treated to kill the adult fleas living on them.

Adult fleas, larvae, and pupae will be present in the bedding, which must be destroyed, and in the crevices of cages and trays, which must be sterilized. Walls, floors, fittings, and equipment must also be cleaned and treated with insecticide.

LICE

Lice are about 2 mm long, and vary in colour from creamy grey (unfed) to dark red (newly engorged with blood). Lice never leave the host animal and tend to be specific in their choice of host. The louse starves to death if it is separated from a host. The eggs are less than 1 mm long and are stuck to the hair of the host, on which they are just visible as a silvery scurf. Nymphs hatch from the eggs after five–eight days and undergo three or five moults to reach maturity. The whole life cycle is accomplished in two–three weeks.

Lice may be divided into two main groups—the blood-sucking lice, *Anoplura* (which occur only on mammals), and the *Mallophaga*, which feed on scurf, epidermis, and feather.

Lice are known to transmit murine typhus from rat to rat.

They may occur on all the common laboratory animals. Lice cause intense irritation to the host animal, which loses condition rapidly and may even die as the result of a heavy infestation. Lice spread from host to host by direct contact, and the infestation spreads quickly among overcrowded animals.

Eradication

The host animal must be treated, but clean cages and bedding should also be given as a precautionary measure.

BEDBUGS

Bedbugs are rare today, but are a potential danger, because a colony of considerable size might become established before its existence was suspected.

The bedbug is dark brown in colour, and is a flat oval in shape, about 3 mm × 4 mm. Bugs live and breed in cracks in walls or behind loose wallpaper or flaking paint. They emerge from their hiding-place only for a short time at night to feed. Bugs feed only on the blood of mammals. Eggs are laid in

the hiding-places. Nymphs hatch from the eggs and undergo five moults to reach sexual maturity. The nymphs also feed by sucking the blood of mammals. The life cycle of the bug may be completed in any period from one month to one year, depending on the environmental temperature and the opportunities for feeding.

Bedbugs found in this country are not known to transmit disease, but they can cause severe anaemia in the host, and their bites are intensely irritating.

Bugs can become established only in dirty, neglected premises.

Eradication

Control measures should be focused on locating and treating the hiding- and breeding-places.

MITES

Mites may be classed in two main groups: (i) *blood-sucking mites*, those which live and breed in dirt and crevices and visit the host only to feed, and (ii) *mange mites*, which live and breed on, or in, the skin of the host.

Mites lay eggs from which larvae hatch. After two or three moults the adult form is reached. The whole cycle may be completed within about twelve days (Psoroptes) or about seventeen days (Sarcoptes).

Mites are barely visible to the naked eye. They tend to be host specific, and are most troublesome on poultry, mice, and rabbits, but they also occur on other species.

The blood-sucking mites (*Liponyssus bacoti*) prefers to feed on the rat, but will also feed on other rodents and on man. The bite is extraordinarily irritating. This mite is said to be a vector of tropical typhus.

The 'Red Mite' (*Dermanyssus gallinae*) is an important pest of domestic fowls. These mites feed only at night, and hide in the crevices of poultry houses at or during daylight.

Mange mites are of the family Sarcoptidae, and for mammals, may be subdivided into three groups—mites which live *on* the skin (Psoroptes), those which burrow *in* the skin (Sarcoptes and Notoedres), and those which burrow in the sebaceous glands and hair follicles (Demodex).

Mange mites living and breeding on skin debris cause flaking of the skin. Ear canker in rabbits is caused by such a mite (*Psoroptes communis cuniculi*). Control of surface-living mites is relatively easy.

Burrowing mites can be more difficult to eradicate, because their eggs are laid, and hatch, and mature, and die within the burrows in the skin. Some mites have a preference for certain areas of the host body, such as the nose, ears, legs, or tail. Scabies (now almost non-existent) in rats is due to such a mite (Notoedres). Rat scabies is transmissible to man. *Cnemidocoptes mutans* causes 'scaly leg' in poultry, and *C. laevis* causes 'depluming itch', so called because irritation from mites living in the skin round the base of the feathers causes the birds to pull at, and break, the feathers.

Mites which enter the sebaceous glands and hair follicles (Demodex) are very difficult to eradicate. These mites may affect any of the common laboratory animals.

Eradication

The presence of scabs, 'warts', pustules, or flakes of dead skin may indicate an infestation with mites. These excretions must be removed before treatment, to permit good penetration of the medicant.

Animals suspected of mite infestation must be isolated and not returned to the general stock before it is certain that treatment has been completely successful.

Infestations are spread by direct contact. Overcrowding greatly facilitates the spread of infestations.

TICKS

Ticks are very rare on small animals, but are occasionally found on larger animals, such as dogs, sheep, and horses. They are large, 3 or 4 mm long. Ticks affix themselves to the host animal, and because they are so easily seen, a severe infestation could not develop unnoticed. Ticks may be killed quickly by touching them with sheep dip or other suitable preparations. The temptation to pull the tick out must be resisted, as it causes much pain to the host animal.

FLIES

Flies can act as the vector of any disease which can be picked up from excreta and transported to contaminate food or water.

The larvae of some flies burrow in the skin of mammals, including man, e.g. *Bot* and *Warble fly* larvae affect sheep, cattle, and horses, and the infestation can occur on man.

Blood-sucking flies visit their hosts only to feed. Horse and cattle flies breed in marsh land. Blood-sucking flies are not troublesome in laboratories in this country, but they are much to be feared in the tropics, where they are vectors of trypanosomiasis (sleeping sickness).

See also:
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CONTROL OF PESTS

The chemicals DDT (dichlordimethyl trichloro-ethane) and gamma-BHC (gamma-benzene hexachloride) have been widely and successfully used for controlling pests. These chemicals are highly toxic to pests, and are also toxic to small laboratory animals when fed or injected in large doses. DDT is more toxic than BHC. Young or weak animals are more susceptible to the toxic effects of DDT and BHC than are other animals, and are more safely treated with pyrethrum.

The modern method of controlling pests is by the use of *continuous-flow aerosols*. An AEROSOL is a semi-permanent suspension of solid or liquid particles in a gas. An aerosol can be produced by heating a suitable insecticide to a temperature just below its boiling point. Apparatus for producing aerosols

Host	Parasite		Treatment	
	Common Name	Latin Name	Active Constituent	Commercial preparation
General	Cockroaches	*Blatta orientalis; Periplaneta americana; Blatella germanica*	1. 50% pyrethrum plus 50% powdered NaF 2. 0·5% γ-BHC * 3. 10% DDT † 4.	Dieldrin insecticidal lacquer
	Bedbugs	*Cimex lectularius*	1. 0·5% DDT † 2. 10% β-thiocyanoethyl laurate 3. 5% DDT in kerosene 4. HCN	Lethane
	Flies		1. 5% DDT in kerosene 2. γ-BHC 3. 4. DDT and γ-BHC	Chlordane
	Lice	Various	1. Pyrethrum 2. Rotenone 3. DDT 4. γ-BHC 5. DDT and γ-BHC	Derris
	Fleas	Various	1. Pyrethrum 2. Rotenone 3. 0·5% DDT 4. 0·5% BHC	Derris
Food	Weevils Flour moths Mites Beetles Cockroaches	*Calandra granaria* *Ephestia kuhniella* Various Various Various	1. Carbon disulphide 2. Ethyline dichloride and carbon tetrachloride 3. Hydrogen bromide 4. Methyl bromide 5. Heat 6. DDT 7. γ-BHC	
Mouse	Louse	*Polyplax serrata*	1. Sodium fluoride 2. Pyrethrum 3. Kerosene 4. 2-(p-tert-Butyl phenoxy) isopropyl-2-chloroethyl sulphite	Aramite—15w

Method	Dosage	Frequency of treatment	Authority	Notes
Dust into and around hiding sites and crevices		Weekly	1	Poisonous to man
Paint around nesting and hiding sites as a lacquer		One treatment lasts 12–18 months	2, 3	
Spray hiding sites			1	For light infestations
Spray			1	
Fumigation		For 2 days	1	Requires expert handling; for control of bedbugs treatment of the rooms and cages is more important than treatment of the animals
Spray walls				
Spray walls				
Spray walls				
Aerosol		Continuous	2, 3	
1. Dust		Twice, at 10–14-day intervals	4	Dusting and dipping animals probably better than spraying ‡
2. Spray				
3. Dip				
Aerosol		Continuous	2, 3	
1. Dust			1, 4	Important that rooms and cages are thoroughly treated
2. Dip				
3. Spray				
Fumigation				For small-scale food treatment in bins
Fumigation				
Fumigation				For large-scale treatment requires expert handling
Heat—140°F		Few minutes	5, 6, 7	Heat must penetrate all food
1. Dust		1 several times according to severity		
2. Spray				
3. Smoke				
1. Dust		Twice at an interval of 2 weeks	8	
2. Dip				
3. Spray				
Dip	2% suspension and wetting agent	Twice, as above	9, 12	

| Host | Parasite | | Treatment | |
	Common Name	Latin Name	Active Constituent	Commercial preparation
	Mites	*Myocoptes musculinus*	1. γ-BHC 2. ββ-Dithiocyano diethyl ether 3. 2-(p-tert-Butyl phenoxy) isopropyl-2-chloroethyl sulphite	RID-O Aramite—15w
	Mites	*Myobia musculi*	1. As 3 above 2. Pyrethrum 3. Tetraethyliuram monosulphide 4. DMC§	Aramite—15w Tetmosol
	Ear Mites	*Psorergates simplex*	1. Dibutyl phthalate	
Rat	Louse	*Polyplax spinulosa*	1. See general section 2. Malathion	
	Mange and mites Rat scabies	Notoedres sp.	1. γ-BHC 2. Tetraethyliuram monosulphide 3. Benzyl benzoate	Lorexane Tetmosol
	Blood-sucking mite	*Liponyssus bacoti*	1. Rotenone 2. γ-BHC	Derris
Guinea pig	Lice	*Gyropus ovalis* *Gliricola porcelli*	1. See general section 2. γ-BHC and DDT	
Rabbit	Ear canker	*Psoroptes communis* *Chorioptes cuniculi*	1. 2% Phenol in liquid paraffin 2. Benzyl benzoate	
—	Body mange	*Notoedres cuniculi* *Sarcoptes cuniculi*	1. γ-BHC 2. Benzyl benzoate	
Poultry and birds	Scaly leg	*Cnemidocoptes* sp.	1. See above	Tetmosol
	Mites	*Liponyssus* sp. *Dermanyssus gallinae*	1. Rotenone 2. γ-BHC	

Method	Dosage	Frequency of treatment	Authority	Notes
Dust		Once or twice	10	
Dust		Once or twice	11	
Dipping	2% suspension and wetting agent	Twice in 3 weeks		
Dipping	As above	Twice in 3 weeks	9, 12	Other methods probably more effective
Spray			8	
Dipping	Dilute to 1·4%	Once	10	
Dipping	0·2% in 50% ethyl alcohol	Two or three times at 5-day intervals		The alcoholic solution appears to increase the effectiveness of this miticide
Paint over affected ears	Neat	Once, possibly twice	14	
Dust		Twice in 2 weeks	15	
⎱ Local treatment of infected areas			16, 1	
Dust		Every 7 days until cured	1	
Aerosol		Continuous	1, 3	
Smear or brush on affected areas			1	Hard scabs may need to be softened and removed with oil before treatment
Paint		Every 7 days until cured	1	
Paint			1	Needs to be well rubbed in
Dust				
Dust				

Host	Parasite		Treatment	
	Common Name	Latin Name	Active Constituent	Commercial preparation
Dog	Mange Fleas Lice	Various Various Various	1. See general section 2. Benzyl benzoate 3. Rotenone 4. DDT 5. γ-BHC 6. Dieldrin 7. Tetraethyliuram monosulphide 8. Selenium sulphide	
Cat	Lice	*Felicola subrostrata*	1. 10% Na proprionate 2. γ-BHC 3. Pyrethrum	Procid
	Mange	*Notoedres cati*	1. Benzyl benzoate emulsion	
	Ear mites	*Otodectes cynotis*	1. 5% piperonyl butoxide	
Hedgehog	Ticks	*Ixodes* sp.	1. Rotenone 2. Arsenical cattle dip	Derris
	Fleas		1. Rotenone	Derris
Snakes	Mites	*Ophionyssus serpentinum*	1. Rotenone	

Reproduced from *Journal of Animal*

* γ-BHC = the γ-isomer of benzene hexachloride.
† DDT = dichlordiphenyl-trichloroethane.

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Method	Dosage	Frequency of treatment	Authority	Notes
Dust Paint			1	
Dust Dust Dust		Twice, a few days apart	1	DDT liable to be toxic
Paint		Twice, a few days apart		Scour affected area with soap to remove scabs first
Paint		Twice, a few days apart		
Paint–touch with emulsion			1	
Dust			1	
				Said to be toxic to snakes

Technicians Association, **11**, No. 3 (1960).

‡ DDT is definitely toxic if too much is used, especially if young rats and mice or sick and weakened animals are treated. In these cases pyrethrum may be advisable.

§ DMC = (dichlorphenyl) methyl carbinol.

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of DDT and BHC and other insecticides are available commercially. The installation of such equipment in animal rooms and food stores is probably the most efficient way to control pests.

Aerosols MUST NOT BE USED if the presence of insecticides would vitiate the experimental work of the laboratory. For this reason an aerosol must not be installed, or an existing aerosol apparatus operated without first ascertaining that it is safe to do so.

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PART TWO

PESTS OF FOODSTUFFS

The storage of foodstuffs presents many problems, one of which is to keep it free from insect and other pests. The food of laboratory animals (which is rich in cereal products and is often fortified with proteins) is particularly susceptible to infestation by pests.

The common invaders of the food store can be grouped under the orders to which they belong, namely:

Lepidoptera	(moths)
Coleoptera	(beetles)
Orthoptera	(cockroaches)
Acarina	(mites)

LEPIDOPTERA

The adult moths are short-lived. The body and wings are covered with scales, and the mouth is adapted for sucking up juices. The larva of the moth is a grub or caterpillar. The mouth parts are adapted for biting, hence only the larvae damage foodstuffs. Some of the more common moths are:

Ephestia kuhniella (The mill moth or Mediterranean flour moth)

This moth usually infests meals and flour. The adult moth is about 13 mm. long, and is a pale grey colour with wavy markings on the fore-wings. The female lays 50–350 eggs, which hatch in seven–fourteen days, when the young caterpillars crawl about the foodstuffs trailing a silken thread which is produced from an opening near the mouth. In about ten weeks the larva is fully grown. It then leaves its source of food and prepares a cocoon, usually in the angles of walls and ceilings, or in any other protective crevice. The larva pupates in the cocoon and the adult emerges after about twenty days.

Foodstuff invaded by moths is often caked together by the silken web produced by the larvae.

Ephestia elutella (The cacao moth)

This moth is another common invader of grain products, such as wheat and wheat offals; it is also found in cacao beans, ground nuts, linseed, and other oilseeds.

The adult moth is about 7–9 mm long and is grey in colour, with a pair of lighter bands across the fore-wings. The eggs are laid loose on the surface of the grain or seeds. The larvae attack and penetrate the germ of grain. Foodstuffs which have been attacked by *Ephestia* smell sour and are contaminated by the droppings and webbing of the caterpillars.

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COLEOPTERA

Beetles have two pairs of wings, the hind pair are used for flight and the front pair form a hard, protective cover. The front, protective wings (elytra) are joined together in beetles which have lost the power of flight.

The larvae vary in type from active mealworms to sluggish, fleshy grubs. Both larvae and adults have mouth parts for biting, enabling them to feed on and damage foodstuffs.

Calandra granaria (The grain weevil)

This is a polished, dark-brown or black insect about 2·5–5 mm long. The hind-wings are not developed, and it is unable to fly. The adult lives for seven to eight months. The female lays about 100 eggs, which are deposited in small holes bored into the grain. The larva is a small, white, legless grub which tunnels into the endosperm of the grain. After several moults it becomes a pupa within the grain, and the adult finally eats its way out of the grain. The whole life cycle takes from twenty-eight to forty days, according to the environmental temperature. This weevil not only attacks wheat, oats, barley, maize, rye, rice, and other seeds but it can also breed in flour.

Calandra oryzae (The rice weevil)

This has a similar life history to the grain weevil. It can be distinguished by its four red or yellow spots on the wing-cases, and by the presence of hind-wings under the elytra. It has been known to fly in Britain in warm, sunny weather. The rice weevil will attack the same range of grains as will the grain weevil.

Stegobium paniceum (The biscuit weevil or bread beetle)

The adult beetle is from 1·75 to 3·75 mm long, is cylindrical in shape and a light-brown colour. The life cycle occupies two to seven months according to temperature. The larvae are active, and when fully developed form cocoons from a gelatinous secretion mixed with the surrounding food. The larva turns into a pupa and finally into an adult beetle which bites a hole in the cocoon and emerges. This beetle has been found in a variety of foodstuffs.

Dermestes lardarius (The bacon or larder beetle)

The beetle is dark brown in colour, 7–9 mm long, and has a fawn, six-spotted band on the elytra. The female lays up to 175 eggs, which hatch out into brown, hairy grubs in about twelve days. The fully grown grubs leave the food and tunnel into surrounding material, such as woodwork, where they pupate, to emerge as adult beetles after about ten days. The bacon beetle requires food of animal origin to complete its life cycle, which takes about fifty to sixty days. An adult beetle may live for twelve months. Damage is caused to foodstuff by the tunnelling of the larvae, and by contamination with skin casts and faeces.

ORTHOPTERA

The cockroach, popularly called the black-beetle, though it is neither black nor a beetle, is a pest found in bakeries, kitchens, and food stores. The two species

commonly found in Britain are the Common or Oriental cockroach and the German cockroach. Both are natives of Africa; the common cockroach reached England about the time of Queen Elizabeth I and the German cockroach some time later.

The females lay large numbers of eggs in cases; these cases are carried around by the females. The young are called nymphs; they resemble the adult cockroach, but are wingless. Wings grow in the final moult, except in the case of the female, *Blatta orientalis*. Cockroaches develop slowly and have a long life. They require moisture and heat for satisfactory development. They infest heated buildings, living behind hot-water and steam pipes and in other suitable crevices, only emerging at night in search of food. They contaminate foodstuffs with their faeces and may carry infection. Cockroaches are the intermediate host of a nematode parasite of rats, *Gigantorhymmochus*.

The three common species are:

Blatta orientalis (Oriental cockroach)

A large dark-brown insect, the so-called black beetle, with a life cycle of some 300 days. The female is wingless.

Blattella germanica (German cockroach)

A small, yellowish-brown insect known as the steam fly is the most troublesome of the cockroaches. It develops rapidly, completing its life cycle in about fifty days.

Periplaneta americana (American cockroach)

A large, dark-brown insect with a life cycle of approximately 200 days.

ACARINA

These are minute animals related to spiders, but without the well-marked division of the body seen in the latter. Probably the most troublesome mite found in foodstuffs is *Tyroglyphus farinae* (the flour mite). It infests stored wheat, flour and offals, and other cereals and their products. A greyish dust is the first sign of infestation, but careful examination will reveal mites. Food is damaged in several ways: the germ is eaten away, grain is eaten or bored, and the food is fouled by excreta, cast larvae skins, and dead mites. The adult female mite lays twenty to thirty eggs scattered about the foodstuff. After four days the six-legged larvae emerge from the eggs, and after a few days feeding they become inert and moult to become eight-legged nymphs; this stage lasts from six to eight days. After further moults the adult is produced.

The life cycle takes from seventeen to twenty-eight days, but is influenced by both temperature and humidity. Mites breed much more rapidly in damp conditions, and cannot thrive on material containing less than about 12 per cent moisture. However, this can be a local condition, and the bulk of the sample may have a much lower moisture content than that of the portion where the mites are living. The optimum temperature for mites to breed lies

between 64° and 77°F (18° and 24°C), but mites are very resistant to cold and cease to feed at only a few degrees above freezing point. Below this temperature they hibernate.

PREVENTION AND CONTROL

Most of the pests mentioned require both warmth and moisture to enable them to complete their life cycles satisfactorily. Further, they require some form of cover in which to hide if the food is removed or disturbed. It follows that a food store should be cool, dry, and well ventilated, and should be free from crevices or corners where food and dirt can accumulate and remain undisturbed.

The room and all food containers should be kept clean, and spilled foodstuff should not be allowed to remain lying on the floor.

Food containers should be emptied and sterilized before refilling.

Unnecessarily large stocks should not be carried, and all foodstuffs should be used in strict rotation of delivery.

All empty food sacks should be returned or burnt as soon as possible, and surplus foodstuffs should be burned. Both these items offer suitable and secure places for breeding. All fresh deliveries of foods as well as existing stocks should be inspected for signs of infestation.

To prevent insect infestation of food stores is almost impossible, and some methods of control must be undertaken.

Both heat and fumigation can be used to destroy insect invaders. Exposure to temperatures of 140°F (60°C) will kill many of the common pests, but it may be necessary to expose a mass of food to temperatures of 212°F (100°C) for 1 or 1½ hours to get complete heat penetration. At such temperatures some of the constituents of the food may be so altered, or even destroyed, as to render it useless as a feeding material.

Dusting powders are useful in preventing infestation, and in cases of slight infestation. Powders containing gamma-BHC are very effective.

Sprays of various types are useful, and insecticides containing pyrethrum are particularly suitable against moths. Continuous-flow aerosols prevent the spread of infestations.

In cases of heavy infestation it may be necessary to fumigate the whole food store. For this purpose hydrogen cyanide and ethylene oxide are used commercially.

The former is very poisonous to man, and should only be used by an experienced specialist operator.

Ethylene oxide has the advantages of having a marked odour and of being less toxic to man than hydrogen cyanide; but has the disadvantage of being highly inflammable, and a mixture of the vapour with air is explosive. The gas is used with an excess of carbon dioxide to reduce the explosive risk, but again it should be used only by an expert.

Small quantities of material may be fumigated by placing it in a suitable bin and pouring in a mixture of ethylene dichloride and carbon tetrachloride, sealing it up, and leaving it for 48 hours. Such food cannot afterwards be fed to animals.

Smoke canisters of various sizes containing gamma-BHC are available. They are suitable for use in lofty buildings.

It should be remembered that prevention is better than cure. A careful watch should be kept on all stored foodstuffs, and at the first sign of infestation immediate action should be taken to eliminate the pest. It is often easier, cheaper, and more effective to destroy a batch of infested food than to try to disinfest and use it.

Humane Killing

Untrained personnel should, in no circumstances, be permitted to kill or administer barbiturates to animals. The animal technician must have complete confidence in his ability to kill cleanly, swiftly, and humanely, and, therefore, must be thoroughly conversant with the correct methods of handling animals and must have the requisite knowledge of the equipment and materials at his disposal.

The need for killing animals may be classified in four groups:

(i) to alleviate unnecessary or prolonged suffering because of disease or accident;
(ii) because an animal has become redundant;
(iii) as part of an experiment;
(iv) slaughtering for food.

In the third case the decision to kill is the experimenter's, although it is the duty of the animal technician to keep the experimenter informed of any change in the animals' condition. Slaughtering for food is carried out by slaughtermen at licensed premises, in accordance with the laws relating to food hygiene and humane killing.

In groups (i) and (ii) the onus of taking the decision to kill falls upon the animal technician.

Euthanasia

This is the term for painless killing. If an animal passes quickly and quietly into the unconscious state and death ensues before consciousness is regained, then it may be assumed that the animal has been correctly and humanely killed.

The following basic principles should be adhered to:

(i) Handle the animal carefully and gently, taking care not to frighten or antagonize it unnecessarily.
(ii) Remove the animal from the animal room.
(iii) Do not kill in the presence of another live animal.

Physical methods

Such physical methods as stunning are distasteful to the operator, but these methods, if applied efficiently, are often less distressing to the animal than

some more complicated method. After stunning the animal usually bleeds from the nose and/or mouth. It is important that blood is cleaned from all surfaces, especially the hands, before another animal is touched, so that it may not be distressed by the smell of blood. Physical killing may also be carried out by dislocation of the neck, thus breaking the continuity of the spinal cord in the cervical region.

Inhalation of poison gases

Strict precautions must be observed when killing by means of poison gas, because these gases present a health hazard to other animals and to personnel. Lethal chambers should therefore be used. These should be situated in a room apart, completely isolated from the animal rooms. The room itself must have ample ventilation and contain no naked lights. Carbon monoxide, in the form of coal gas, is probably the most commonly used lethal agent, and its inhalation causes little or no distress to animals or human beings. It should be noted that the minimum of distress is caused to the animals if the lethal chamber is filled with coal gas before the animals are placed in it. Because of its characteristic smell, any escape of coal gas is easily detected, though it should be remembered that carbon monoxide itself is odourless. Where coal gas is not readily available carbon monoxide may be obtained from the exhaust gases of a four-stroke petrol or diesel engine. The amount of carbon monoxide gas in exhaust fumes from a normally running engine is negligible, but if the air/fuel ratio is altered (i.e. if the choke is used) the carbon monoxide content of the exhaust fumes can be raised to about 14 per cent. Exhaust gases are hot and dirty, so they must be cooled and filtered by passing the fumes through a large volume of cold water and a metal gauze and a cloth filter. This method is in common use at poultry stations for killing large numbers of unwanted chicks.

Nitrogen may be used, but for general purposes it has no great advantage over coal gas, and is much more expensive.

Inhalation of volatile anaesthetics

Ether and chloroform are the two volatile anaesthetics most commonly used. Ether is highly inflammable, and a mixture of ether and air may be exploded by a naked flame. The use of ether should therefore be confined to cases of necessity. These gases should be used in a lethal chamber. A large, lidded glass jar or a glass dessicator is satisfactory as a chamber for killing small animals. A chloroform-soaked pad is placed at the bottom of the jar and a fine-mesh wire grid placed over and above it. Chloroform is a skin irritant, and therefore on no account should the liquid anaesthetic come into contact with the animal's skin.

A metal (or even wooden) chamber is used for larger animals, such as guinea pigs, rabbits, or cats. This chamber should be fitted with a large *glass* (*N.B.* Perspex is soluble in chloroform) observation panel and must have an inlet point through which a chloroform-soaked pad can be inserted. It should also be fitted with an adjustable air vent. A wire-mesh grid must be fitted on the floor of a metal chamber, as the animal may become panic-stricken if it cannot retain its foothold. The animal is placed in the chamber and the lid closed but the air vent left fully open, and the chloroform-soaked pad is then

inserted. In a few moments the animal sinks into unconsciousness and falls on its side; the air vent is then closed. Note the difference between the gradual administration of chloroform and the instant administration of undiluted coal gas. The animal should be left in the chamber until death is certain; preferably until rigor mortis is established.

A mixture of equal parts of chloroform and carbon tetrachloride may be used instead of chloroform alone.

The room temperature should be about 70°F (21°C) to obtain the quickest results.

Inhalation of a non-toxic mixture of air and carbon dioxide

Recent advances in humane killing of large numbers of pigs has shown that they may be passed through a tunnel containing an odourless, non-toxic mixture of air and carbon dioxide. In this way each pig drops peacefully asleep in less than half a minute and stays in this condition until the animal can be bled-out in the conventional manner used for normal bacon pigs.

The oral administration of barbiturates

Barbiturates in capsule form may be given to cats and dogs, but only under qualified supervision. The method is fairly satisfactory, but the animals' reaction is largely dependent on the amount of the stomach contents. Thus, this method should be avoided if the animal has been fed recently. Sodium pentobarbitone is widely used for this purpose, and is packed specially in capsules containing $1\frac{1}{2}$ grains of the drug.

Killing by means of injections

Animals which can be conveniently injected intravenously may be killed swiftly by means of the injection of a saturated solution of magnesium sulphate. The size of the dose depends on the size of the animal and may vary from 5 to 20 ml. Magnesium sulphate is much cheaper than most other killing agents, so it is common practice to sedate the animal with sodium pentobarbitone and then kill with an intravenous or intracardiac injection of saturated magnesium sulphate solution. No other route for the injection of magnesium sulphate solution is effective.

Intravenous or intracardiac injections of barbiturates are much swifter in effect than intrathoracic or intraperitoneal injections, but it will be realized that the former are the more skilful operations. The intraperitoneal injection of sodium pentobarbitone apparently causes little discomfort to the animal and, providing a large enough dose is injected, the animal will quickly become unconscious and death will ensue. The speed of the response of animals to intraperitoneal or intrathoracic injections of sodium pentobarbitone varies considerably, but the simplicity of these methods commends them.

Electrocution

A method of killing which has come into more common use during recent years is electrocution. It has proved satisfactory for dogs, but is not recommended for cats. Cat fur is a poor conductor of electricity, and therefore it is

difficult to ensure positive contact between the electrodes and the skin surface. Since a transformer is needed to raise the voltage, adequate insulation is essential to protect the operator.

Electric stunning

The electric stunning of larger animals as an integral part of humane killing is an important feature of killing for food. At present this method is mainly used for stunning pigs. Sheep and calves may be dealt with in this manner, and research is being carried out to include its use for cattle, horses, and large mammals in general. After exhaustive tests it has been found that in about 10 per cent of the animals stunned in this manner there has been some slight lung haemorrhage. Otherwise all organs and muscles were undamaged. The animals usually remain unconscious for about 150 seconds, but researchers found that the ideal time to dispatch the animals is from 4–6 seconds after stunning. Prior to this time and after there is likely to be convulsive movement, which makes the task of killing more difficult. There are various instru-
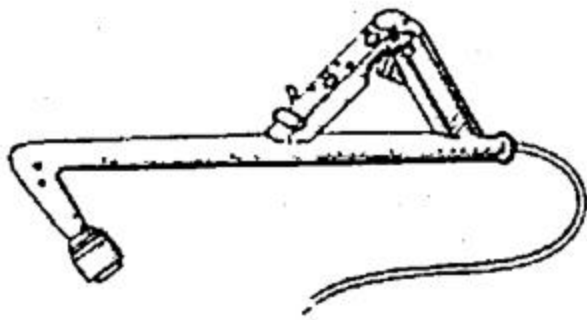
FIG. 1. Triangular shaped electric stunner.
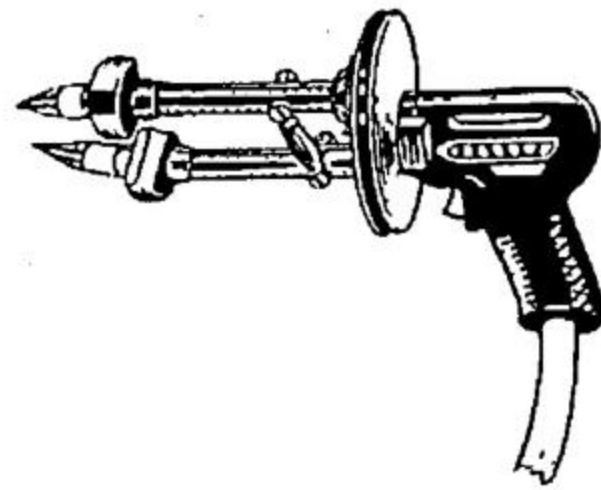
FIG. 2. Pistol-grip type of electric stunner.

ments on the market for electric stunning. One is triangular in shape, with one leg extending to the applicator head (Fig. 1), another is of the pistol-grip type with twin electrodes running parallel and separate (Fig. 2). These instruments nearly all utilize tattooing needles to ensure contact with the skin of the animal, and may be applied to any part of the head with equally effective results.

Compressed-air stunning

Stunning by compressed-air gun. This may be accomplished by using equipment which consists of a compressed-air system, furnishing a working pressure of approximately 180 lb per sq. in., and either a captive-bolt or a concussion-knob instrument (Figs. 3 and 4). In the captive-bolt instrument a sharpened, hollow bolt is driven through the skull into the brain by a piston activated by compressed air. The concussion-knob stunner consists of a solid head bolt activated in the same manner, which renders the animal unconscious purely by means of a high-velocity impact with the skull, causing concussion.

Captive-bolt pistol

Penetrating captive-bolt pistol. This instrument resembles a pistol in appearance and is fired by the detonation of a blank cartridge (Fig. 5). It is called the 'Cash X' pistol and is widely used. It weighs less than 6 lb, and the muzzle has

a castellated face to reduce the possibility of movement when in the firing position. The cartridge is detonated by squeezing the trigger. The captive bolt is projected into the brain to a depth of 3–3½ inches, as it is with the compressed-air-gun captive bolt. Cartridges are ·22 calibre and are made in several strengths. It is advisable to use the correct strength cartridge designed for the
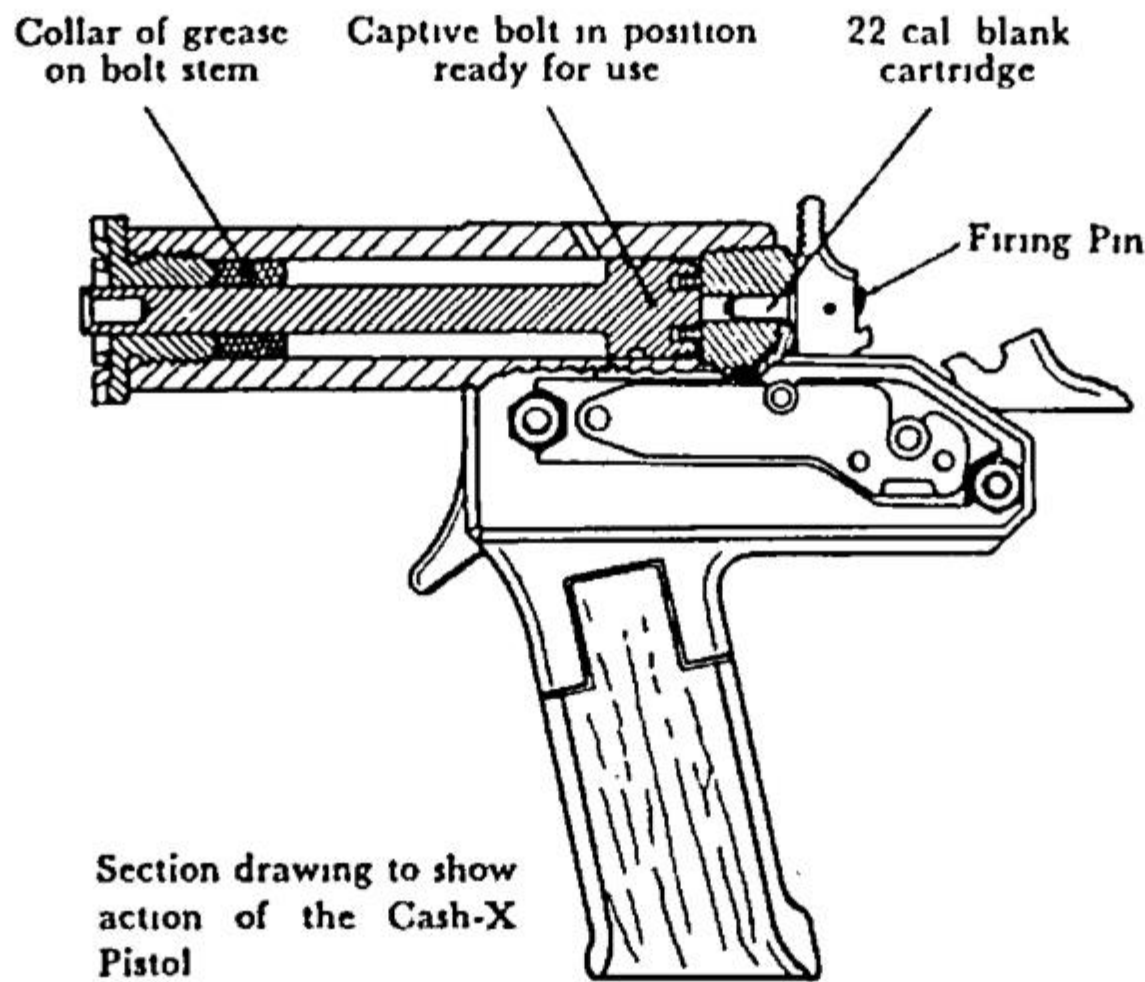
Collar of grease on bolt stem Captive bolt in position ready for use 22 cal blank cartridge

Firing Pin

Section drawing to show action of the Cash-X Pistol

FIG. 5. Penetrating captive-bolt pistol.

specie and size of animal to be killed, as the continued use of unnecessarily heavy charge cartridges causes needless stresses to the bolt and barrel of the pistol. There is also a cylindrical-type stunner similar in action to the pistol type, but the cartridge is detonated by means of a side lever (Fig. 6). This instrument may be fitted with a long handle having the detonating lever at its base, thus allowing the operator to work at a distance from the animal.

The 'knocker' stunner

This is a modern interpretation of the older, hammer-method of stunning (Fig. 7). It is a pistol-like instrument having a flat stunning bolt (the impact mechanism) projecting slightly so that it touches the animal's head before the stunning bolt. The instrument is swung in the manner of a hammer, and on impact with the animal the firing mechanism drives the stunning bolt at high velocity against the beast's skull. This does not cause permanent unconsciousness, but will deprive the animal of all feeling for about 5 minutes, thus allowing sufficient time for the animal to be bled out. The force of the blow can be adjusted to suit the species and weight of animal to be killed. This method demands considerable skill and experience, and is not recommended for the novice.

Humane stunning cartridges

These are ·22 calibre rimfire cartridges which may be fired from a ·22 calibre rifle. These cartridges, which should never be used in any cartridge-powered or piston-type stunner, have a velocity of 2,000 feet per second, and they disintegrate on impact with hard surfaces. This means that the hazard from a stray bullet is somewhat reduced. They are also lead-free, which is an extremely important factor in the food industry.

FIG. 6. Cylindrical type stunner with or without long handle.

FIG. 7. The 'Knocker' stunner.

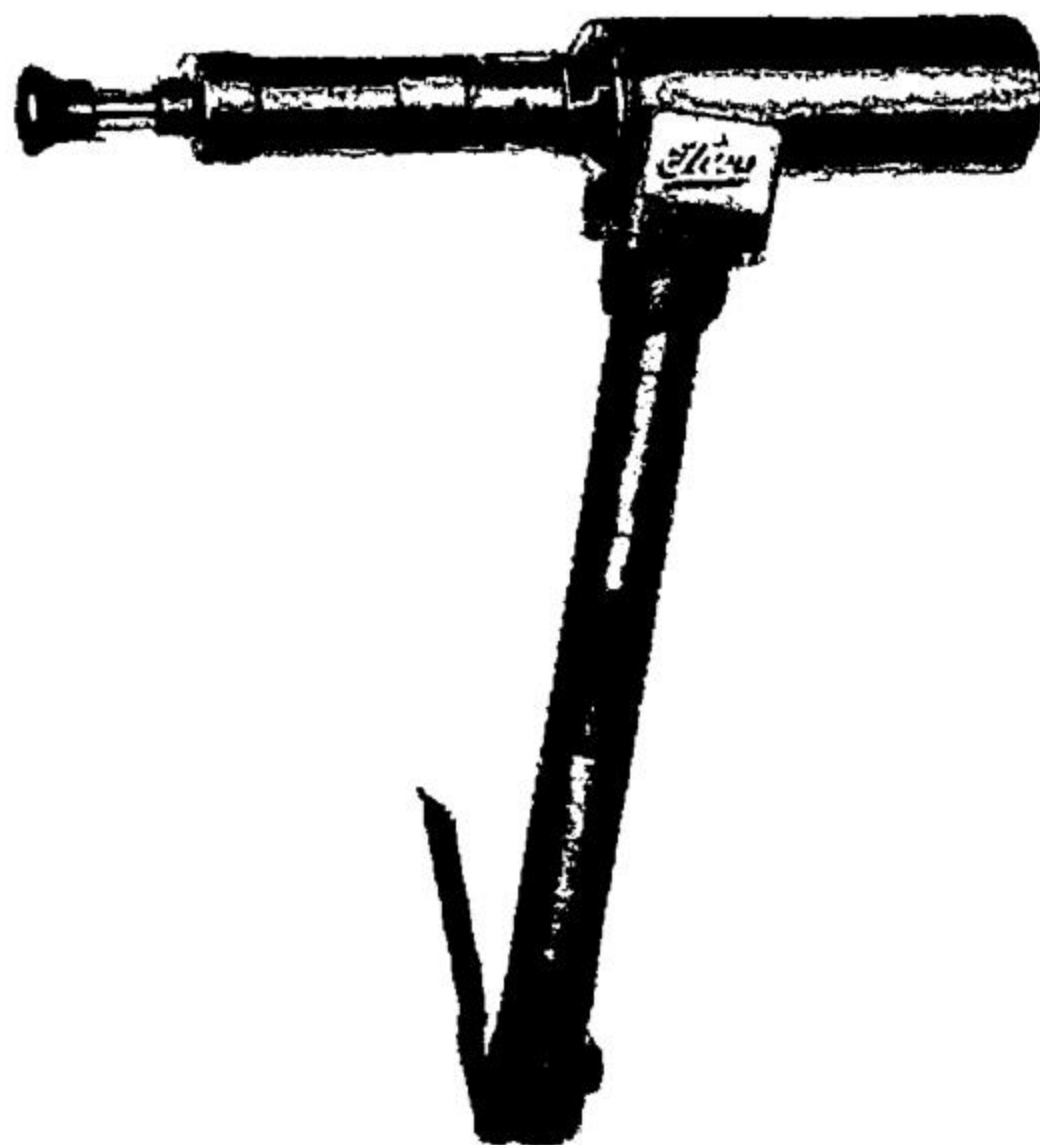FIG. 4. Compressed-air gun stunner. Quarter actual size.

FIG. 3. Compressed air gun stunner.

It should be noted here that any of the guns, stunners, captive-bolt pistols, or the humane stunning cartridge, or, indeed, any instrument using cartridges as their means of power, must be covered by a firearms licence, and should be used only under qualified supervision.

Scientific requirements

These should always be taken into account when the question of killing experimental animals is considered. The method of killing should be such that tissues, organs, or blood are not damaged in a manner that would render them useless for further scientific investigation.

RECOMMENDED METHODS FOR KILLING

MICE

Small numbers of mice may be killed by dislocation of the neck. Place the mouse on a flat surface, press a pencil or similar object across the back of the neck, then grasp the base of the tail and give a jerk to dislocate the neck.

Larger numbers of mice may be killed in a glass jar or dessicator containing a chloroform-soaked pad, or in a lethal chamber with coal gas.

RATS

Small numbers of young rats may be killed by physical stunning or by dislocation, or by the inhalation of chloroform or coal gas in a lethal chamber.

HAMSTERS

Inhalation of chloroform or coal gas. Intravenous or intraperitoneal injection of sodium pentobarbitone, the minimum lethal dose being 13·5 mg per 100 gm body weight.

GUINEA PIGS

Kill by dislocation of the neck. Place the guinea pig on a flat surface facing the operator. Place one hand over the top of the head with the first and second fingers on either side of the neck. Increase the pressure on the fingers and swing the animal so that the body is vertical and then let the arm drop swiftly downwards to the side. The weight of the falling guinea pig's body will cause dislocation. Guinea pigs may be killed by physical stunning, but this method is crude compared with the dislocation method. They may also be killed by the inhalation of chloroform or coal gas.

RABBITS

These may be killed by a sharp blow at the back of the neck either with the edge of the hand or a short stick. Alternatively, the neck may be dislocated. This is done by holding the hind-legs in the left hand and the head in the right hand, then, simultaneously, the head is bent sharply backwards and the legs and body wrenched downwards. Inevitably there are some post-mortem convulsions. This method is not suitable for novices, and skill in it should be obtained by practice on dead animals. Chloroform and coal gas administered

in a lethal chamber, though slower than physical methods, is quite satis-
factory, and is preferable for injured or diseased animals.

Intravenous injection of a saturated solution of magnesium sulphate or
sodium pentobarbitone may also be used.

MINK

These animals should be killed in a lethal chamber with coal gas or nitrogen.

CATS

Cats may be killed by administering barbiturates, in capsule form, by mouth;
this method is especially satisfactory for kittens. The capsule or capsules may
be passed over the back of the tongue with the aid of a finger, the blunt end of
a pencil, or forceps. 'Nembutal' is supplied in capsules containing $1\frac{1}{2}$ grains
(100 mg) of sodium pentobarbitone. A lethal dose will vary from two capsules
upward, so that a medium-size cat may need from four to seven capsules to
kill it by this method. Alternatively, sodium pentobarbitone may be given by
intrathoracic or intraperitoneal injection at the rate of 60 mg (in 1·0 ml) per
3 lb body weight. Probably the easiest method is to administer either chloro-
form or coal gas in a lethal chamber. Young kittens may be killed by stunning
by striking the base of the skull on a hard surface, such as the edge of a sink or
bench. Though this method sounds barbaric, it is, in fact, swift, and is cer-
tainly preferable to killing by drowning.

DOGS

Sodium pentobarbitone may be injected intraperitoneally, intravenously, or
intrathoracically. Alternatively, a sedation dose of sodium pentobarbitone
may be followed by the intravenous injection of a saturated solution of mag-
nesium sulphate. The dog may be killed with a captive-bolt pistol, but this
method is not recommended for persons unskilled in the use of these weapons.
Coal gas may be administered in a lethal chamber. Chloroform is not recom-
mended for use with dogs, and should only be resorted to in an emergency.
A very large dose of chloroform is needed to kill a dog.

MONKEYS

Monkey cages are often fitted with a movable, vertical back grid, which can be
pulled forward to trap the animal against the front of the cage. It is then quite
a simple matter to inject sodium pentobarbitone intraperitoneally. After the
injection the back grid is released to its normal position, and the monkey
becomes unconscious in a few minutes. Providing the cage is fitted with a
movable back grid, this is undoubtedly the simplest method, and, though it is
comparatively slow, the monkey dies peacefully. The animal may be killed
swiftly by an intravenous injection of sodium pentobarbitone, given either as
an overdose or as a sedative followed by the intravenous or intracardiac in-
jection of a saturated solution of magnesium sulphate. An intravenous in-
jection entails handling the animals, and great care should be exercised
(especially with newly imported stock) to avoid bites, which can transmit
disease from monkey to man.

FOX

The fox may be killed by the oral administration of sodium pentobarbitone to produce sedation, and then chloroform or coal gas may be administered in a lethal chamber. The intravenous or intrathoracic injection of sodium pentobarbitone is a swift method of killing, providing the animal can be adequately restrained while the injection is given.

FERRET

The animal may be killed with chloroform in a lethal chamber, or sodium pentobarbitone may be injected intraperitoneally or intravenously.

HEDGEHOG

These animals are best killed by the intraperitoneal injection of sodium pentobarbitone.

To unroll a hedgehog hold it close to the back of the head and lift it clear of the table, and, by moving the wrist, rock the animal gently up and down; it will then unroll. When the hind-legs touch the table, place a pencil across them and hold it in position. The head may then be lowered until the animal is lying on its back. The hedgehog cannot roll itself up while its head is held and the pencil is kept across its hind-legs.

FOWL

Physical dislocation of the neck is the most common method of killing fowls. The legs are taken in the left hand and the head between the first two fingers of the right hand with the thumb under the beak. A sharp stretching movement, pulling the head backwards over the neck, will part the spinal cord in the cervical region. Older birds, or large birds, such as turkeys and geese, may first be stunned by means of a sharp blow from a short, heavy bar on to the base of the skull. They should then be hung, head downward, and the throat cut with a sharp knife.

CHICKS

Chicks in small numbers may be killed by pressing the neck against the sharp edge of a table to part the vertebrae. Alternatively, they may be killed by the administration of chloroform or coal gas in a lethal chamber.

LIZARDS, SNAKES, CROCODILES

Lizards, snakes, small caymen, or crocodiles are best killed by administering ether or chloroform in a lethal chamber.

FROGS AND TOADS

These should be placed in a dessicator or jar over a chloroform-soaked pad. They may be anaesthetized before killing by placing them in a beaker containing 1–2 per cent Urethane solution; the heads should be left above the level of the liquid, but the beaker must be covered, as the solution is volatile.

FISH

Small fish in aquaria may be anaesthetized by putting them in a 1–2 per cent Urethane solution, or they may be killed by being thrown forcibly on to a hard floor. Larger fish should be struck on the head with a stone or heavy stick. Eels should be decapitated after stunning.
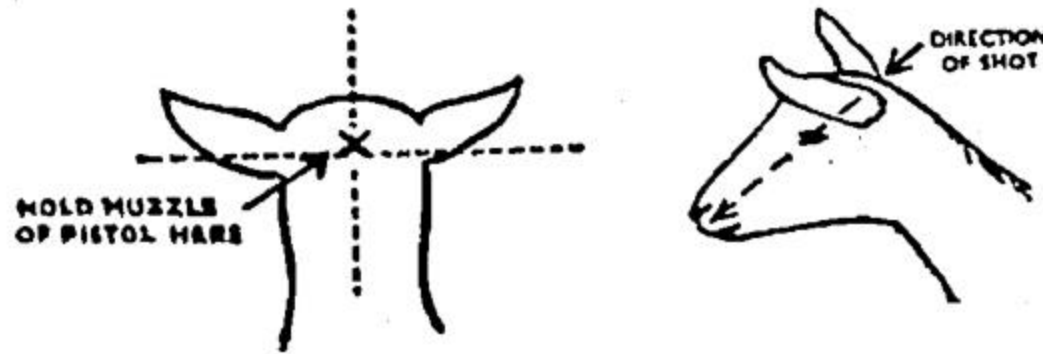

FIG. 8. The Kid: using captive-bolt pistol.

KIDS

These may be humanely killed by the administration of chloroform. This task may be done single-handed, but it is easier if two people are available—one to restrain the kid and one to administer the anaesthetic. The kid's head is restrained with one hand, and a wide-mouthed jar containing a chloroform-soaked pad is held loosely over the animal's face. There is little struggling if the anaesthetic is given slowly. When the animal becomes insensible the jar should be placed tightly against the face to exclude air.

A captive-bolt pistol may be used. The firing position is illustrated in Fig. 8. Sodium pentobarbitone may be administered intravenously or intrathoracically either as a lethal dose or as a sedative, followed by the injection of a saturated solution of magnesium sulphate.

CALVES

Electric stunning methods may be used. Alternatively, the captive-bolt pistol, compressed-air gun, or humane stunning cartridge methods are satisfactory, but, of course, the animal must be bled out immediately after stunning to kill it.

FIG. 9. Calves: using captive-bolt pistol.

N

CATTLE

The captive-bolt gun is used for killing cattle, but the firing position should be slightly higher than for calves. When shooting bulls or other thick-skulled beasts the muzzle of the gun should be placed ½ in. to one side of the ridge that runs down the centre of the face.

FIG. 10. Cattle: using captive-bolt pistol.

PIGS

Animals small enough to be restrained adequately can be killed by an injection of sodium pentobarbitone solution into an ear vein.

Pigs may be killed by bleeding out immediately they have been stunned or anaesthetized. The use of an anaesthetic tunnel containing a mixture of air and carbon dioxide is described above. Other methods, such as electric stunning, compressed-air gun, or captive-bolt stunners, are used extensively. Special care must be taken to position the captive-bolt pistol accurately when killing pug-nosed pigs, as the brain surface is rather small.

FIG. 11. The Pig: using captive-bolt pistol.

HORNLESS SHEEP

Captive-bolt guns or electric stunners may be used for these animals.

FIG. 12. Hornless Sheep: using captive-bolt pistol.

HORNED SHEEP OR RAMS

A captive-bolt gun may be used, but the muzzle must be placed behind the long ridge that runs between the horns.
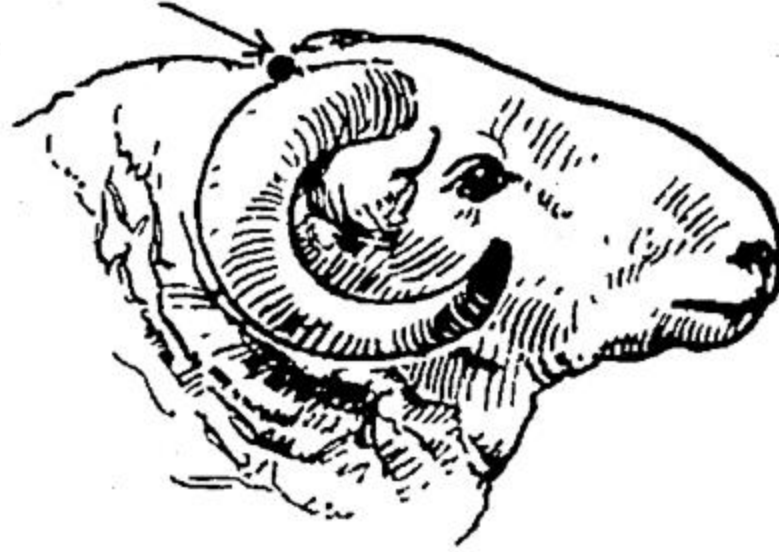
FIG. 13. Horned Sheep or Rams: using captive-bolt pistol.

GOATS

Electric stunning or captive-bolt pistols may be used as for sheep, but the muzzle of the gun should be directed towards the mouth of the animal instead of the gullet.

FIG. 14. Goats: using captive-bolt pistol.

HORSES AND DONKEYS

A captive-bolt stunner may be used, the muzzle being placed immediately below the roots of the forelock and firing roughly parallel to the line of the neck. The operator should stand to one side of the animal, as it will almost certainly fall forward on shooting.
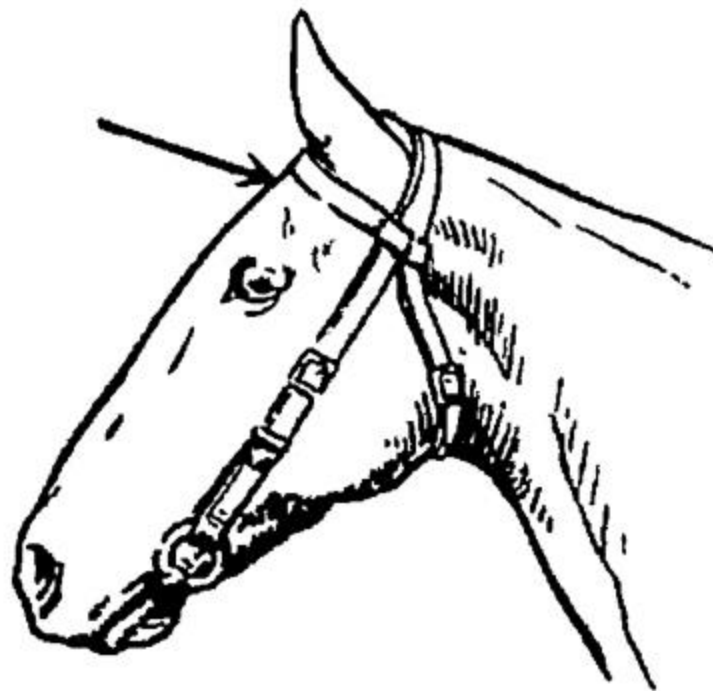
FIG. 15. Horses and Donkeys: using captive-bolt pistol.

Techniques and Practice in the Use of Radioisotopes

INTRODUCTION

Although the emphasis in this chapter will be on the practical aspects of handling radioactive animals, it is first necessary to give a simplified explanation of the physics of the atomic nucleus.

THE ATOM

Three fundamental particles make up the structure of the atom:

(*a*) The proton, which has a mass of 1·0076 atomic mass units (a.m.u.) and is positively charged.

(*b*) The neutron, with a mass of 1·0089 a.m.u., but no charge.

(*c*) The electron, with a mass of 0·00055 a.m.u. and a charge equal in magnitude to that of the proton, but negative in sign.

The atomic mass unit is defined as $\frac{1}{16}$ of the mass of the oxygen-16 atom, and is equivalent to $1·6603 \times 10^{-24}$ gm.

The model suggested by Bohr and Rutherford provides a satisfactory picture of atomic structure. This supposes a small central nucleus of protons and neutrons, surrounded by electrons travelling in fixed orbits, the orbits being grouped together in successive shells. Since the protons and neutrons are much heavier than the electrons, the nucleus represents by far the major part of the mass of the atom. The protons in the nucleus are positively charged, and this is balanced by an equal number of negatively charged orbital electrons, so that the atom is electrically neutral. The number of protons present in the nucleus is known as the Atomic Number Z, and this also defines the position of the element in the Periodic Table. The mass number A is the sum of the number of neutrons and protons in the nucleus.

Isotopes

This is the term given to the atomic species (or nuclides) having the same Atomic Number Z, but a different Mass Number A (i.e. the number of neutrons differs). Since Z governs the chemical behaviour of the atom, different isotopes will behave identically from the chemical point of view.

Radioactivity

The variation in the relative number of neutrons to protons in the nucleus gives rise to 'stable' or 'radioactive' atoms. Certain combinations of protons and neutrons are stable, but as the excess or deficiency of neutrons increases, so does the probability of the atoms being unstable or radioactive.

The unstable nucleus undergoes a rearrangement of its particles and emits its excess energy in one of several forms. If the nucleus has an excess of neutrons the emission of an electron (or β-particle) from the nucleus accompanies the change of a neutron into a proton. This process is β-decay, and it is often the case that further energy in the form of electro-magnetic radiation (γ-ray) is emitted from the nucleus. The isotope decaying in this manner is one often to be encountered in normal isotope work.

In certain nuclides of high atomic weight, e.g. radium, disintegration occurs with the emission of a heavy particle having a positive charge twice that of the electron and a mass of about 4 a.m.u. Such α-particle emitters are not in wide use, and will only be referred to briefly.

Nuclides with a deficiency of neutrons can decay by the emission from the nucleus of a positively charged electron (positron), which rapidly interacts with the electrons of surrounding matter, suffering annihilation with the subsequent emission of 2 γ-rays. Alternatively, the nuclide can capture an orbital electron (electron capture) and subsequently an X-ray, identical in effect to a low energy γ-ray, is emitted.

Thus to summarize the types of decay, we have:

α-emission—heavy particles emitted. Not in common use.
β-emission. By far the commonest type of decay, and often accompanied by γ-emission.
γ-ray emission. Electro-magnetic radiation.
Positron emission. Usually detected by the annihilation radiation γ-rays.
Electron capture. Detected by the X-rays emitted.

Individual radioactive isotopes are characterized by the energy of the emitted radiation, and the rate at which disintegration occurs.

Energies

These are measured in terms of the electron-volt (eV), defined as the energy acquired by an electron in falling through a potential of one volt. The multiple terms keV (thousand eV) and MeV (million eV) are often employed. The energies encountered with common isotopes can range from that of a very weak β-emitter of 19 keV up to γ-emitters with an energy of 2·8 MeV. Since the latter is difficult to handle, and the former requires special equipment for measurement, it is more usual to use isotopes with energies of 100 keV up to about 1·7 MeV.

Half-life

While it is impossible to predict when any particular atom of a given isotope will disintegrate, the average rate of disintegration of a large number of such

atoms is fixed and invariable. It is convenient to introduce the term half-life, which is the time taken for the number of radioactive atoms present to fall to one-half of the original value. The passage of a second half-life reduces the number to $\frac{1}{4}$ of the original value, and in 7 half-lives we have less than $\frac{1}{100}$ of the original radioactivity present ($2^7 = 128$). The unit of disintegration rate is the Curie, defined as the quantity of any radioactive material disintegrating at the rate of $3\cdot7 \times 10^{10}$ disintegrations per second (d.p.s.). Sub-units of more practical interest are the millicurie (mC) having $3\cdot7 \times 10^7$ d.p.s. and the micro-curie (μC) with $3\cdot7 \times 10^4$ d.p.s. (or $2\cdot22 \times 10^6$ dis. per minute). Animal experiments generally involve quantities ranging from a few up to perhaps several hundred μC.

Half-lives exist in a range from less than a second up to thousands of years. The very short ones are of no practical use, but some long-lived isotopes are of immense biological importance, a particular case being Carbon-14, of 5,600 years half-life.

A few of the commonly used isotopes, with their main characteristics are listed below:

SYMBOL	HALF-LIFE	β-ENERGY	γ-ENERGY
H-3	12·3 years	18 keV	—
C-14	5,600 years	158 keV	—
S-35	87 days	167 keV	—
P-32	14·3 days	1·7 MeV	—
I-131	8 days	610 keV	640, 364 keV
I-132	2·3 hours	1·53, 1·16 MeV	0·96, 0·76, 0·67 MeV
Fe-55	2·7 years	—	6 keV
Fe-59	45·0 days	460 keV, 270 keV	1·3, 1·1 MeV
Co-60	5·3 years	310 keV	1·3, 1·2 MeV
Na-24	14·8 hours	1·39 MeV	2·8, 1·4 MeV

Applications

The name isotope means 'same place', implying the same chemical properties. Thus a radioactive isotope will follow the same chemical route as the stable nuclide, but the radiation it emits makes it an easily detectable label. Usually small tracer doses are used to follow the metabolism of a substance or the distribution in tissue of a drug or other compound. The label's behaviour in the body can be followed without the necessity of killing the animal. The excretion or retention of a substance can equally easily be followed, such experiments requiring tracer amounts of isotope only.

In studies on the effect of internal radiation on animals much larger amounts of radioactivity are injected, but this type of work is unusual, and will not be considered here.

While radioisotopes are undoubtedly powerful tools of research, they can present handling hazards which need to be balanced against these advantages. It is easy to exaggerate the dangers, and even easier to forget them.

BIOLOGICAL EFFECTS OF RADIATION

When ionizing radiations are absorbed in living tissue, either from an external source or from an internally deposited isotope, profound changes can occur in individual cells. Although the absolute amount of energy involved is very small, its effect is concentrated in a few molecules only. Cell death or damage may result, and gene mutations may also occur. The results of this damage may manifest itself in the individual, or the effect may be a genetic one, inherited in future generations.

PROPERTIES OF RADIATIONS

In order to appreciate the relative hazards of different isotopes, consideration must be given to the behaviour of the radiations emitted.

α-PARTICLES, having a relatively large mass and being doubly charged, have an extremely short path length in tissue. Along that path, however, considerable ionization and damage can occur. Although a sheet of paper will completely stop α-particles, so that they cannot be an external radiation hazard from an injected animal, they can be extremely dangerous if once deposited and retained in the body.

β-PARTICLES have a well-defined range in matter, their small mass causing them to follow a tortuous path, analogous to a ping-pong ball ejected into a set of billiard balls. The range of 1·7-MeV β-particles in air is about 650 cm, or in tissue about 7 mm. Their ionizing power is less than that of α-particles by a factor of about 150.

Their larger path in tissue means that an animal injected with mC amounts of a high-energy isotope could deliver a measurable β-dose during handling. This, however, would be an extreme case, and it is difficult to imagine a tracer experiment using a pure β-emitter where the handling time of an animal would be sufficiently long for any significant total dose to be delivered to the hands.

γ-RADIATION, AND X-RAYS. These are the most penetrating and least ionizing of the radiations. The ionization produced depends on the original γ-ray energy, but is of the order of 100 times less per centimetre of path than a β-particle. There is no definable range, their absorption by matter causing an exponential fall in intensity. Where the intensity is reduced to one-half by a given thickness of material (half-thickness), the addition of a second similar thickness reduces it to one-quarter. A 100-keV photon will have a half-thickness in tissue of about 4·0 cm. In this case the injection of mC amounts of a high-energy isotope into several small animals could produce an external level of radiation which might require additional shielding if personnel are to remain close to such animals for long periods. This, again, would be an unusual situation in normal tracer experiments.

Dose units

The units of dose measurement are still subject to discussion, though from a practical point of view the several units in existence may be considered as identical.

THE ROENTGEN (r) is defined in terms of the ionization produced in 1 cc of dry air at normal temperature and pressure (N.T.P.), and by definition is applicable to X-rays and γ-rays. It is a measure of exposure dose.

THE RAD is defined as the absorption of 100 ergs in each gramme of tissue, following exposure to ionizing radiation. It is the release of energy in tissue which eventually causes radiobiological effects, but this effect also depends on the nature and energy of the incident radiation. α-particles produce a dense distribution of ions along a short track, and to allow for such differences between particles a factor known as the Relative Biological Effectiveness (R.B.E.) is used to modify the rad. For α-particles the R.B.E. is 10, so that the dose in rads $\times$ R.B.E. gives the Roentgen Equivalent Man (rem), i.e. rad $\times$ R.B.E. = rem. For β-rays, and moderate energy γ-rays, R.B.E. = 1, so that the rad and rem are equivalent for these cases.

Small differences also occur between the values of the roentgen and the rad, since materials of different chemical composition receiving the same exposure dose will experience different absorbed doses. Although academically these differences are important, from a protection point of view they may be ignored.

Control of dose

The dose actually received by an individual from a given source depends on three factors:

TIME. i.e. the duration of the exposure.

DISTANCE. Where air absorption is unimportant, i.e. with γ-rays, the dose rate at a distance d from a point source is inversely proportional to the square of the distance:

Dose rate $= \dfrac{K}{d^2}$, so that doubling the distance will reduce the dose rate by a factor of four.

SHIELDING. β-particles are completely stopped by light materials, the thickness required depending on the β-ray energy. Below are a few values of absorber required for complete shielding.

SUBSTANCE	ENERGY MEV		
	0·5	1·0	2·0
Perspex	2·0 mm	4·0 mm	7·0 mm
Glass	1·0 mm	2·0 mm	4·0 mm
Wood	4·0 mm	7·0 mm	14·0 mm

A γ-ray shield needs to be of a material which is both dense and of high atomic number. Lead is probably the commonest, but by no means the cheapest, and in tracer experiments where an occasional shield is required

steel can often be an adequate substitute, although greater thicknesses are needed. The half-thicknesses for lead for a few isotopes are given below:

ISOTOPE	HALF-VALUE LAYER CM LEAD
Bromine-82	1·0
Chromium-51	0·2
Cobalt-60	1·2
Copper-64	0·4
Gold-198	0·3
Iodine-131	0·3
Iron-59	1·1
Potassium-42	1·2
Sodium-24	1·5

HEALTH HAZARDS

The health hazards to be encountered by personnel may be divided into two types:

(a) External radiation, principally from γ-ray sources.
(b) Contamination of the body, either externally or by ingestion or inhalation.

As stated previously, the tracer amounts normally used in animal experiments are unlikely to present a radiation hazard.

The greater hazard is undoubtedly from contamination. External contamination from handling of excreta, cages, or animals can easily be avoided by the use of rubber gloves, or plastic disposable ones. Operation gowns should be donned before any radioactive work is commenced, and removed after monitoring when work is complete. Rubber gloves should be washed and monitored before setting aside, and care should be taken not to contaminate the inside when removing them. Heavily contaminated gowns, such as might arise following an accidental spill, or urination, should be set aside for decay if the half-life of the isotope is not too long. Rinsing with water will often remove the major portion of activity before sending the gown to the laundry. It is to be emphasized that spills are usually the result of poor technique, and as such are avoidable. Finally, rubber boots worn in the laboratory are easily washed down, and reduce the risk of spreading contamination.

Monitoring

Frequent monitoring with a suitably sensitive detector will prevent the build-up of unsuspected contamination. It should be carried out after any procedure where there is the slightest possibility of any contamination occurring, since besides the potential danger to personnel, an experiment can also easily be ruined.

Internal contamination by breathing radioactive gases or vapours is more difficult to control, but less likely to occur. Although radioactive carbon-14 and tritium-3 can be expired by injected animals, the concentrations reached,

when mixing with the laboratory air has occurred, are normally so low as to be negligible. Isotopes excreted in urine are usually chemically bound and unlikely to create a breathing hazard, but it must be the responsibility of the worker concerned to point out any special precautions necessary if such products are likely to be volatile.

It is to be remembered that much higher levels of radiation obtain during the dispensing of an isotope, and also during its actual injection into an animal, than during the subsequent feeding and handling. Separate facilities are required for dispensing, and shielded syringes may be necessary for injections, but these aspects are the responsibility of the scientific worker planning the experiment. Once an animal is injected, the hazards are reduced to those stated above.

Film badges

Both the total body radiation and the levels of isotopes in the air which will deliver small permitted maximum doses are laid down in the Report of the International Committee for Radiation Protection. These levels are maxima for a 40-hour week, and in a well-run laboratory it should never be necessary to approach these figures.

In order to check radiation levels film badges should be worn by persons in contact with γ-emitters and high-energy β-emitters. They also serve as a useful indicator of splashing or spraying which occurs but is not detected by normal monitoring. Badges are supplied by the Radiological Protection Service, Sutton, Surrey, who also process them and report the result.

RADIOACTIVE ANIMAL LABORATORY

Wherever possible, a separate laboratory should be set aside for the use of radioactive animals. The concentration of such work in one area minimizes the spread of contamination and generally makes control easier. Preferably it should not be too close to counting-equipment laboratories.

Basically, such a laboratory needs to be easy to clean and needs to be kept clean. For this purpose, it should have smooth, non-absorbent surfaces to benches, and walls should be painted with a high-gloss paint. Cracks in any surface whatever, which are difficult to clean, must be avoided. The junction of floor and walls should be suitably faired, and a gulley in the floor provided, since at least one daily wash of the floor is necessary. The floor surfaces must also be smooth, and painting with an acid-proof paint has been found to be satisfactory.

A changing lobby at the entrance, with a hand-basin for washing, complete with arm-operated taps, should be provided. There should be a supply of paper towels, and sufficient space for a trolley to carry monitoring equipment.

Sinks and drains

For cleaning purposes a deep sink with arm-operated taps is desirable. A rubber hose attached to the water inlet and reaching to the sink floor will considerably reduce splashing. The sink U-bend should have an easily openable

trap, and experience has shown that normal lead plumbing does not lead to a build-up of contamination in the trap if small amounts of activity are always flushed away. It is preferable to use only one sink for active waste, and if more than one is necessary in the laboratory the 'active' one should be so marked.

Cage-washing tank

An open water tank (100 gallons) with a high-level outlet pipe is useful for washing contaminated cages. These may be left to soak in continuously running water, the large volume of which is also an effective shield against external radiation. Cages must not be re-used until completely clear of activity, as a small residual contamination can completely vitiate a subsequent experiment.

Furniture

This is best kept to a minimum. A cupboard for the storage of syringes, chemicals, etc., is needed, and an operating table and lamp are usually necessary. The table is most suitably made in stainless steel throughout, and the cupboard is also best made of metal. Any surface to be used for handling animals or radioactivity should be freshly covered with two layers of blotting-paper or large squares of Whatman filter-paper. This simple precaution saves a considerable amount of time should any spillage occur. When sources for injection are being handled the operator should use a second containment vessel on the table in case of breakage.

Daily routine

The day-to-day routine of a radioactive animal laboratory differs only little from an ordinary animal room. It must be kept spotlessly clean, being washed down daily. The change of clothing must be used, and smoking, eating, drinking, and the application of cosmetics strictly forbidden.

Metabolism cages are often required, or at least cages with open-mesh bases, so that faeces and urine fall into suitable collection pans or bottles. It must be the responsibility of the worker to warn technical staff if highly active excreta is to be anticipated in any experiment.

Coprophagia is always a possibility, even in metabolism cages. Its importance in any experiment obviously depends on whether any of the metabolites occur in faeces. If necessary rabbits may be collared, but smaller animals are more difficult, and may have to undergo whole body restriction.

If total recovery of the isotope is required it may be necessary to trap expired carbon dioxide or water vapour.

Finally, hay or sawdust is to be avoided in radioactive animal cages.

Waste disposal

There is no entirely satisfactory method of disposing of radioactive wastes. The matter has been discussed in the Stationery Office publication *Control of Radioactive Wastes* (HMSO Cmnd 884, 1959), and reference should also be made to the Radioactive Substances Bill (8 and 9 Eliz 2. Ch. 34, 1960), the 'appointed' day of which is 1st December 1963. When the Act comes

into force all premises disposing of radioactive waste will need to be registered with the Ministry of Housing and Local Government.

Liquid wastes

Currently the Local Authority must give agreement before any radioactive wastes are discharged. The quantities involved must be discussed with the Authority, but the following approach is a useful one. A level of $10^{-6}\,\mu C/ml$ is below the maximum permissible figure for drinking-water for all β-emitters except strontium-90. If the amount of water used daily in the building is such that the average concentration in effluent does not exceed $10^{-4}\,\mu c/ml$ there will be no significant radiation problem, since no one regularly drinks sewage, and also since further dilution occurs. The concentration quoted amounts to about 5 mC per 10,000 gallons, and this is usually far beyond the figure required.

Since transient figures will be higher, it is best to ensure that at the sink the concentration does not exceed $0\cdot1\,\mu C/ml$. An inactive 'carrier' solution, i.e. containing the inert form of the substance disposed of, should be added to every solution, and followed by copious amounts of water.

Solid wastes

Waste paper, glassware, etc., should be temporarily placed in lined steel buckets, clearly marked 'radioactive waste'. The activity levels of solid wastes are usually lower, and combustible materials should be carried directly to the incinerator and burnt. Some storage facilities may be necessary for glassware contaminated with the shorter-lived isotopes.

In most animal experiments the level is sufficiently low for combustion of the body to be safe, although a storage method has been described by Boursnell and Gleeson White.

All incineration methods require that gaseous products are carried above surrounding buildings, and that a heavy ash is produced. The organization of a safe procedure during and after combustion is the responsibility of the Radiation Protection Officer.

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Techniques for Infected Animals

Infections in the animal house may arise from diseases occurring in the normal animal population or from agents used in diagnosis or in research projects, which have been introduced experimentally in the animals. Of all the possible infections encountered in the animal house some may be dangerous to man and animals, while others may be dangerous only to the animal population. The precautions which have to be taken are therefore not only for the safety of the animal technicians but also to reduce the risk of infection spreading among the animal population. The latter precautions are essential for the maintenance of a strong and healthy animal population. Because of the very wide range of infectious agents which may arise in handling different kinds of animals or may arise from the type of diagnostic or experimental work, no attempt will be made in this chapter to deal with the subject systematically. Instead, the basic principles which can be applied to any of the infections likely to be encountered in a small or large animal house will be outlined. In addition, a general description will be given of special isolation-units which can be used for housing large numbers of infected animals, where the risk to the animal technicians or other animals is greater. For the successful running of a special isolation-unit I will relate our own experiences during the last ten years at the National Institute for Medical Research, London, where we have safely handled many thousands of animals experimentally infected with tuberculosis.

HOW INFECTIONS ARE TRANSMITTED

An infection is a disease which is caused by a germ or infectious agent which may be a fungus, a bacterium, or a virus. In the animal house these infectious diseases can spread or be transmitted from animal to animal, animal to man, or even man to animal. The method of spread depends on the type of disease. It may be direct contact, and is then often referred to as a contagious disease, as occurs in many skin infections, for example ringworm. On the other hand, the disease may affect only the gut or intestine, as in food poisoning, diarrhoea, or dysentery, and then the germs enter the body in already infected food or water at the time of eating or drinking. Or again, if the hands are soiled there is a serious risk of introducing the germs when smoking a cigarette or handling food. A third route of infection is that of inhaling the germs into the lungs. This method of spread occurs when small droplets of moisture or dust containing the germs enter the lungs during breathing, and is most likely to occur

when infected dust from the floor or animal cages is disturbed. An example of this type of infection is tuberculosis of the lung.

From these examples of how infection can be spread it is seen that preventive measures for the animal-house personnel depend on common-sense measures of hygiene. Similarly, the spread of infection among the animals can also be prevented by the same simple principles of hygiene which must be applied by the animal technician. The animal technician therefore can not only safeguard his own health but can play an essential part in maintaining the health of the animals in his care.

GENERAL PRECAUTIONS FOR ANIMAL TECHNICIANS

The animal technicians should be given simple instructions in bacteriological principles with particular reference to the type of infections handled in their own animal house. It is essential that the animal house be provided with adequate washing facilities, including soap, scrubbing brushes, and disposable towelling. A number of skin conditions which arise from the animals can be prevented by thoroughly washing the hands after handling these animals. Rubber boots and gowns should be worn in the room housing infected animals. These gowns should not be taken out of the infected rooms. There should be no smoking, eating, or drinking in the infected part of the animal house. No unauthorized persons or casual visitors, such as friends or relations, should be allowed in rooms housing infected animals. All injuries which occur in the animal house, particularly bites from animals or scratches from cages, should be reported to the senior animal technician or, if available, to the medical staff. Any cuts on the hands or other exposed parts of the body should be adequately covered during the time of work in the animal house. Where special infections are handled protective vaccination should be given to the animal personnel. For example, in units handling poliomyelitis or tuberculosis the specific vaccines should be administered to the personnel before employment in the unit.

GENERAL PRECAUTIONS FOR THE ANIMALS

1. Ventilation

That part of the animal house in which infected animals are maintained should be especially well ventilated.

2. Cleaning of animal rooms

It should be accepted that all dust, animal food, and bedding on the floors is infected, and therefore all such dirt should be removed regularly. All floors should be kept clean by hosing with water and if necessary by swabbing with disinfectants, such as 1 per cent Tego in hot water or 5 per cent Lysol (see chapter on Sterilization and Disinfection for further details), but never by dry-sweeping. Similarly, wet-dusting should be used for cleaning racks, bench tops, window-sills, and all other furnishings and equipment. Dry-sweeping will automatically produce in the air particles of dust which may be loaded with germs and which could be a hazard to both the technicians and the

animals. Wet-sweeping with or without disinfectant will prevent the formation of potentially dangerous dust.

More complete disinfection should be carried out in the rooms when an experiment is completed and before starting another experiment. During this procedure the walls and ceilings should be disinfected, most conveniently undertaken using Tego applied from a spray-machine (see chapter on Sterilization and Disinfection).

In all operations where disinfectants are being used in rooms housing animals it should be remembered that some disinfectants can damage or be toxic to the animals, and therefore care should be taken to avoid splashing the animals with the disinfectants.

3. Cleaning and sterilization of cages

It is important to keep the cages clean by regularly changing the soiled bedding and removing stale green or root vegetables. Bedding and food heavily soiled with excreta will favour the spread of disease among the animals. Furthermore, all excreta from experimentally infected animals are likely to be contaminated. It is important that the litter from these cages should not be disturbed, and therefore at the time of cleaning the animals should be carefully placed in a clean cage. The dirty cages, trays, and bedding should be sterilized by steam *before cleaning and disposal of the litter*. If the autoclave is in another building the infected cages should be transported to the autoclave in suitable metal bins with properly fitting lids. If no steam-sterilizing apparatus is available the infected cages should be soaked in disinfectant before cleaning and disposing of litter. Unless the infected bedding is first treated by steam-sterilization or by antiseptics there is a serious risk to the animal personnel from contaminated dust particles.

4. Feeding and watering animals

In order to avoid the possible contamination of food by infected excreta, the standard diet should be provided in hoppers. Where it is necessary to supplement with fresh vegetables these should be changed regularly. Water should be provided freshly from bottles. It is also important that each cage should have its own bottle in order to avoid the carrying over of infection from one cage to another.

5. Reporting of sick or dead animals

Depending, of course, on the type of investigations, any unexpected sickness or deaths among the animals should be reported to the Officer in Charge. A prompt notification of sickness or death may avoid a serious outbreak of infection among the animals.

6. Eradication of wild animals

Mice and rats are the most likely wild animals to be encountered in the animal house. These must be eradicated, because there is a serious risk that they may bring disease to the experimental animals or spread infection from cage to cage. If such wild animals are present in the animal house there is the added risk that they may contaminate the main stocks of food.

ISOLATION TECHNIQUES

The same basic principles dealt with in the previous sections apply equally to the more specialized animal houses dealing with larger numbers of infected animals. Nevertheless, the greater risks involved in dealing with larger-scale work, particularly when handling several different experimental infections in the same animal house, require additional safety precautions and isolation techniques. The precautions to be taken are again not only for the safety of the animal technicians but also for the well-being of the animals and the success and reliability of the experimental work. Where several different experimental infections are being studied there is a greater risk of cross-infection among the animals, which if it was allowed to occur would lead to completely erroneous results. Unless strict isolation techniques are enforced cross-infection is likely because many of the species of animals are highly susceptible to the particular experimental infections, and furthermore, there may be in the same animal house similar species of normal animals which could also become infected.

General isolation precautions

A separate room should be provided for each experimental infection. Rubber boots and gowns should be worn in the infected areas. A large tray containing Lysol or some other suitable disinfectant should be placed in front of the door in the infected room so that the technician can wash his boots before leaving. The doors to each room should be self-closing, using a strong spring device, and should preferably be of the 'push-open type' (from the inside), thus avoiding the use of a handle on the infected side of the door. Bins should be provided for the dirty gowns, which should not be taken outside the infected area. Furthermore, where the same animal technician is responsible for the care of rooms housing different infections, a clean gown should be used in each infected room. The same gown should never be worn when handling animals with different infections. Cages and water bottles must never be taken from one infected room to another room housing a different infection. Similarly, any apparatus or materials that are likely to be contaminated with one infection must never be used in a room with a different infection.

Type of cage

Although no type of cage will eliminate completely the escape of infected material to the outside, the risk can be considerably reduced by housing mice in metal boxes and rabbits or guinea pigs in cages with the back and side and bottom 3 in. of the front made of sheet metal. The larger animals are kept most conveniently in cages with a wire-mesh base standing on or above a detachable tray. In this type of cage only the tray is changed, and the animals can remain in the same cage throughout the experiment. In order to reduce the risk of infection from dried excreta the trays can be kept moist with dilute disinfectant. By using this simple device the dirty trays can be changed without disturbing or handling the animal and therefore avoiding the spread of infection by contact or by disturbing the dusty bedding.

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Cage sterilization

In the more specialized infected units all infected cages, trays, and bedding should be sterilized by steam before cleaning and disposal of the litter.

Post-mortem examination of animals

Post-mortem examinations should be carried out in sterilizable trays, and the fur of the animals should be kept damp with diluted disinfectant. After examination of the animal all the instruments should be sterilized by heat, and the carcase should be placed in a waterproof bag and incinerated.

Syringes and needles

In order to avoid the risk of a needle becoming detached from the syringe during animal inoculation of infected material a Luer–Lock type of syringe should be used. If it is necessary to remove air from the syringe which has been filled with infected material the air should be extruded into a tightly packed wedge of cotton wool.

In the foregoing sections the general principles concerning the spread of infection and the general and special safety precautions which should be used when handling experimental infections have been outlined. The methods that have been suggested are all those that should and can be applied in the ordinary type of animal houses where this more specialized type of experimental work is undertaken. Where larger-scale work is undertaken or where new animal houses have been built for this type of work, special isolation units have been designed. In the last section is a general description of the type of isolation units used at the National Institute for Medical Research, Mill Hill, and particularly the one used by the Tuberculosis Research Unit.

SPECIALLY DESIGNED ISOLATION-UNITS

The rooms housing the animals infected with tuberculosis are situated in a self-contained unit in the main animal house. The unit is provided with its own double-ended autoclave and its own changing room for the animal technicians. The rooms housing the infected animals have their own ventilation system providing fresh air to each room and extracting the stale air. Because the extracted air may contain living virulent tubercle bacilli, it is sterilized by dry filtration, followed by ultra-violet irradiation before being discharged to the outside. The rate of extraction from the isolation unit exceeds the rate of inflow of fresh air in order that the whole isolation unit is placed at a slightly negative pressure to the rest of the animal house. This ensures a general flow of air towards the isolation unit, so that when the main door of the unit is opened air will not pass from the isolation unit back into the normal animal house.

Safety screen for the inoculation of small animals

Mice infected intravenously with suspensions of virulent tubercle bacilli are used on a large scale in many of the experiments. In order to protect the operators against the hazard of splash with infected material during the

inoculations, a simple screen has been designed. This apparatus is shown (Fig. 1), together with the wire-mesh cage for holding the mouse and the Luer–Lock type of syringe used for injecting the bacilli into the tail vein of the mice.
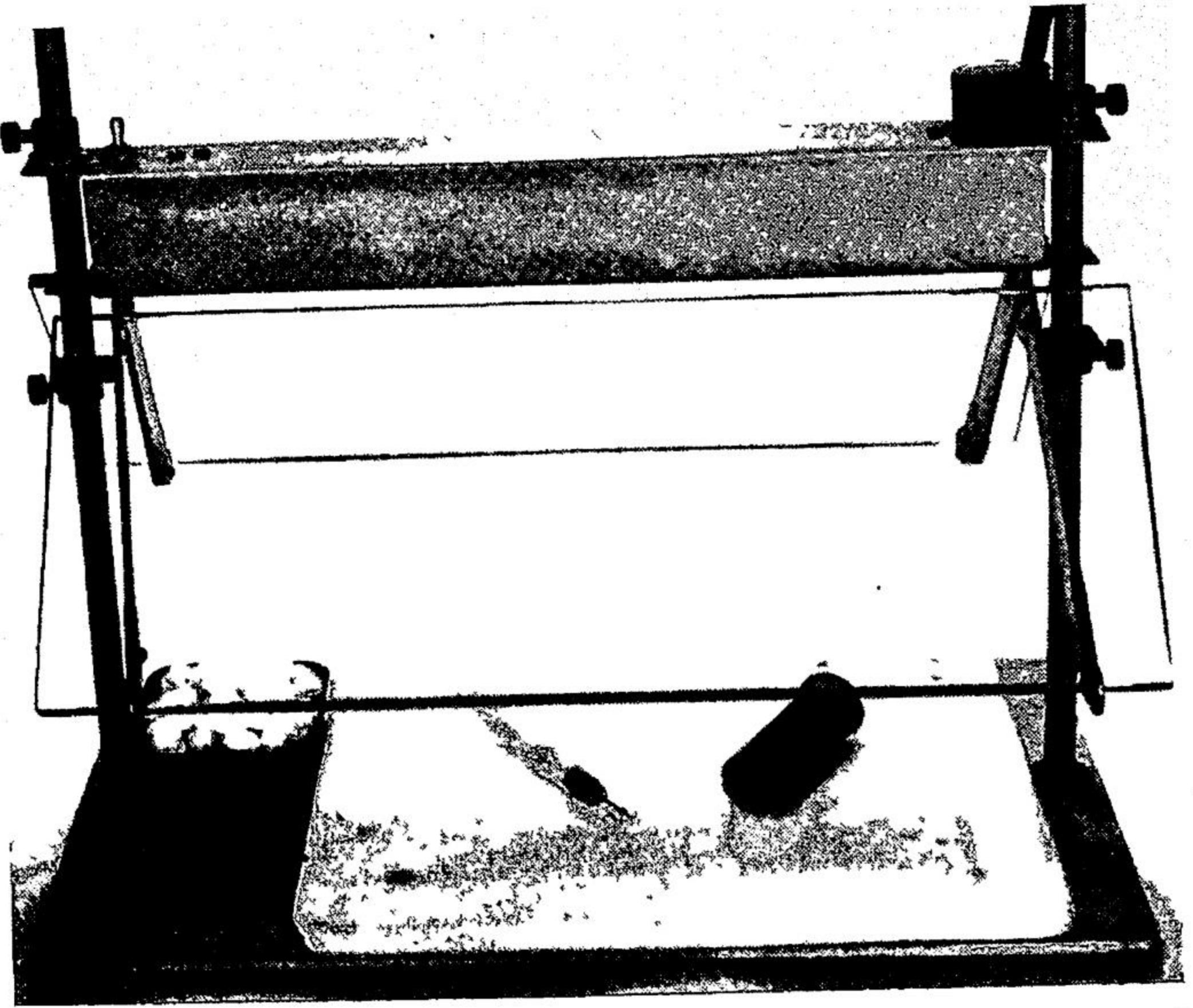

FIG. 1. Safety screen for protection against splash during the inoculation of small animals.

Method of housing the animals

When large numbers of animals, such as rabbits, guinea pigs, rats, and mice, are heavily infected with virulent tubercle bacilli the danger of their contaminating the environment is very considerable. This risk has been reduced by housing the animals in special cabinets which are connected to the extract system. The type of cabinet is shown (Fig. 2). It is a metal-framed cabinet with three shelves for animal cages, and each shelf is provided with a pair of sliding glass windows. The upper part of the cabinet is connected to the extract duct, and the lower part houses a trough of Lysol. Fresh air enters the room through the grille shown in the top right-hand corner of the plate. When all the windows are closed there is a sufficient flow of air between the panes of glass for the animals. As soon as one of the glass windows is opened the resistance in the cabinet is reduced and air enters freely through the open window. The ventilation system has been balanced so that when one of the

The type of *post-mortem* hood is illustrated (Figs. 3 and 4). The cabinet, with a glass front, is connected to the extract duct, shown entering the top of the cabinet. A 6-inch-wide opening below the edge of the glass screen admits the operator's arms for carrying out the *post-mortems*. The air velocity through this opening is not less than 300 ft per minute. Two cork-covered turn-tables are provided for pinning out the dead animals (mice, rats, or guinea pigs).
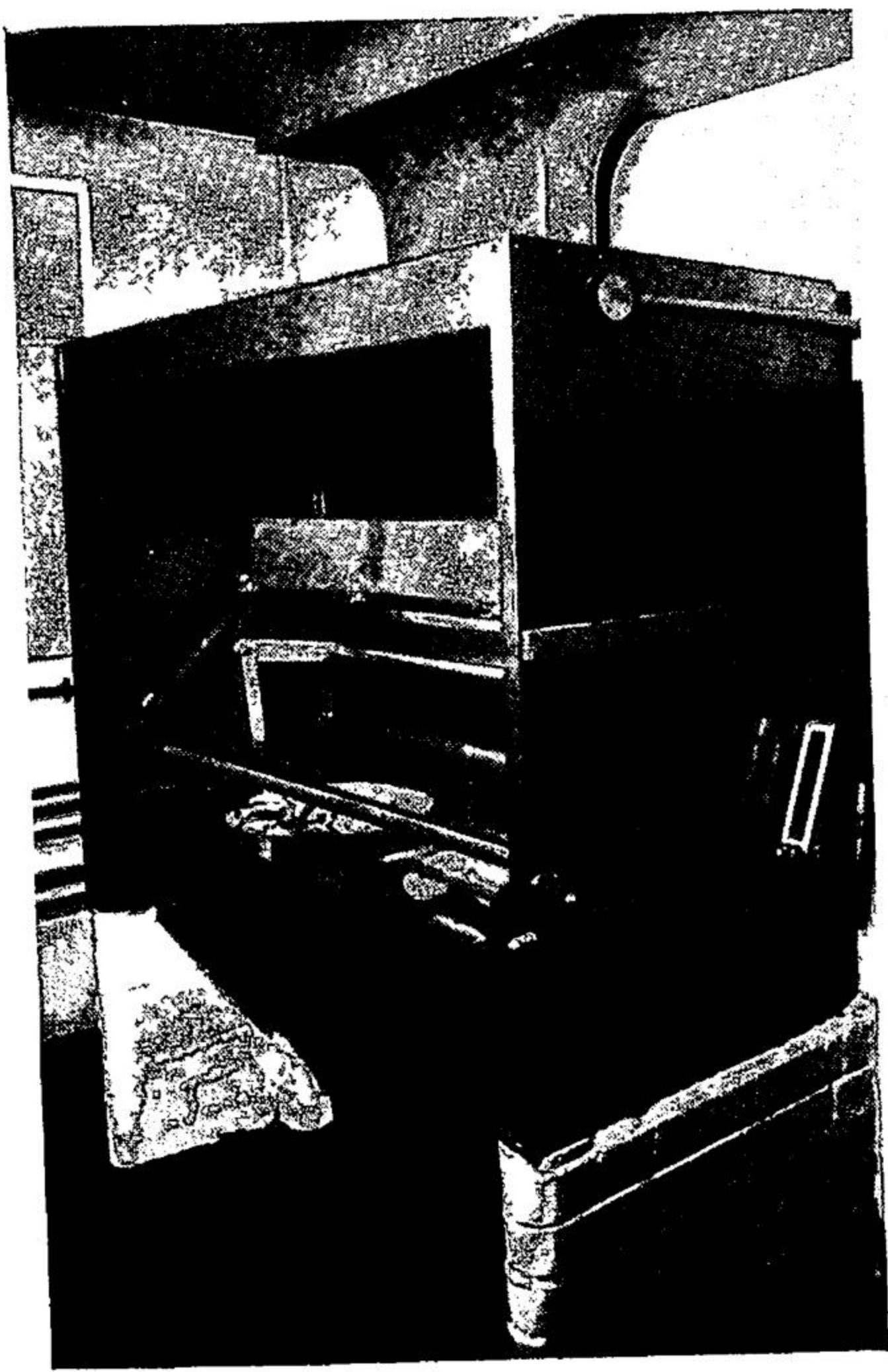

FIG. 3. Ventilated cabinet for the *post-mortem* examination of infected animals showing mice pinned to cork-covered turn-tables.

Each animal can be brought in front of the operator by adjusting the turn-table. When the examination of the animals is completed the turn-tables with the attached animals can be immersed into a bath of Lysol by turning the handle shown on the right-hand side of the cabinet (Fig. 4). The animals are left immersed in Lysol for 2 hours before being removed. The cabinet is provided with ordinary strip-lighting and also ultra-violet light (Fig. 4), the latter can be switched on for a predetermined time by the time-switch shown in the switch box at the right-hand side of the *post-mortem* cabinet (Figs. 3 and 4).
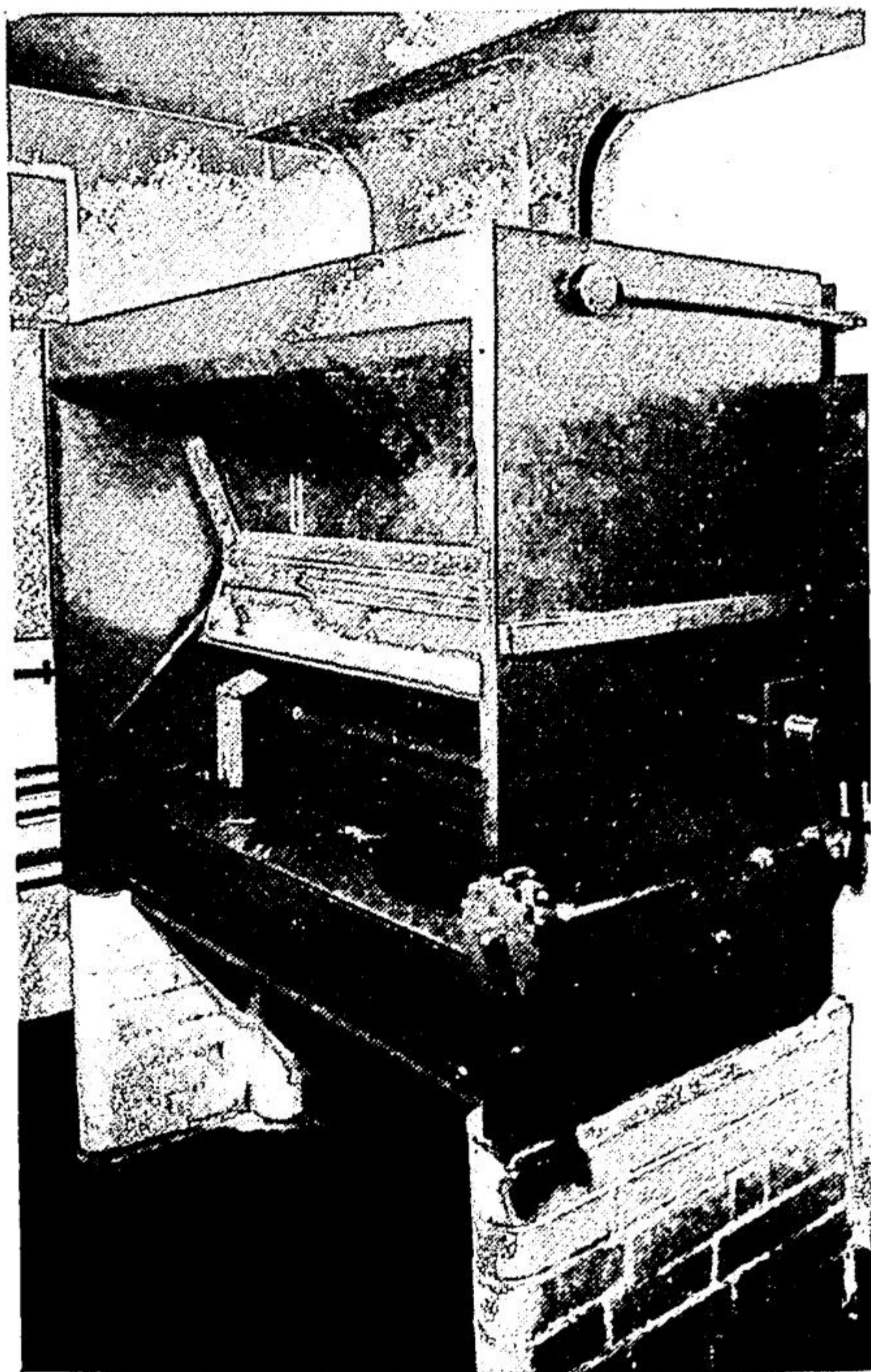
Fig. 4. *Post-mortem* cabinet with screen raised show-
ing turn-tables lowered into tank of Lysol.

Cage sterilization

All infected cages, trays, and bedding are sterilized in the autoclave provided
for the unit. The autoclave is a double-ended type with one end opening on
the infected side and the other end on the clean side of the unit. The cages are
finally cleaned and their contents disposed of only after autoclaving. Every
effort is made to pass most of the items from the isolation unit out through the
autoclave, including animal carcases, notes, and syringes and tubes containing
the suspension of tubercle bacilli used to inoculate the animals. No living
infected animals are allowed out of the isolation unit, and when the experi-
ments are completed the animals are killed, autoclaved, and then after leaving
the unit the carcases are incinerated.

SPECIAL PRECAUTIONS FOR PERSONNEL

There remain two additional precautions that should be taken for animal technicians and personnel working in animal houses where there is a risk of contracting tuberculosis. This applies to personnel dealing with experimental or diagnostic animals and also personnel handling large numbers of monkeys. The latter particularly applies to special units involved in the production or the testing of poliomyelitis vaccine. First, all such personnel should be given the opportunity to have periodic X-ray examination of their chests. The frequency of these examinations will depend on the degree of risk, but should be every six or twelve months. Periodic X-ray examination is, of course, a benefit to the staff whatever their risk, and there is the possibility that an animal technician might have active disease and, in fact, be a hazard to the experimental animals! This hazard is of considerable importance in animal houses dealing with large numbers of monkeys.

In addition, the resistance of the worker against contracting tuberculosis can be increased by vaccination with BCG. BCG vaccination can give very considerable protection to those who are most susceptible, namely those who are tuberculin negative. It is therefore important that all personnel, before being employed in the animal houses, should be tuberculin tested and if they are negative they should be vaccinated. In fact, tuberculin negative workers who refuse to be vaccinated should not be employed in the animal house where there is a risk of contracting tuberculosis from the experimental animals. The decrease in the incidence of tuberculosis in the population of this country is associated with a steadily increasing proportion of tuberculin negative reactors in our younger population. It is therefore more than likely that younger new recruits for the animal house will be tuberculin negative, and therefore they will be at greater risk than their predecessors, who were more likely to be tuberculin positive.

Although in the foregoing section the description of a specially designed isolation-unit for housing animals infected with tuberculosis is given, the general principles apply equally for other infections.

4. INTRACUTANEOUS OR INTRADERMAL INJECTION. Hair is removed using a depilatory or clippers. A short hypodermic needle is used, and care must be taken to see that the needle passes only into the dermis, as near the surface as possible, and not into the subcutaneous tissue, by holding the syringe nearly parallel to this surface.

5. INTRAMUSCULAR. Substance injected directly into muscular tissue.

6. INTRANASAL. Substance injected directly into the nose.

7. INTRATHECAL. Substance injected directly into the spinal canal. Care must be taken to avoid damaging the spinal cord.

8. INTRAOCULAR. Usually substance administered as eye drops; in special cases injected into the cornea.

9. INTRATRACHEAL. Substance, in small quantities, is usually injected into the trachea by means of a fine catheter which is inserted through the nose.

10. INTRACEREBRAL. Injected directly into the brain through the skull.

11. PERCUTANEOUS INJECTIONS. The substance is placed on the surface of the skin for a given time and protected from the animal's reach.

The common laboratory animals will now be considered.

FIG. 1

(a) Mice

The most common routes of injection are subcutaneous, intraperitoneal, intravenous, and intracerebral. For intraperitoneal injections the loose skin at the nape of the neck is held between the thumb and index finger and the tail is held between the third and fourth finger (Fig. 1). Subcutaneous injections are made into the loose skin of the back.

Intravenous injections are normally made into the lateral veins of the tail as near to the tip as practicable. Before attempting this injection the whole mouse should be *warmed* to dilate the tail veins.

For intracerebral injections the mouse is first anaesthetized and the substance introduced to the crown of the head slightly to one side of the centre.

(b) Rats

Common routes of injection in the rat are intraperitoneal, subcutaneous, and intravenous. Intracutaneous injections are again made into the loose skin of the back.

For intraperitoneal injections the rat is immobilized by grasping gently, but firmly around the shoulder region, the tail is held with the other hand and the animal kept taut (Fig. 2). Intravenous injections are made into the tail vein or the saphenous vein in the hind-limb. For percutaneous injection the substance is protected by either placing the animal in a special cage, or by the use of collar, sleeve, etc.

FIG. 2

(c) Guinea pig

Common routes of injection are subcutaneous, intraperitoneal, intracutaneous and intravenous. An assistant holds the animal during the operation, holding the shoulders firmly with one hand and restraining the lower part of the body with the other hand, so that the required portion is exposed. For the intravenous route, the saphenous vein in the hind-leg is used, and for both intracutaneous and intravenous injections the skin is shaved.

For oral dosing, the guinea pig is best held wrapped in a cloth. A gag and flexible polythene cannula, with a well-rounded smooth tip, are used.

(d) Rabbits

A subcutaneous injection is normally made into the loose tissue of the flank or at the back of the neck. The marginal vein of the ear is the most convenient site for intravenous injections, for which the ear is shaved and the vein is dilated. Injections are made towards the head of the rabbit. For intracerebral injections the animal is first anaesthetized for an incision in the skull using a trephine. After injection the skin is sutured.

Although these are the most common ways of handling animals for injec-

tion, it may be that the animal technician can adjust these methods if he finds a method which affords greater comfort to himself and the animal.

Injections can be made difficult if hypodermic needles are not in good condition. Blunt or hooked needles cause damage to the tissue into which they are forced, pain to the animal and strain on the temper of the operator. The needle point should always be examined (even if the needle is a new one); for this purpose a small hand-lens or binocular microscope should be used. For rapid examination it may be found useful to draw the needle across the back of the hand, before sterilization. The MRC Memorandum No. 41 *The Sterilization, Use and Care of Syringes* give practical advice on the sharpening of needles. Consideration of the shapes of needle points is discussed in a paper by Franz and Tozell in *Anaesthesiology*, 17, No. 5, 726 (1956).

The MRC Memorandum, already mentioned, also gives details of the different ways by which syringes may be sterilized. Many people now use sterile disposable plastic syringes which may be used once and then thrown away.

Preparation for Surgical Procedures and Post-operative Care

An operation with subsequent recovery of an animal can be carried out only by those holding not only a licence from the Home Office but also the necessary certificate or certificates.

Certificate B only is required for any operation with subsequent recovery for all animals except cats, dogs, and horses.

Certificates B and EE are required if cats and dogs are to be used.

Certificates B and F are required for horses.

The following details must be considered for all operations:

(i) Preparation and premedication of the animal.
(ii) The antiseptic or aseptic precautions which will be needed.
(iii) Choice of anaesthetic.
(iv) General care immediately after operation.
(v) Any special treatment.

General matters relevant to the five headings will be considered, and then different species of animals will be dealt with.

All animals used for operations with recovery must be perfectly healthy.

They should be housed in the animal house for some time prior to operation so that they become accustomed to conditions and surroundings. Cats and dogs should be cared for and handled by the same person before and after the operation.

Cats should be examined to see that they are free of fleas, and as a precaution can be treated by rubbing into the fur a little insecticide powder.

Dogs should be bathed a day or two before operation using soap and water to which Dettol or Cetavlon may be added.

Removal of hair

This is essential for all species. For mice and rats it is sufficient to clip the hair very carefully around the area at which the incision is to be made. The site of operation can then be washed with Dettol or surgical spirit (70 per cent ethanol), and a little weak solution of iodine may be applied. For other species this is not sufficient; the skin must be absolutely free of hair if the wound is to heal properly. The long hair should be clipped with good electric clippers or blunt-nosed scissors. The remaining hair can then be removed either by shav-

ing or with a depilatory paste. Both methods are liable to damage the skin, but in the hands of a skilled operator shaving with a sharp open hollow-ground razor is less harmful than a depilatory paste.

The usual formula for a depilatory paste is:

Barium sulphide	35 gm
Flour	35 gm
Talc	35 gm
Powdered soap	5 gm

The powder keeps indefinitely, and a sufficient quantity can be mixed into a paste with water, when required. It should be applied carefully and washed off with warm water *even more carefully*. The skin may easily become sore, and healing will be impaired. The paste has an unpleasant smell, and if cost is not important 'Sleek' (Elizabeth Arden) can be used. This is smoother and more pleasant to handle. After shaving or depilation the skin should be washed with Dettol or Cetavlon and a weak solution of iodine may be applied.

Weak solution of iodine:

Iodine	2·5 gm
Potassium iodide	2·5 gm
Distilled water	2·5 gm
Alcohol (90 per cent) to	100 cc

Cetavlon (CTAB. Cetyltrimethylammonium bromide). Solutions of Cetavlon are especially active against gram-positive organisms and to a somewhat lesser degree against gram-negative organisms. Use a 0·1 per cent solution in 70 per cent alcohol for skin sterilization. Stronger solutions may be used, and a 1 per cent aqueous solution is useful when scrubbing up before operation.

Preparation of syringes, instruments, etc.

When an operation is performed from which the animal is to recover care must be taken to see that organisms which will set up infection are not present, and do not enter the wound. If the site of the wound becomes infected healing will be retarded, and at the worst a general infection will occur and the animal will die.

Not all organisms are pathogenic to all species in the same way. For instance, much less care is needed for operations on rats and mice than is required for abdominal surgery in the cat or dog.

Antisepsis

This is the prevention of sepsis by the destruction of micro-organisms and infective matter. To achieve such a condition various methods can be used. Not all will produce complete asepsis, but they will generally be sufficient to prevent infection.

It is not essential to have an operating theatre for mice, rats, rabbits, and even cats. For abdominal surgery in the dog, or brain surgery in the monkey, if space is available an operating theatre is desirable.

Sterilization of syringes

This is of much greater importance when human treatments are being carried out, but it is sometimes necessary to have sterile syringes for animal experiments. It is not very satisfactory to sterilize syringes in alcohol or a disinfectant; the only efficient method is to use heat. For most animal experiments it is sufficient merely to boil the syringe. It should be taken apart, wrapped in gauze, and put into cold water. Bring the water to the boil, continue boiling for 15 minutes. Another method is to place the syringe in a hot-air oven at 212°F (100°C). Instruments can be placed in 70 per cent alcohol, in 1 per cent or 10 per cent Cetavlon in alcohol or aqueous solution, or they may be boiled in a steamer. Scalpels and scissors should not be boiled, since this destroys their cutting edge. They should be placed in surgical spirit or Cetavlon, or when required for deep surgery in strong Lysol. When instruments have been immersed in alcohol or Lysol they should be dipped in sterile saline before being applied to the wound.

Cloths to cover the wound can be baked in an oven, or wrapped in grease-proof paper and put in a steamer.

These precautions are usually sufficient for most animal operations. Not all organisms will be killed, however, and when carrying out abdominal or thoracic surgery in the dog or monkey the instruments, cloths, gowns, gloves, etc., must be autoclaved.

THE AUTOCLAVE

An autoclave is a pressure boiler fitted with a pressure gauge. It must be used properly or it will be no more, and may even be less, effective than boiling.

(1) Check that there is sufficient water in the bottom of the autoclave in excess of that which will be turned into steam.

(2) Instruments, towels, gowns, etc., can be packed in a cylindrical drum which is specially made for the purpose. The panels of the drum should be left open. Place drum in the autoclave, shut the lid, and screw it down, but leave the stopcock open.

(3) Turn on the heat supply and wait until steam has issued for some time —i.e. *until all the air has been driven out*. Shut the stopcock.

(4) Set heat regulator and pressure gauge to the required pressure. The length of time for autoclaving is taken from the time at which the required pressure is reached. The whole process depends on a pressure–temperature relationship, using wet-heat. The atmosphere in the autoclave must be entirely steam, with no air admixture. If the correct conditions have been produced a pressure of 10 lb in excess of atmospheric pressure will produce a temperature of 239°F (115°C), 15 lb in excess a temperature of 249·8°F (121°C).

(5) When the necessary time has elapsed turn off the heat and allow the pressure indicator to return to zero.

(6) Open the autoclave and slide the panels on the drum to close the holes through which the steam has entered. The materials in the drum will now remain sterile until the drum is opened. The drum can therefore be prepared beforehand. Swabs can be rolled from cotton wool, boiled in saline,

and used wet. Various sizes and shapes of swabs can be bought, or gauze cut into different sizes may be used. These can be baked or autoclaved and used dry.

It is advisable to withhold food for 12–18 hours from an animal which is to have an operation, but water should be available. This will reduce the chance that the animal will vomit. If this does occur there is a danger that the vomitus will get into the trachea and the animal will be suffocated.

Atropine

Another danger, particularly when volatile anaesthetics are being used, is excessive salivation and bronchial secretion. The saliva blocks the trachea and the animal may be asphyxiated. This may also occur over a period even with the basal anaesthetics, particularly if recovery is slow. If the animal is not asphyxiated excessive saliva in the trachea and bronchioles may start pneumonia. To prevent this *atropine* is usually given before operation. Wilberg (1914)[1] gives a list of the total tolerated dose of atropine for many species. Nothing like this amount is required to prevent salivation, but the ratio is of the same order. Birds can be given as much as 10 mg; rabbits 2 mg per kg, cats 0·5 mg per kg, dogs 0·1 mg per kg, and the maximum dose for a man of 60–70 kg is 1 mg. The larger the animal, the less atropine it tolerates. Mice and rats do not require atropine, but it can be given, 2–5 mg for a rat of 200 gm.

Atropine is given by subcutaneous injection.

Morphine is sometimes given prior to operation, especially to dogs, in order to produce some degree of narcosis before the general anaesthesia is started. The effect of morphine is variable, and it may produce complications in so far as the amount of anaesthetic required in addition is also very variable. Its use has no very great advantage in small animal work. It is more widely used in the veterinary field.

CHOICE OF ANAESTHETIC

ETHER is a good anaesthetic, and easy to use.

There are four stages of anaesthesia:

Stage 1 is the period from the time induction is commenced until the patient becomes unconscious.

Stage 2 is the excitable period during which the animal may struggle and make a noise.

Stage 3 is the fully anaesthetized condition; full relaxation has been obtained and respiration is regular.

Stage 4 is the toxic stage when respiration becomes shallow and may stop, resulting in death.

Stage 3 is the one which is required, and this should be reached as rapidly as possible.

Ether should not be given too slowly, but a high concentration should be produced in the early stage. This can be reduced and anaesthesia can be maintained with a much lower concentration. If the animal does become too deep the concentration can easily be reduced by removing the mask, and after

a few breaths the animal will have become much more lightly anaesthetized. Care must be taken when ether is being used. It is very volatile, highly flammable, and ether–oxygen mixtures are explosive. When using ether see that all sterilizers and heaters are turned off and do not use an electric cautery.

A simple way to anaesthetize small animals (rats and mice) is to place them under a bell jar or large funnel and either to drop ether on to a cotton-wool pad or to blow air through ether in a Woulff's bottle. When unconscious the animal can be restrained in some way on a board and the anaesthetic can be maintained with a small pad or funnel over the nose. Administration of ether to guinea pigs and rabbits is more difficult, they get a laryngeal spasm very easily and resist the anaesthetic. It is not advisable therefore to put them into a box, as they will struggle a great deal and may harm themselves. They should be gently restrained by an assistant, and ether should be administered with a mask, using a small amount at first. The concentration should be increased as rapidly as possible allowing some air intake from time to time. The anaesthetics given by injection are much more suitable for guinea pigs and rabbits.

Cats and dogs and monkeys may be put into a box, and anaesthetic can be pumped in by blowing air through ether in a Woulff's bottle. They can also be held provided skilled assistance is available. Anaesthesia for cats and dogs can be maintained by using a mask, but for operations of any length in dogs it is better to pass a Magill's cuffed endotracheal tube, which when the cuff is blown up will remain in position, and the animal will then breathe ether by itself. The endotracheal tube can be attached to a Woulff's bottle, or if available to an E.M.O.[2] (the latest form of vaporizer). With this apparatus known concentrations of anaesthetic can be given.

ETHYL CHLORIDE is a volatile anaesthetic sold in tubes which are graduated. It is highly toxic to the heart. It is used chiefly in cats and kittens to produce unconsciousness rapidly. About 5 ml and not more than 10 ml should be sprayed on to the mask. When anaesthesia has been produced ether should then be used, since ethyl chloride cannot be used to maintain anaesthesia.

TRILENE is a volatile anaesthetic which, like ether, is flammable. It is fairly safe to use and is short-lasting. Its main use is as an analgesic in childbirth. It has no advantage over ether for animal experiments.

CHLOROFORM is a volatile anaesthetic which is not explosive. It produces deep anaesthesia, but is dangerous because of its toxicity. In comparable concentrations it is ten times more toxic on the heart than ether, and it also produces bad effects on the liver. It is not much used for small animal work, but is still used in the veterinary field, particularly for horses, and sometimes for pigs. It does not follow that chloroform is safer in large animals, but rarely is a full and deep anaesthesia required, and chloroform produces a state of narcosis. Ether is not used for horses and cattle, because very large amounts would be required to produce a comparable narcosis and the risk of explosion would be present.

HALOTHANE (FLUOTHANE) is a new volatile, non-explosive anaesthetic. It is being used in human surgery. Induction is smooth and fairly rapid, and it is rapidly excreted. It is particularly suitable for dogs; anaesthesia can be maintained through an endotracheal tube very easily. Care is needed, because

it causes a fall of blood pressure which if the concentration is too high may continue for some time. Because it is non-explosive it is safer to use, but it is much more expensive than ether.

CYCLOPROPANE is a gas, which must be administered together with oxygen. Since the amounts must be controlled, a flow meter is necessary and the mixture is explosive. It is expensive, but can be given in a closed circuit, i.e. a certain amount of a known mixture is put into a bag, which can be attached to the animal. Some system for absorbing excess CO_2 must also be included, and a method for forced breathing must be used. Unless absolutely essential, there is no general indication for its use in animal surgery.

NITROUS OXIDE is a gas which is of low anaesthetic potency. When used to produce unconsciousness it is given without oxygen, and part of its effect is due to anoxia. Anaesthesia cannot be maintained with it. It has been used in monkeys, which when unconscious can easily be given an intravenous anaesthetic. For this purpose it is safer and less irritant than ethyl chloride.

ANAESTHETICS GIVEN BY INJECTION

THIOPENTONE SODIUM (PENTOTHAL). The average dose required is 50 mg per kg. It can be injected intravenously. It lasts only a short time, and it was generally thought that this was because it was rapidly detoxicated and excreted. It has been shown, however, that thiopentone is rapidly absorbed from the blood into the fat, and although anaesthesia will have passed off, a great deal of the original dose is still present in the fatty tissue. When giving thiopentone it is important to see that the injection is given properly into the vein. It is very painful if any gets into the tissue surrounding the vein.

PENTOBARBITONE SODIUM (NEMBUTAL). The average dose required is 29 mg per kg. It can be given intravenously or intraperitoneally. It will produce a greater depth of anaesthesia than pentothal and lasts much longer. When giving an injection intraperitoneally be sure that the needle is properly into the peritoneal cavity, particularly if the animal is fat. If the injection is given by mistake into the peritoneal fat no proper anaesthesia will be produced.

It should be realized that the doses given for thiopentone and pentobarbitone are the average recommended doses. There is a great variation in the response of an animal; one rabbit, for instance, may be anaesthetized with pentobarbitone in a dose of 20 mg per kg, another may require 40 mg per kg.

When a barbiturate is injected intravenously it must be given slowly, the rate and depth of the respiration being the anaesthetist's best guide. The depth of anaesthesia can be tested by pinching the heel; if the animal does not withdraw its leg surgery can be commenced.

The rabbit, which has a good marginal ear vein, is the easiest animal to inject intravenously. Always use a sharp needle, which should not be too fine, e.g. SWG 23.

Guinea pigs, hamsters, and ferrets can be injected intraperitoneally, or if an intravenous route is required the external saphenous vein can be used.

Cats and dogs can also be injected intravenously in this way if properly restrained, but it is not easy. They can also be injected intraperitoneally.

P

Barbiturates can and have been used in larger animals. The amount required to produce complete anaesthesia is large and therefore very expensive. They are not very successful in animals if given by mouth, though this would seem to be the ideal way to administer it to a dog. It is rapidly destroyed, and if enough is not given a hyper-excitable state may be produced.

When a volatile anaesthetic is being used the removal of the mask will immediately cause a reduction in the concentration of anaesthetic in the blood provided the respiration is satisfactory, but when an injected anaesthetic has once been given it cannot be taken out again. For this reason the intraperitoneal route creates difficulties. Because of the variation in the amount required for different animals there is no certainty about the depth of anaesthesia which will be produced for any given dose. The intravenous route is safer; some control can be exercised by carefully watching the respiration.

Stimulants

If respiration ceases artificial respiration must be applied, preferably by a rocking motion. Raise and lower the back legs so that the viscera fall against and away from the diaphragm at regular intervals. When respiration has been restarted it may be stimulated after an excess of a barbiturate by Picrotoxin B.P. or Bemegride (Megimide).

If an operation was needed on the nose or eyes and a volatile anaesthetic could not be given, *bromethol, paraldehyde,* or *chloral hydrate* were often given per rectum. With the introduction of the barbiturates these anaesthetics are not used in small animal work, but they are still used to produce narcosis in large animals.

Urethane (ethyl carbamate) and chloralose should not be used for recovery operations; their excretion is very slow, and the animal may develop respiratory complications before recovery from the anaesthetic.

After the operation has been completed the wound must be sewn up. This is done either with catgut for the muscle, and silk for the skin, or with nylon throughout. Michel clips should not be used.

Healing is helped and the risk of infection is reduced if a little penicillin–sulphathiazole powder is sprayed between the muscle layers and the skin. For most operations dressings should not be applied—Nubecutane or some other form of collodion dressing can be sprayed over the site of operation. If abdominal operations have been performed on cats or dogs a coat of some smooth material can be made with tapes which tie on the back. This will keep the wound clean and helps to support the weight of the abdominal contents and prevents a breakdown of the stitches.

POST-OPERATIVE CARE

All operations produce a degree of shock. The animals must therefore be kept warm—preferably at about 70°F (21°C). Rats and mice should not be put on sawdust, but on woodwool.

Guinea pigs, rabbits, etc., should be on a bed of hay.

Cats must have a cage large enough so that they do not injure themselves during recovery. The bedding can be hay or wood wool. A large metabolism cage is the most suitable housing for a dog. A blanket on the grid of the cage

will provide a comfortable place on which the dog can lie. Faeces and urine will pass through the grid and avoid the dog becoming dirty in the recovery stage.

Rats, mice, guinea pigs, and rabbits do not require much treatment after operation; they do not easily develop infections at the site of operation. Guinea pigs should be watched for respiratory infections. Rabbits will usually eat greenstuff quite soon after operation. Cats should be given milk on the day of the operation, and afterwards food which can be taken easily. A cat will eat what it can smell; fish is therefore preferable to meat at this stage, and if any difficulty is encountered sardines will often tempt a cat to eat. If difficulty is experienced a cat can be fed daily with warm milk containing dextrose by means of a pipette: 80–100 cc of milk can be given during the day. Eyes and nose must be kept clean by wiping with warm saline, or weak Dettol solution. Eye infections can be treated with Albucid eye drops.

Dogs need more personal care than any other animal. They must be kept warm, and clean, and as with cats, it is essential to persuade them to eat. Warm milk with dextrose can be given instead of water, but it is not so easy to forcibly feed a dog, unless a stomach tube is used. Both cats and dogs may be injected intramuscularly with procaine penicillin for three days after operation; 25,000 units per day for a cat and 50,000 units per day for a dog will usually prevent any infection developing at the site of operation, and will also reduce the possibility of secondary pulmonary infections.

For details about the general use of anaesthetics in large animals, and for details of specialized techniques in veterinary work, Wright's *Veterinary Anaesthesia*[3] should be consulted. A general survey of the use of anaesthetics has also been produced by Dr Croft for *UFAW*.[4]

GENERAL SUMMARY OF REQUIREMENTS

GUINEA PIG, HAMSTER, FERRET, RABBIT

Clip hair with electric clippers, remove remaining hair by shaving or with depilatory paste. Wash site of operation with Dettol or CTAB. Apply weak iodine. Instruments—boil in steamer or bake in oven at 212°F (100°C). Cover animals with sterile cloths. Scissors and scalpels in surgical spirit or CTAB. Dip in sterile saline before use.

Atropine 2·0–2·5 mg per kg subcutaneously 15 minutes before the anaesthetic is commenced.

Anaesthetics

Ether may be used, but is not easy to administer to guinea pigs and rabbits.

Thiopentone sodium (Pentothal) for short operations intravenously to rabbits.

Pentobarbitone sodium (nembutal) intravenously or intraperitoneally.

Close wound with catgut for muscle, and silk for skin or nylon throughout. No dressings are required, the site of operation may be sprayed with Nubecutane.

Recovery usually rapid. Keep warm and give good-quality green food. Ten per cent glucose saline may be given intravenously or subcutaneously if necessary. Watch for respiratory infections, particularly in the guinea pig.

MOUSE AND RAT

Clip hair, wash site of operation with surgical spirit or Dettol, apply weak iodine. Instruments in surgical spirit (70 per cent alcohol) dipped in warm sterile saline before use. Atropine not required.

Anaesthetics

Ether. Pentobarbitone sodium, intraperitoneally. Close wound with silk or nylon. Recovery usually rapid, keep warm. Feed on a little bread and milk with dextrose if necessary for two days. Watch for respiratory infections. No special treatment required unless necessary as a result of the operation, i.e. cortical extract to suprarenalectomized rats.

CATS

House for some days before operation. Dust fur with insecticide powder. Clip long hair with electric clippers, then shave or use a depilatory paste. Wash site of operation with Dettol or CTAB. Apply weak iodine solution. Instruments and cloths—boil in a steamer or bake in oven at 212°F (100°C) for simple operations such as nerve section. For abdominal and thoracic surgery pack in surgical drum and autoclave. Scissors and scalpels in CTAB or Lysol. Dip in warm sterile saline before use. Remove food 12 hours before operation.

Atropine 0·5 mg per kg 15 minutes before the anaesthetic is commenced.

Anaesthetics

Ethyl chloride followed by ether, or ether alone.

Pentothal sodium intravenously, followed by a little ether. (This is not usually very satisfactory; for short operations it is better to use ether only.)

Pentobarbitone sodium, intravenously or intraperitoneally.

Close wound as usual, spray penicillin–sulphathiazole powder between muscle layers and skin. Spray site of operations with Nubecutane, no dressing required, except in case of abdominal operation, when a coat which ties on the back may be put on for two or three days. Keep warm, feed milk and dextrose if the cat will not take food by itself. Give penicillin (procaine penicillin) intramuscularly 25,000 units per day for three or four days.

DOGS

House for some time before operations so that they are quiet and can be easily handled. Bath with soap and water with a little Dettol. Remove food 18 hours before operation, but water should be available. If abdominal operations are to be done sulphasuxidine can be given twice daily for three days before operation. Removal of hair and preparation of instruments as for cats.

Atropine 0·1 mg per kg 15 minutes before operation: 0·1 mg per kg morphine may also be given.

Anaesthetics

Ether or Halothane. When induction is complete pass a Magill's endotracheal tube and allow the animal to breath from a Woulff's bottle or ether inhaler. Pentobarbitone intravenously may also be used. Closing of wound and dressings as for cats. Keep dog warm after operation. Give water, or water

and milk with dextrose on the day of the operation. Give solid food on second day. Give penicillin (procaine penicillin) 50,000–100,000 units per day for three or four days.

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