STUDIES ON THE NEURAL CONTROL OF FEEDING IN THE RAT

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INTRODUCTION

Part of the normal behaviour of any animal is the ingestion of food. Whether it be a coelenterate polyp in a coral colony filtering continuously from the sea, or an amoeba ingesting tiny particles whenever it encounters them, the intake of food is essential for the continued life of the organism. As we proceed up the evolutionary scale, behaviour in general tends to become more complex and the ingestion of food has evolved *pari passu*. From direct observation of any mammal, including the human, we can see an obvious rhythmic pattern of feeding behaviour.

Little is yet known about the mechanism exercising control over the cycle. From recent reviews (Mayer, 1955; Brobeck, 1960*a*, *b*) it would appear that both humoral and nervous factors are involved. The relationship between these two systems remains unsolved and work on either one or other has continued. Recently, all the obvious lines of investigation have either yielded their results or seemed too difficult for study with existing techniques.

It is more than likely that the cerebral cortex plays its part in controlling feeding. But does the cortex act through the hypothalamus or the hypothalamus through the cortex? There is no doubt, however, that the hypothalamus plays an important role in regulating food

intake. During the past two decades, experiments on the nervous control of feeding have all provided results, some of them quantitative, to substantiate this.

The usual approach has been to destroy regions of the hypothalamus and to observe the effects. Although other behaviour patterns may be affected and thus confuse the picture, the feeding behaviour of some species is so well defined that any change in feeding pattern is easily detected.

The regions of the hypothalamus thought to be involved in ingestion are the ventromedial nucleus and the lateral area. Bilateral destruction of the ventromedial nucleus causes an increased intake of food (hyperphagia) and bilateral destruction of the lateral areas causes total cessation of feeding (aphagia). The effects observed have been studied qualitatively and quantitatively but how they are induced is still obscure.

The aim of the present work was to study the changes in the feeding behaviour of rats before and after destruction of the lateral areas of the hypothalamus. The resulting aphagia meant that no food was ingested. But behaviour is more than the simple ingestion of food. Feeding behaviour is presumably determined and accompanied by sensations in the animal. Localised damage to the brain may well interfere with the motor activity of feeding at the effector level, i.e. preventing eating. On the other

hand, the damage may be to the regions of the brain sensitive to blood-borne signals giving rise to hunger sensation and the urge to eat. If it were possible to provide the animal with a means of signalling such an internal change then it might be possible to differentiate between the motor activities of feeding and the internal drive to feed.

A specialised technique is used in the present experiments to provide records of feeding patterns of rats. A study was made of the daily oral feeding patterns before and after a series of operative procedures culminating in destruction of the lateral hypothalamic area. A period of aphagia resulted.

It is obvious that the various surgical procedures incidental to producing localised lesions in the hypothalamus may well have produced specific effects themselves. The stages in the operative procedure have been examined one by one and the effects of the various parts of the operations studied as control measures.

The rats were trained to eat normally by initiating a series of mechanical events and so obtain a small amount of food each time. By a modification of this apparatus specially prepared rats could obtain food during a 24 hr period via the stomach. Although such feeding was a conditioned response to hunger, the normal motor activities

of eating were by-passed. Those rats were also suitable for study after destructive hypothalamic lesions were made.

The training of rats to feed themselves intragastrically was originally intended to be merely a preliminary stage for subsequent work. Development of the technique required in this preliminary training took up much of the time of the investigation. The time spent in this work threatened to impair the subsequent stages of the programme. As a consequence, the later work was done on rats feeding orally. Nevertheless, the development of the intragastric feeding technique was a major part of the investigation, and, although the results obtained have strict limitations, they are definite and valuable. The lack of any equivalent method, in my opinion, justifies drawing conclusions from such results as we have.

The work was carried out in co-operation with Dr. S.D.Morrison, from whom came the initial idea for the experiment. The conclusions drawn from the results obtained are, however, my own.

REVIEW OF THE LITERATURE

Hypothalamic Control of Feeding

Most of the work dealing with hypothalamic control of feeding has taken place in the past 20 years. As the work developed, many reviews of the literature were produced. Where reviews are adequate they will be referred to in preference to individual publications. Important conclusions from original work, however, will be covered in some detail.

As much of the present study deals with rather new and unusual aspects of this branch of Physiology, importance had often to be placed on little emphasised parts of the literature. Many of the better known papers have had to be looked at in a different light.

Ventromedial area of hypothalamus

In 1921 Bailey & Bremer reported that a dog with a surgical lesion of the hypothalamus became obese. At that time there was no real evidence of central nervous control of feeding. Only when Hetherington & Ranson (1939) observed that 12 rats with lesions in the hypothalamus all became obese was nervous dysfunction considered likely. In the same paper pituitary damage was also shown to cause obesity. The effects of lesions in the hypothalamus were repeatable only when a very localised area was damaged.

Accurate placement of the lesions was achieved by using a stereotaxic instrument. Hetherington (1943) provided proof that the hypothalamic damage could produce obesity before and after hypophysectomy thereby disposing of a possible pituitary action.

Histological studies of the localisation of the successful bilateral lesions showed them to be restricted to the ventromedial nucleus of the hypothalamus, although the nucleus was not always completely destroyed (Hetherington & Ranson, 1940). These findings were confirmed by Tepperman, Brobeck & Long (1941).

Hetherington & Ranson (1942) were of the opinion that the great deposition of fat resulted from decreased activity of the animals. On the other hand, increased food intake was the reason for the positive energy balance proposed by Brobeck, Tepperman, & Long (1943).

Reviewing the subject, Brobeck (1946) came to the conclusion that the main cause of the obesity was increased food intake, i.e. hyperphagia. While a decrease in energy output was also of importance, Brobeck was quite certain that malfunction of the regulation of energy intake was the prime defect. That was to say that something had gone wrong with the control of feeding.

One inference which Brobeck made was that the lesions could have changed the rate of reactions at the level

of intermediary metabolism. Hetherington & Weil (1943) reported a decrease of phosphorus and calcium when calculated as proportions of body weight in obese animals. They concluded that the decrease was due to disordered fat metabolism. Brobeck, however, interpreted their results as merely showing an apparent 'dilution' of phosphorus and calcium due to the increased adipose fraction of the carcass. Since the pituitary is not involved it is not easy to imagine how purely nervous lesions may affect the rate of metabolism.

Although not stressing its importance in controlling food intake, Brobeck (1946) appreciated that the ventromedial nucleus of the hypothalamus regulated energy equilibrium and ad hoc food intake. He did not think it likely that energy output had changed following the lesion operation.

Energy output resulting from activity was known to be associated with food intake. Wald & Jackson (1944) found that starved rats became more active, while Richter (1922) found that rats increased activity before eating. Mayer, Marshall, Vitale, Christensen, Mashayekhi, & Stare (1954) subjected rats to varying degrees of exercise and found that food intake increased to compensate for the increased output of energy. Body weight was maintained except when the animals exercised for 6 hours in 24, when food intake dropped.

Although the decreased activity reported by Hetherington & Ranson (1942) may have caused part of the positive energy balance, it is unlikely to account for much of the change in body weight. Mayer et al.(1954) found that normal non-exercised mice showed a slightly higher rate of weight gain than exercised mice; obese exercised mice gained even more weight, eventually "plateauing" at a higher level of body weight. The changes in energy balance following destruction of the ventromedial hypothalamus would then appear to be due to a change in the level of food intake.

Rats normally show a diurnal variation in feeding activity which is more pronounced during the night (Richter, 1927; Anliker & Mayer, 1957). Hyperphagia of hypothalamic origin has the effect of increasing feeding during the day so that the activity during the day and the night is the same. Moreover, there is apparently an increase in the rate of feeding i.e. an increase in the avidity with which the food is ingested. This holds for both rats and mice (Brooks, 1946; Brooks, Lockwood & Wiggins 1946; Miller, Bailey & Stevenson,1950; Teitelbaum & Campbell, 1958; Hervey, 1959; Anliker & Mayer, 1957).

The importance of the change in body weight caused by obesity was investigated by Brooks (1946). It was

observed that after 120 days of hyperphagia due to lesions in the hypothalamus the body weights of those rats ceased to rise. Brooks thought that the increased energy expenditure caused by obese rats performing normal movements accounted for energy balance being regained. Activity during the day increased and, even before gain in weight ceased,the diurnal variation in activity had disappeared. During the whole 24 hrs the rats were very active.

Brooks, Lockwood & Wiggins (1946) tried to correlate activity, feeding and the cessation of the gain in weight. An important fact emerged from those long term studies of hyperphagic rats. The obesity developed quickly until, at about 120 days post operatively, gain in weight ceased and the body weight remained steady. This increase in body weight has been called the dynamic phase and the obese plateau the static phase.

The size of the meal increased in the dynamic phase and the diurnal feeding rhythm disappeared, more food being eaten during the day. As the conclusions of Brooks (1946) had been that the energy equilibrium was due to increased energy utilisation, the possibility of damage to the centres controlling feeding behaviour was considered. One of the most important observations was overlooked until Kennedy (1950) reported that as the body weight became steady then the

hyperphagia was lost. The importance of changes in activity as a cause of the "static" phase of obesity seemed to diminish. By starving "static" obese rats to reduce the body weights, and then giving access to food once more Brobeck, Tepperman & Long (1943), and Kennedy (1950) renewed the hyperphagia but only until the "lost" body weight had been regained. The additional energy expended by many obese animals is regarded by Mayer (1955) as far too small to produce any significant increase in food intake.

Both on liquid and on solid diets the "dynamic" hyperphagic rats ate 150 - 200% of the diet consumed by both the "static" obese and normal rats (Teitelbaum & Campbell, 1958). The reduction in food intake of "static" obese rats from hyperphagic to normal levels appeared to suggest that regulation of food intake had been regained once more. Kennedy (1950) concluded that satiety had been regained at a different level, depending on the amount of adipose tissue laid down.

Many investigators thought that damage to the ventromedial nucleus brought about changes in metabolism. Brobeck (1946) considered it possible and the results noted by Lundback & Stevenson (1947) prompted them to reach the same conclusions. When given high-fat diets, followed by high-carbohydrate diets, hypothalamic obese rats reduced

their food intakes. When the change was from high-carbohydrate to high-fat diets no change in food intake occurred. Lundbaek & Stevenson concluded that the lesions caused a reduction in the rat's ability to metabolise carbohydrate.

Changing from a high-fat to a high-carbohydrate diet caused a decrease in food intake in normal rats. Hyperphagic rats in the "dynamic state" showed a greater decrease in food intake but "static" obese rats showed a still greater decrease. No increase in food intake was observed in hyperphagic animals changing from a high-carbohydrate to a high-fat diet (Lundbaek & Stevenson). If both regulation of food intake proper and of metabolism had been affected, the normal level of food intake of "static" obese rats should not have resulted in a lower level of carbohydrate intake.

Although Mayer (1953) suggested in his review that it is likely that a metabolic disturbance resulted from hypothalamic lesions, the evidence was flimsy. Much of the support for his claim depends on acceptance of the interpretations of Lundbaek & Stevenson (1947). Kennedy (1953), moreover, was more inclined to the view that the lesions caused changes in satiety. Metabolic disturbances could possibly change the number of meals eaten but the hyperphagic rats ate the same number of meals as normal rats (Teitelbaum

& Campbell, 1958). Alternatively, a change in the level at which satiety is signalled would be in keeping with the results.

Kennedy (1953) concluded that Lundbaek & Stevenson's results were due to a change in the palatability of the diets. The results of Strominger, Brobeck & Cort, reported by Brobeck (1955), verify those of Lundbaek & Stevenson. Brobeck was also of the opinion that metabolic changes take place and affect food intake.

Hypothalamic hyperphagic rats made increasing use of a lever-pressing system in order to obtain extra food. After a period of starvation they did not operate the lever as frequently as starved normal rats and so showed a decreased intake of food (Miller, Bailey & Stevenson, 1950). The authors concluded that although the rats were hyperphagic there was not the same hunger motivation causing them to feed. Teitelbaum (1957) extended this investigation and found that when required to press more often to obtain the same amount of food, hyperphagic rats failed to respond and hence the intake decreased. Mice made hyperphagic by lesions in the hypothalamus continued to eat in a definite meal pattern which was abnormal in that there was no difference between night and day (Anliker & Mayer, 1957). This deviation from normal pattern was confirmed by Teitelbaum

& Campbell (1958) who found that, on a liquid diet, the number of meals and rate of ingestion of hypothalamic rats did not change although the sizes of the meals increased.

In order to explain the loss of hyperphagia in obese rats, Kennedy (1950) suggested the possibility of the existence of a "satiety centre". The evidence for a change in satiety rather than for a change in metabolism is not inconsiderable. Damage to areas of the hypothalamus sensitive to changes following on feeding or on starvation could well explain the behaviour of animals with hypothalamic lesions.

By diluting the diet with inert kaolin, Adolph (1947) caused rats to increase the total bulk eaten and the calorie intakes remained constant. They could maintain their normal nutrient intake until the kaolin was 75% of the bulk eaten. Similarly the animals could regulate the amount of diluted milk taken to maintain a constant calorie intake. Kennedy (1950) confirmed the results with kaolin dilution, and Bruce & Kennedy (1951) obtained similar results with fluid diets even in rats suffering from diabetes insipidus. Teitelbaum (1955) used cellulose as the diluent and found that adjustment of intake in normal rats could take place up to 50% dilution of the diet. One interpretation of these results is that the normal rat continues to eat until

a certain required amount of nutrient is consumed: then feeding stops.

Further work by Teitelbaum and by Kennedy and his colleagues showed that rats with ventromedial hypothalamic lesions were unable to regulate the intake of food when it was diluted. Kennedy, and Bruce & Kennedy, found that the total bulk of diet eaten by hyperphagic rats remained the same after 50% dilution. Calorie intake thus dropped. Teitelbaum measured intakes of both "dynamic" and "static" obese rats at various levels of dilution. Hyperphagic "dynamic" rats maintained intake of nutrients up to 25% dilution, but with greater dilution failed to compensate. Obese rats could not compensate at all when cellulose formed more than 50% of the diet.

By postulating the existence of a "satiety" centre in the hypothalamus many workers were satisfied that the theory fitted the results. Indeed damage to such an area could explain the hyperphagia and lack of control of nutrient intake, by removing the animal's ability to experience satiety. Kennedy's (1953) suggestion that the change from "dynamic" to "static" phase could be the result of satiety being regained at a higher degree of adiposity, indeed fits well into the picture.

Localisation of the centre in the ventromedial

nucleus of the hypothalamus seems undisputed. Mayer (1953) believes that both surgical lesions and injections of gold-thioglucose cause obesity of a regulatory rather than of a metabolic type. In both rats and mice the gold-thioglucose is known to damage the ventromedial nucleus (Mayer & Marshall, 1956). However, obesity has been produced by injection of gold-thioglucose only in mice: it may be that the dose required to damage the ventromedial area of rats is also lethal.

Lateral area of hypothalamus

It was shown by Anand & Brobeck (1951) that bilateral destruction of a region in the lateral hypothalamus of rats and cats caused chronic cessation of feeding i.e. aphagia. The site of successful lesions was limited to a very well defined area of the lateral hypothalamus, at the same rostro-caudal level as the ventromedial nucleus.

Anand & Brobeck called this lateral area the "feeding" centre and ascribed to it the ability to induce the 'urge to eat'. It was proposed that the ventromedial area acted via the lateral centre. Unilateral destruction of the lateral area alone caused no effect, while subsequent destruction of ventromedial centres caused hyperphagia. Combined bilateral lesions in the medial and lateral areas, together, produced the same effect as lateral lesions alone i.e. aphagia.

These results were repeated in cats and monkeys (Anand, Dua & Schoenberg, 1955). Aphagia resulted from destruction of both lateral and ventromedial centres. When lateral lesions were placed in hypothalamic hyperphagic rats aphagia immediately resulted.

Stimulation of the lateral area in cats produced confusing results. Stimulating for one hour per day, Delgado & Anand (1953) produced no effect on that day. On the following day, increased feeding resulted and was of the order of magnitude of 10 times normal. Miller (1960) showed that rats, satiated, but deprived of water for some time, will feed when electrical stimulation is applied to the lateral hypothalamus. If water is subsequently provided, and then stimulation is commenced the rats will stop drinking and seek food. The food will even be provided by lever-pressing activity which is conditional to providing food.

While stimulation of the lateral hypothalamic areas of sheep and goats produced immediate feeding, it was associated with licking of the lips and rumination (Larsson, 1954). Stimulation of the vagal dorsal motor nucleus in the medulla of sheep and goats produced pronounced polyphagia. Not only did those animals eat large quantities of food, but also food contaminated with urine and faeces.

A possible connection between vagal outflow, gastric contractions and feeding activity cannot be dismissed.

The location of two centres controlling feeding within the hypothalamus fits well into Stellar's (1954) hypothesis. Motivation resulting from excitation by a drive and inhibited by satiation was the theme. In the case of hunger being the drive then the evidence, both anatomical and physiological, is in agreement with Stellar's theory. Regulation of motivation is by internal changes.

The nature of these changes is the subject of much dispute. Evidence has been collected and shown to fit into various theories. These theories, on the whole, oppose one another and often in great detail. The observations cannot be disputed but as yet no hypothesis can make use of them all. The hypotheses have been the subjects of lengthy discussions and reference will be made to only the most comprehensive accounts.

The main hypotheses postulate either fluctuations in concentrations of metabolites, or of temperature, in the blood stream. These fluctuations either inhibit or stimulate to maintain a proper balance between intake and expenditure of energy.

Thermostatic hypothesis

It is known that, when the environmental temperature

rises, rats eat less. Brobeck (1948) has proposed that the hypothalamic centres are sensitive to heat production. This thermostatic hypothesis depends essentially on the known Specific Dynamic Action (S.D.A.) of foodstuffs to explain meal patterns (Brobeck, 1960,b). S.D.A. can also be used to explain the changes in food intake which follow changes in dietary composition.

Mayer (1955) criticised the theory on many scores but some of his arguments were not strictly valid. Although the thermostatic hypothesis cannot explain changes in appetite following insulin injection, this hypothesis can account for the frequency of meals. Defending his theory against Mayer's assertions that it was untenable, Brobeck (1960 b) stated that Kennedy's (1953) results revealed that animals adjust their food intake to changes in temperature, but that the ventromedial nuclei were not essential. Decreased intake of food by hyperphagic rats in a hot room, and increased intake to maintain body weight in a cold room were observations which caused Kennedy (1953) and also Mayer (1955) to reject the possibility of regulation of food intake by a thermostatic mechanism.

Brobeck thought that the increased food intake during exposure to cold was due to the heat of S.D.A. being more quickly dissipated. As a consequence eating continued

without interruption. His rejection of the conception of a satiety centre does not appear altogether wise at this stage: so many of the results of recent work can best be explained on such a hypothesis.

Morrison (1959) has summarised aptly what are perhaps the greatest objections to the thermostatic hypothesis: the theory is difficult to put to the test, and S.D.A. itself is a conception far from completely clarified.

Glucostatic hypothesis

Availability of carbohydrates is known to decrease utilisation of fat and to influence general metabolism. The sensitivity of the ventromedial nucleus of the hypothalamus of the mouse to gold-thioglucose suggested the existence of receptors readily permeable to glucose compounds (Mayer & Marshall, 1956).

The relationship of glucose utilisation to hunger was the basis of Mayer's (1955) glucostatic hypothesis. The hypothalamic centres were thought to be sensitive to changes in glucose utilisation, the arteriovenous glucose difference (Δ glucose) being the signalling factor. Much support for this theory has been collected by Mayer and co-workers. Even connections between gastric contractions and glucose have been shown by administration of glucagon

to inhibit contractions (Morrison, Lin, Eckel, Van Itallie & Mayer, 1958; Mayer & Sudsaneh, 1959).

The failure of glucose given intravenously to inhibit gastric contractions in rats is noteworthy (Morrison et al., 1958). The postulation that glucose itself is the factor concerned is thus considerably weakened. It is not inconceivable, however, that circulating carbohydrates in some form, or phosphorus compounds affect receptors in the hypothalamus. After lesions of the ventromedial nuclei, glucagon could no longer inhibit gastric contractions (Mayer & Sudsaneh, 1959).

Forssberg & Larsson (1954) found that the feeding centre had a greater uptake of labelled phosphate and of glucose in hungry than in recently fed rats. Mayer (1955) concluded, on the basis of the preferential gold-thioglucose uptake, that the ventromedial nucleus has a metabolism unlike that of other nervous tissues.

Brobeck (1960 b) now criticises the glucostatic theory since insulin seems to be required in order to enable the glucoreceptors to respond to glucose. If, as Mayer suggests, the ventromedial cells are atypical and do require insulin then Brobeck's argument falls to the ground. At present there is no direct evidence either way. In all species investigated other than mice, the uptake of gold-thioglucose is not limited to the cells of the ventromedial

nuclei. This does suggest that many regions of the brain may be affected by the concentration of glucose.

Lipostatic hypothesis

Kennedy (1953) proposed that the level of lipid reserves in the body could control food intake. This lipostatic theory is based on the stabilising of body weight following the development of obesity. The disappearance of hyperphagia was thought to be caused by activation of some satiety mechanism which required for its operation, a deposition of fat in the body greater than normal.

This hypothesis fails to explain the daily meal pattern. Teitelbaum & Campbell (1958) have shown that meals occur with the same frequency in hyperphagic, obese and normal rats. Mayer (1955) was willing to accept the lipostatic theory as a long-term control of body weight. If it be true, the lipostatic hypothesis has little bearing on the work to be reported in this thesis.

Hervey (1959) studied parabiotic rats. He destroyed the ventromedial nuclei of one partner: this animal became hyperphagic. The other partner failed to eat and became emaciated. The obvious conclusion would appear to be that a humoral factor is conveyed from the "dynamic" hyperphagic to the normal parabiont, causing increased satiety. Brobeck (1960 b) is not of this opinion. He concludes that the heat

production of the hyperphagic member was transferred to the partner, reducing food intake. There is then no need to postulate the transference of the humoral metabolite with no nutrient value.

Multifactor theories

Controversy over the actual nature of the factor signalling bodily state to the brain does not detract from the evidence which has been accumulated regarding feeding mechanisms. No doubt, the proof that one (or more) factor (s) actually stimulated brain centres controlling feeding, would enable many further experiments to be made concerning the mechanisms involved. However, investigations, not only into the nature of the signalling factors but also into the neural functions can continue with profit. Tentative theories have been proposed, mainly as working hypotheses, to allow some further investigations to be made.

Morrison (1959) proposed that the ventromedial area functioned as a sensory centre responding to the signal at a high level, outflow being limited to an inhibitory effect on the lateral area. The fact that meal patterns remain after destruction of the ventromedial area (Teitelbaum & Campbell, 1958) implies that the lateral area can also be inhibited by the signal, but at a level higher than that exciting the ventromedial centre. Morrison concludes that the motor outflow controlling feeding originates solely in

the lateral area. Sensitivity of both centres to sight, to taste, and to smell, and of the lateral area alone to hunger contractions, completes his hypothesis.

Although the lipostatic mechanism can in theory act through the lateral area alone, Brobeck (1960 b) argues that, on the thermostatic hypothesis also, the ventromedial area is not required. He maintains that Hervey's findings with parabiotic rats could well be used as support for the S.D.A. theory: the warmed blood of the hyperphagic animals signals satiety in the brain of the partner. This hypothesis would demand very considerable interchange of blood between the parabiotic rats. In fact the interconnection of the circulations was rather slight. In parabiotic rats a signal, of whatever nature, would in theory no longer be effective in the rat with destroyed ventromedial nuclei, but the partner with intact centres would be sensitive and satiety would result. We must not dismiss the possible significance of the ventromedial area.

A 'multifactor' concept of regulation has been proposed by Brobeck (1955, 1960a, b). It is based on the idea of appetite causing hunger which resulted by a variety of factors, in satiety. The factors, gastric distension, tissue dehydration, relief of hypoglycaemia, and S.D.A. were all considered to facilitate reflexes necessary for feeding.

Morrison (1959) found it convenient to accept this "multiple factor" concept although he points out that such theories, being entirely empirical, are always unsatisfactory.

Interaction of mechanisms

Brobeck (1960 a) puts forward some interesting ideas. By analogy with pulmonary ventilation he points out the possibilities of hypothalamic feeding centres being inhibitory or facilitatory to reflexes occurring at lower levels of the brain. There is also the possibility (Brobeck, 1955) that the receptive cells sensitive to the blood-borne signal are not localised in the hypothalamus.

Facilitatory effects upon a feeding reflex could only be inhibited by bilateral sources. The need to destroy both lateral areas in order to induce aphagia fits well the hypothesis that the lateral areas have facilitatory actions. Anand & Brobeck (1951) made unsubstantiated reports that the ventromedial areas had to be destroyed bilaterally to produce obesity. Mayer & Barrnett (1955) found that, in rats at least, that was not so. When unilateral lesions were placed in the ventromedial area noticeable hyperphagia resulted, although it was only sufficient to produce a weight gain 50% of that in rats with bilateral destruction of the medial areas.

If the ventromedial areas produce their effects

normally by inhibiting their corresponding lateral areas, then the observations of Mayer & Barrnett fit well into Morrison's (1959) general theory. The nature of the signal to which the ventromedial nuclei are sensitive is not altogether important as long as the result which it produces is constant. The inhibition of lateral areas by these ventromedial centres is not beyond the limits of belief. The method by which the lateral areas produce feeding behaviour and control ingestion is a much more inviting question.

Localisation of effects

The possibility that the aphagia following lateral lesions might be the result of cessation of drinking was considered by Montemurro & Stevenson (1955-56). They found that the adipsia always occurred with the aphagia. An observation on one rat showed that water intake could decrease without affecting food intake after lesions were placed in the lateral hypothalamus (Montemurro & Stevenson, 1957).

Neither complete food deprivation nor complete water deprivation in sham-operated animals is capable of simulating the feeding patterns of animals with hypothalamic lesions (Morrison & Mayer, 1957a). In the same investigation it was found that water administered by stomach tube did not

cause the aphagia to disappear from operated animals. Thus, it was concluded that aphagia and adipsia could be produced independently and are separate pathological entities.

While it has been generally accepted that lesions in the lateral areas produce permanent aphagia and adipsia this is not altogether true and the recovery sometimes observed has been investigated by a few workers (Teitelbaum & Stellar, 1954; Morrison & Mayer, 1957a; Mayer & Morrison, 1958; Williams & Teitelbaum, 1959).

Aphagic rats which are tube fed with water during the aphagic period have an increased chance of recovering from the effects of the lesions. In almost all chronic experiments, occasional animals recover spontaneously before their condition deteriorates too far. The table below shows the frequency of occurrence of such recovery or 'escape' in the papers of various workers. The feeding behaviour of 'escaping' rats has been recorded in order to provide clues to the nature of the lesion.

Following tube feeding, rats recovered the ability to eat various foods in a definite sequence (Teitelbaum & Stellar, 1954). Concentrated milk, sweetened fluid diet, mash, and solid diet were accepted in that order. From this, Teitelbaum & Stellar concluded that the eating behaviour was elicited by the ingestion of fat because milk, corn oil,

Table (1).

'ESCAPE' OF ANIMALS WITH LATERAL HYPOTHALAMIC LESIONS

Spontaneous	Following tube feeding	Authors
1 Rat		Anand & Brobeck, 1951
Cats		Anand, Dua & Schoenberg, 1955
9 out of 40 rats	14 out of 40 rats	Teitelbaum & Stellar, 1954
	4 out of 6 rats	Morrison & Mayer, 1957a
8 out of 14 rats		Morrison, Barrnett & Mayer, 1958
1 out of 12 rats	2 out of 12 rats	Mayer & Morrison, 1958
	9 out of 23 rats (revised coordinates)	Williams & Teitelbaum, 1959

chocolate etc. were more readily accepted than other foods.

Williams & Teitelbaum (1959) used different co-ordinates to produce lesions from which the rats recovered relatively quickly. These animals would not eat solid diet, although they consumed larger amounts of fluid diet than controls. That increase, however, may have been due to the previous days of starvation (Kennedy, 1950). The refusal to eat solid diet appeared of some importance. Morrison & Mayer (1957 a) reported that all six rats with hypothalamic lesions died when no water was administered while four of the six, to which water was fed by tube, recovered. The other two died at an even earlier stage than those which received no water. From these results the authors incline to the view that the increased chance of recovery in the intubated group was due to the hydration and smaller weight loss: the rats survived for a length of time sufficient to allow recovery from the lesion. Morrison & Mayer suggest that all rats may theoretically recover from the operation given time. Either the effect of the lesion did not destroy the entire regulatory mechanism permanently or some other part of the brain assumed the function.

By re-inflicting the lesions, using the same stereotaxic co-ordinates, 'escaped' rats could be made completely aphagic and adipsic (Mayer & Morrison, 1958).

The longer the time between operations (50 as against 12 days) the more persistent the result of the second operation. The authors think that 'escape' is due to recovery of mildly damaged parts of the lateral area. These eventually recover and assume functional control.

It is not inconceivable that if such a recovery took place quickly it could well explain the spontaneous recoveries observed. Reduction of dehydration by intubation and administration of water might allow the animals to remain "reversible" longer. A positive correlation between initial body weight and length of survival of starved hypothalamic obese rats has been shown by Montemurro & Stevenson (1960). Morrison & Mayer (1957a) proposed that the sensation of water in the gut might re-establish pathways for feeding. The implication was that the nervous lesions were such as to interfere with the motor mechanisms of the lateral hypothalamus rather than with the discriminative mechanisms of the sensory signal for hunger and thirst.

Destruction of a motor outflow suggests that damage has been inflicted on an anatomical fibre tract. Unfortunately, although Kreig (1932) has described the fibre tracts of the rat hypothalamus their functions are virtually unknown.

Anand & Brobeck (1951) mentioned that the lateral

hypothalamic region contained fibres of the median forebrain bundle, amygdalo-hypothalamico fibres, and fibres from the lenticular region. Since only lesions placed in a very circumscribed region caused aphagia these authors ruled out the possibility of injury to motor fibres controlling feeding. They therefore postulated the existence of a "centre" in terms of a discrete group of neurons lying among the fibres.

On the other hand, Morrison & Mayer (1957a) were more inclined to the belief that tracts rather than "centres" were affected. The transitional effect, which usually followed sham operations where only the electrode was passed, suggested to them that indeed very small areas were involved (1957b). Three animals showed complete adipsia and aphagia following sham operations. However, Morrison, Barrnett & Mayer (1958) later showed histologically that the quite extensive damage resulted from passage of the electrode. On one occasion an extensive lesion resulted simply from passing the electrode, with resulting damage to the median forebrain bundle and also to the lateral area. They were of the opinion that the median forebrain bundle was as important as the lateral area in the control of food and water intake.

By placing lesions along the median forebrain bundle

of the rat, Morgane (1960) found that only at the level of the "feeding" centre did any effect on food intake result. It was noted, however, that the extent of the lateral nucleus was difficult to determine histologically, as was the extent of the damage to it (Morrison et al., 1958). As Mayer & Morrison (1958) considered that migration of neurones undamaged by the lesion to replace the destroyed area could cause the 'escape' phenomenon, it is not inconceivable that lateral nucleus cells are involved.

Bilateral destruction of the lateral and medial amygdala of rats does not change food intakes (Anand & Brobeck, 1952). It was further noted that, when ventromedial hypothalamic lesions followed, rats, in which the amygdala had been destroyed, showed hyperphagia and obesity. Stimulation of amygdalar regions did not result in increased food intake (Anand & Dua, 1955).

Working on cats, Morgane & Kosman (1960) verified that hyperphagia followed destruction of both ventromedial and amygdalar regions, thus removing the possibility of the amygdala acting through the ventromedial nucleus. They reported that hyperphagia also resulted when lesions were placed simultaneously in the lateral, ventromedial and amygdalar regions. From this finding it was concluded that the lateral area did not function as a 'feeding' centre in the cat. Their results with lateral lesions

showed a flaw in the argument. Neither with their lateral lesions, nor with combined lateral and ventromedial lesions, did they produce aphagia. Evidence of previous workers (Anand & Brobeck, 1951; Anand, Dua & Schoenberg, 1955) points to the fact that the co-ordinates used by Morgane & Kosman did not provide the correct placement for their lesions.

Other Factors Affecting Food Intake

Factors, other than those just discussed, which may affect the hypothalamus, and control food intake, have been investigated. Mechanisms other than hypothalamic central control have been proposed. As the division into hypothalamic and non-hypothalamic systems is difficult to make, a brief review of the various theories will be made under separate headings.

Regulation of water intake

One would expect a rather obvious connection between the controls of food and water intakes. Recent work on the "drinking centre" of goats does suggest a control of water intake similar to that for food intake, and by a hypothalamic centre (Andersson, 1957; Andersson & Wyrwicka, 1957; Andersson, Larsson & Persson, 1960). But thirst appears to be quite a separate entity from appetite and the controls can be separated (Morrison & Mayer, 1957b).

Nevertheless, Adolph (1947) found that water intake could depend on food intake. When dilution of the nutrients by kaolin was made, water intake was decreased to correspond to the decreased nutrient intake. Strominger (1947) found that rats, whose food intake was decreased, reduced their water intakes, again to a degree corresponding to the change in food intake. Complete deprivation of food resulted in

little or no water being drunk (Adolph, 1947; Strominger, 1947). Those findings were not confirmed by Cumming & Morrison (1960) who found that in a 2-day fast water intake in rats was not always reduced to zero, and sometimes increased. Bruce & Kennedy (1951) found that when intake of water was restricted to 50% of normal, the intake of food initially fell but slowly recovered over a 10 day period.

The findings of Cort reported by Brobeck (1955) seem to indicate that food intake depends to some extent on changes in the sodium chloride content of the blood. Increased salt load resulted in decreased food intake. It may well be that osmoreceptors are involved in regulation of feeding. On the other hand, no measure of thirst was made, and from the previous data it would appear that water and food intake are interdependent. Andersson (1957) has induced drinking in goats by injecting hypertonic saline into the hypothalamus close to the drinking area.

Bruce & Kennedy (1951) reported that rats with hypothalamic diabetes insipidus could not prevent dehydration when fed on milk. This was because ingestion of diet was regulated by calorie intake and so fluid intake was insufficient to maintain a water balance.

As changes in appetite might result from ingestion of dry food, with or without accompanying water, it is preferable to minimise changes in the water content of food.

Harper & Spivey (1958) showed that there was an inverse relationship between food intake and the ability of dietary carbohydrate to exert an osmotic pressure. Incidentally, in this present work we have used a fluid diet which allowed food and water intake to be directly related. The rats studied by Adolph (1947) seemed resistant to water loading, even up to 120% of their body weights: the fluid diet was so dilute that in order to obtain the necessary calories, the rats had to take in that volume of water. Bing & Mendel (1934) found water intake varied with the texture of the diet eaten by mice. Working on rats, Harper & Spivey (1958) found that as the capacity of the carbohydrate content of the diet to exert osmotic pressure increased so did the water content of the stomach. Schwartzbaum & Ward (1958) found that hypertonic substances in the stomach reduced food intake and both NaCl and sucrose had similar effects. These results all appear to indicate that water intake may be to some extent governed by the bulk of the stomach contents. Indeed, Lepkovsky, Lyman, Fleming, Nagumo & Dimick (1957) found that the percentage water in the stomach of rats varied little before and after restriction of water intake. The constancy was maintained during deprivation by a reduced intake of food.

Radford (1959) was of the opinion that, under most conditions, water intake will be determined by the minimum

urine water required for excretion of solutes. Lepkovsky *et al*. thought that water could even be transferred from the rest of the body to the gut contents to maintain constant proportions.

Regulation of dietary composition

Changes in the composition of a diet change the amount of the diet ingested. As already mentioned, Adolph (1947) showed that dilution of stock diet with kaolin or cellulose up to 70% of the diet caused increase in the amounts eaten to maintain a constant energy intake. When the nutrient content was less than 25% of the bulk of the diet then the rats seemed unable to eat the quantity of food required to balance expenditure of energy.

Kennedy's (1950) results from hyperphagic rats fed 50% kaolin diets showed they did not ingest more calories per day than unoperated controls i.e. no regulatory increase took place.

Dilution of diets with kaolin by Kennedy (1953) produced little change in calorie intake in normal rats but "static" obese rats had their calorie intakes reduced to zero. "Dynamic" hyperphagic animals had their intake reduced to that of the normal controls. Cowgill (1928) reported that dogs could maintain body weight even when the nutritious component of the food varied widely. These results support

the theory that, in normal animals, adjustments in total food intake are made to maintain a constant calorie intake, even when the nutritive content of the diet is altered.

The changes in intake reported by Lundbaek & Stevenson (1947) seemed to indicate a change in the level of circulating metabolites following changes in the proportion of carbohydrate or fat in the diet. The fact that the bulk of the food intake decreased after a change from high-fat to high-carbohydrate diet and did not differ when the change was in the opposite direction, seemed to indicate changes in sensitivity to a factor increasing in high-carbohydrate feeding. However, the effects upon hypothalamic obese rats did not support this where an increased satiety apparently resulted.

By giving nut oil to hyperphagic rats Kennedy (1953) found that the bulk of their subsequent food intake decreased, but total calorie intake increased, causing increased growth. These changes in food intake, which follow on changes in the nature of the food, do not support the idea that eating is controlled simply by the intake of calories. The control of calorie intake, were the food of constant composition, could be achieved by a mechanism sensitive to the level of one or more metabolites.

Regulation by gastric contractions

The importance of the hypothalamus in feeding seems

now to be well established. The importance of gastric "hunger" contractions, once regarded as the main factor in the drive to eat, has become a matter of dispute since the hypothalamic control came to light.

The classical theory of a control of feeding by gastric contractions had, as its proponents, two eminent physiologists of their day (Cannon, 1915; Carlson, 1916). The statements of them both have been misquoted quite frequently. Cannon attempted to present an integrated explanation of hunger but the main experimental support came from evidence of hunger contractions (Cannon & Washburn, 1911) which occurred along with the sensation of hunger. Cannon (1915) and Carlson (1916) believed those contractions to cause hunger sensations although they both realised that, after 3 days fasting, when contractions were very marked, there occurred a total lack of appetite in man i.e. anorexia. Both men had suggested blood glucose changes might initiate feeding but neither made suggestions as to the mode of control. Cannon's deduction that gastric contractions caused the subjective hunger sensation has been undermined by the observations that appetite and hunger occur in human beings after gastrectomy (Grossman & Stein, 1948). The findings of Morrison, Lin, Eckel, Van Itallie & Mayer (1958) that various metabolites can abolish "hunger contractions" in rats and the similar effects of heat, cold, and various

metabolic hormones (Sudsaneh & Mayer, 1959) all tend to support the hypothesis that "hunger contractions" give an indication of an internal state. Mayer & Sudsaneh (1959) found that hypothalamic aphagic rats showed marked "hunger contractions".

The possibility of using such a method to measure a sensation of "hunger" in a hunger state was consequently considered worthy of investigation in the present work. Abolition of "hunger contractions", after a fast, may be taken as a fair indication of satiety having been achieved.

In previous investigations (Miller, 1955; Morrison et al., 1957; Mayer & Sudsaneh, 1959) the gastric contractions were produced by starvation for 4 - 24 hours: such starvation probably causes greater deprivation than normally occurs.

Regulation by gastric distension

Distension of the stomach has been thought to have some effect on "metering" food intake. The passage of food through the bucco-pharyngeal region has also been regarded as stimulating regulatory receptors but the results of Adolph (1947), Cowgill (1928), and Kennedy (1950) tend to suggest the act of eating is of little importance. Kohn (1951), however, found that orally ingested milk produced greater satiation than a similar volume given intragastrically. A small oral component would appear to exist in regulating food intake.

Gastric distension, on the other hand, has been shown to produce decreased intakes of food. Berkun, Kessen & Miller (1952) reduced the lever-pressing response in rats by feeding them milk. Kohn (1951) observed that milk directly injected into the stomach of rats caused a greater reduction in lever-pressing than the same volume of saline. Milk, however, remains for a longer time in the stomach. Working on dogs, Towbin (1949) had found that gastric distension abolished thirst. Miller & Kessen (1954) showed that simple distension of the stomach with a balloon made the rats avoid food. The animals chose to have a volume of milk injected into the stomach rather than a comparable distension of the balloon. The rats made the choice in a T-maze.

By feeding dogs intra-gastrically with 50%, 100% and 175% of their calculated calorie requirements, Janowitz & Hollander (1955) found that subsequent oral intake was depressed in the first case and almost completely abolished at the 100% and 175% calorie intake levels. This compensatory decrease in food intake did not take place immediately or completely as there remained a small residual intake by mouth even at the 175% level. These authors regard this finding as meaning that a neural mechanism exists, tending to maintain the act of ingestion regardless

of caloric need. The fact that this ingestion decreased over a period of intragastric feeding tended to suggest an adaptation and a gradual predominance of the metabolic mechanism of regulation.

From all the work on intragastric injections it is quite certain that, over a long period, it is possible to feed an animal by that method alone: this indeed is the experience in man. The time factor as demonstrated by Janowitz & Hollander is obviously important and provided animals could be maintained in suitable condition, a stable performance would eventually result. The ingestion of a nutrient material rather than saline, appeared essential for regulation, (Schwartz-baum & Ward, 1958) and a controlled composition of diet and water to avoid osmotic pressure changes must be maintained (Harper & Spivey, 1958).

Although the subsequent response with a lever mechanism following intragastric injection of food has been studied in rats (Kohn, 1951; Miller, 1955; Share, Martyniuk & Grossman, 1952; Janowitz & Hollander, 1955) autoinjection of food intragastrically following lever response has not been reported. Forcing the animal to obtain its calories without involving feeding, mastication, and swallowing, is a technique not available to date but worthy of investigation. Much of the work in this thesis is an attempt to fill this gap in our knowledge.

Terminology

In reading through the literature relevant to the initial stages of alimentation a whole host of terms is encountered. Each describes a condition which can exist in an animal whose feeding behaviour is being studied. Such terms as hunger, appetite, anorexia, and aphagia, may be used by various authors in differing contexts. Sometimes a definition is given clarifying the term. All too often, however, the terms hunger and appetite are confused in the literature. While it is of no direct importance to the outcome of the results of this study, it may avoid confusion to review various meanings given to hunger and appetite and try to decide upon a terminology for ultimate use in the discussion. This very point was made by Richter (1957) but unfortunately his contribution was simply a plea to each author to define his own terms.

According to Quigley (1955) hunger is a sensation which exists during a period of starvation. This sensation can be characteristically relieved by the ingestion of food. Appetite, in Quigley's definition, is a conditioned reflex depending on previous experiences of taste, smell, etc. which results in the disappearance of hunger. Hunger and appetite thus appear quite different.

Cannon (1915), on the other hand, thought that

appetite was the first degree of hunger and was an agreeable sensation. Hunger was considered to be a more advanced condition and painful in character.

Cannon & Washburn (1912) referred to the psychic elements of appetite associated with the taste and smell of food. They were of the opinion that it was appetite which normally accounted for the ingestion of sufficient nutrients for an individual's requirements. In support of this idea, Quigley went so far as to say that in the human it was the major force regulating food intake.

A psychic element, the eager craving after food, was attributed to appetite by Pavlov (1902). Young & Chaplin (1945) considered the Pavlovian definition to apply to the term hunger which could be subdivided into palatability and appetite. We can contend that preference requires a certain amount of conditioning or learned behaviour. It is simply a craving after food in which cortical discrimination is involved. The definitions of appetite of Young & Chaplin, and of Pavlov, are identical when palatability is considered a special type of appetite involving preference.

Young (1957) clarified his own views and referred to appetite as a "specific hunger" for a given foodstuff, as opposed to palatability. The seeking out and selecting of a specific nutrient by an animal which is offered a

choice can only be inferred and is not proven. Results, of which Richter, and Young, have provided many, are simply evidence of the animals' abilities to select a whole diet most beneficial to their bodily conditions. The idea of an animal selecting foods for one vitamin etc., as a conscious desire (Young, 1957), would appear difficult to accept. In addition it only adds confusion to the use of hunger and appetite.

Kennedy (1950) emphasized two components in the urge to eat, a discriminative one i.e. appetite and a primitive urge following deprivation of food i.e. hunger. Bruce & Kennedy (1951) thought that appetite might be a cortical phenomenon while hunger was of hypothalamic origin, since hypothalamic lesions caused anorexia. Again confusion arises. By definition anorexia is a loss of appetite. The term generally used to describe the lack of eating following lateral hypothalamic lesions is aphagia. This, however, has never implied that the lesions did cause a reduction or loss of hunger also.

Grossman (1955) has made fairly acceptable definitions of hunger and appetite based on the difference between sensory and affective states. In his definition hunger is referred to as a conscious sensation resulting from the depletion of body nutrient stores. This in turn

leads to a psychic desire for food i.e. appetite. Much of the acceptability of his terminology stems from the use of behavioural terms in describing feeding. In his discussion, Grossman suggested that appetite results in appetitive behaviour leading to feeding. "Appetitive", is used in the sense intended by Craig (1918). It is a description of all behaviour of a drive, leading up to the consummatory act, in this case the ingestion of food.

Many of the results obtained in this thesis have been derived from a motivation to feed, or hunger drive (Stellar, 1954). In Grossman's terminology, the strength of a hunger drive would be an index of the depletion of bodily nutrients. Since the term "hunger drive" is widely used by psychologists then it is perhaps suitable to adopt Grossman's terminology as little ambiguity can result from its usage.

The use of psychological descriptions to evaluate a physiological phenomenon introduces further difficulties. There is no trouble in referring to behavioural responses in terms used by psychologists. But when these terms have a different meaning for the physiologist, complications arise. Before we proceed to any description of the theories of behaviour involved it is worth defining words which may cause confusion.

In any discussion of behaviour, the conception of a nervous reflex is liable to occur. In physiological terms the reflex is the precise response by an effector to a specific stimulus on a receptor. The nervous connection making this possible is the reflex arc. At spinal levels specificity holds good. By introducing conditioning into the higher connecting neuronal system it is possible to alter completely, a response to a specific stimulus. The idea of the closed route in the reflex arc then disappears.

Skinner (1931), in attempting to surmount this difficulty, suggested that the applicability of the relation of stimulus to response should be the criterion for defining "reflex". The implication of his definition was that "reflex" in behaviour implied predictability. In the physiological system fatigue, for instance, can reduce the predictability. Skinner (1938) appears to have overlooked this in his analysis of behaviour. Lorenz (1950) referred to acquired responses as "conditioned reactions", quite distinct from reflexes. He tends to think of acquired reactions to stimuli as resulting from the more complex neurological mechanisms than the simple reflex arc. His conclusion was influenced by the complex nature of most behavioural responses.

It is difficult to explain the production of a response by no apparent stimulus in terms of the simple reflex

arc. The response to various motivations may stem from no explainable stimulus and in his review of the physiology of motivation, Stellar (1954) avoided the use of "reflex". It would appear reasonable to follow Stellar's example and omit the word "reflex" in a behavioural sense. To avoid confusion the term "conditioned response, or reaction" (Lorenz, 1950) could be applied to the behavioural description only. Then "reflex" can be used solely as a physiological term.

Working in psychologico-physiological fields, Konorski (1950) became aware of some words which were used in more than one context. He attempted to surmount the difficulties in usage which thus arose. He has regarded a stimulus as any compound of agents acting on the receptive parts of the nervous system and evoking their excitation. Konorski defined learning as a process leading to lasting changes in the manner in which an organism reacts to a stimulus. These changes are due to the application of the particular stimulus in definite combinations with other stimuli.

Skinner (1938) adopted Pavlov's (1927) definition of conditioning. This definition denotes a class of reactions which are conditional upon a certain operation being performed upon the organism. Stress is laid upon the reciprocal process of extinction by Skinner. After

extinction, conditioning will return to its previous strength provided the reinforcement is returned. The ability to return to this strength is proof of its lasting effect and so conditioning may be regarded as one form of learning.

Behavioural Studies

From the time of Cannon's treatise on hunger and thirst there has been interest in the mechanism underlying motivations and drives. Cannon's (1915) ideas of stomach contractions and dehydration of the buccal cavity as the prime factors controlling hunger and thirst have long since lost favour. Even while there was some acceptance of his views as a general source of motivation, investigations were being made to attempt to explain such obvious phenomena as hunger motivation.

Richter (1922) studied activity in rats, and gastric contractions in the human, and tried to correlate one with the other. Although invalid by modern experimental standards, it was an attempt to use a behavioural response, of activity, as an index of a physiological mechanism. In a later review Richter (1927) made some further, much more accurate observations on the behaviour of various animals and attempted to describe quantitatively such things as feeding habits over 24 hr periods.

Skinner (1938) described his experiments using rats to explain many examples of laws fundamental to behavioural science. The technique which he adopted as standard, was at that time entirely new. Its theory has a sound physiological basis and, with this in mind, has been used

to ppovide evidence of a physiological nature. The review of the physiology of motivation by Stellar (1954) has shown how the concept of motivation has been built up as a central motive state dependent upon sensory, humoral and neural factors.

The control of hunger by two reciprocal centres was a suggestion fitting well into Stellar's hypothesis about the control of motivational behaviour. The support which the findings of Brobeck, Tepperman & Long (1943), Anand & Brobeck (1951) and Delgado & Anand (1953) gave to this hypothesis put it in a very favourable position. It is possible to use the observable effects of motivation to investigate the control of feeding.

Skinner (1938) published observations which were provided to support an analysis of behaviour. He had used rats which were, at that time, in a novel situation. Since the publication of his monograph and the general acceptance by experimental psychologists of his theories on reflex responses, his technique has become fairly standard.

A detailed description of the lever system in the cage was provided by Skinner (1938). As a result of a rat pressing the lever, a food reward was automatically delivered to the animal. The system has come to be known as the "Skinner box", a description covering both the apparatus and

the technique.

Although the Skinner box has been widely used in behavioural studies, as yet it is not well known to physiologists. It is therefore proposed to review here the general principles of the Skinner box system.

Pavlovian conditioning is known to require a stimulus to elicit a response conditional to an unconditional stimulus first causing that response. Some types of behaviour may be emitted by an organism when no originating stimuli in spontaneous activity can be found in the environment. The first type of conditioning, Pavlovian, is called RESPONDENT behaviour and is correlated with a specific eliciting stimulus. The second type is known as OPERANT behaviour and no stimulus can be found that will elicit it. Indeed, no correlated stimulus can be detected upon occasions when the characteristic behaviour is observed to occur. Any operant behaviour will occur spontaneously at a given frequency and the strength of that operant behaviour is gauged by the frequency of its occurrence.

Another feature peculiar to operant conditioning is that it involves the correlation of a reinforcing stimulus with a response. Skinner proposed the Law of Operant Conditioning stating that if the occurrence of an operant is followed by presentation of a reinforcing stimulus the strength

of the operant is increased. On the contrary, by the Law of Extinction of operant behaviour, if the occurrence of an operant already strengthened through conditioning is not followed by the reinforcing stimulus, the strength will be decreased.

In feeding behaviour, an animal will show a series of responses. In the early stages of the sequence the responses are operants, while the typical Pavlovian "gastric" responses are respondents. Just as the respondent responses prepare the organism for the ingestion of food, the food can only be obtained upon the emission of operant responses.

Skinner saw that the approaching and picking up a piece of food is so common in rat behaviour that it might be looked upon as an unconditioned response. Such behaviour is in fact conditional to the strength of the hunger drive of the animal. By experimentally adding a food-reinforced lever-pressing response to the chain of ingestive events, Skinner found that it was possible to study changes in strength of this new operant. This was done by reinforcing with food and observing the frequency of response.

In a conditioned operant the drive governing the strength is determined by the reinforcement. If pressing the lever is reinforced with food, the strength varies with

hunger. Barnett (1957) considers that there is an important element in exploratory behaviour leading to "latent learning". This factor would be important in training the animal to feed by lever-pressing.

Skinner has listed many features of the lever-pressing as a good example for studying operant behaviour. Of these features, a few certainly support the use of this response in a physiological study of feeding behaviour. When a rat is placed in an experimental cage provided with a lever in the wall, its behaviour will fulfil many desired conditions. The lever-pressing will occur with a convenient frequency and is not part of any other significant behaviour in the rat. Although relatively unambiguous, lever-pressing is a response that cannot be made outside the experimental situation. When reinforcement by food-reward is offered for each response or number of responses i.e. pressings of the lever, the increase in responses raises the rate significantly above the original, or free operant, level. The spontaneously elicited response of lever pressing acts upon the environment to produce food. It is the reinforcing stimulus, and is followed by the reinforced response, which is feeding.

Animals such as the rat can be trained to make lever-pressing an integral part of their feeding behaviour. The act of lever-pressing, although originally not part of

any experimental animal's significant behaviour can become so well established as to be virtually unconditioned.

Skinner also found that reinforcement need not occur every time to maintain the strength of response. Even when reinforcement is gradually reduced the frequency of response may not decline. By introducing a second response conditional to the food reinforcement and gradually diminishing the frequency of food reward, then the second response will eventually act as reinforcement. The importance of this statement will appear when the change from purely oral to purely intragastric feeding is discussed.

Ferster (1953) reviewed the use of the free operant in behavioural analysis. While the system was that applying particularly to pigeons, the theory of the free operant remains the same for all experimental animals. The point was made that it was not necessary for the free operant level to be above zero. Conditioning to the reward was sufficient to increase the frequency of response.

A Skinner box for use with mice was described by Anliker & Mayer (1955). The result of lever-pressing was the delivery of pellets of solid food. By recording the pattern of pressing over long periods, a picture of feeding behaviour was built up and used to show differences in feeding between normal mice and those which had become

obese for different reasons. Anliker & Mayer stressed the suitability of a response using skeletal muscles and acting upon the environment.

A picture of the changes in feeding behaviour during experimental manipulations has been provided by Skinner box systems. The effects of various fluids introduced into the stomach on subsequent lever-pressing was studied by Smith & Duffy (1955). Kohn (1955) studied the effect of similar fluids introduced by chronically implanted fistula, upon a panel-pushing response. Anliker & Mayer (1955) and Teitelbaum & Campbell (1958) recorded changes in feeding response resulting from hypothalamic hyperphagia in mice and rats. Support of the use of the Skinner box as a measure of motivational response is given by Stellar (1955), who used it extensively in this type of study.

MATERIALS

Lever cage systems

Introduction

Before any investigation of the patterns of feeding and of the total food intakes of animals can be undertaken, it must be possible to record them. Such records must provide details of the length of time between meals, the duration of meals and the size of the meals. The obvious solution to this problem is to record the occurrence of meals on a trace paper moving at constant speed, and to demonstrate the magnitude of the meal on the same trace. For this purpose a cumulative recorder was used, whereby a pen traced the occurrence of meals on a moving paper roll. The magnitude of the meal was represented as the total deflection of the pen from the original point, at right angles to the paper movement, as in figs. (58), (59), (60).

In a small mammal such as the rat, meals occur quite frequently during a 24 hr period. It is therefore difficult to find a means of recording the feeding events. Our method was to use a modification of the Skinner box technique (Skinner, 1938), where the animal was trained to press a lever to obtain its food. (The training process will be described later). The lever, apart from controlling

the delivery of food, also triggered the pen recorder, fig. (1).

Teitelbaum & Campbell (1958) described a Skinner box cage for recording meal patterns and frequency in normal and hypothalamic hyperphagic rats. Working in co-operation with Dr. S.D. Morrison, I used a cage of similar layout, but operating by different means. Fig. (2) shows a rat in such a cage, and operating a lever.

In the study on rats with gastric fistulae, which paralleled open cage work, the Skinner box principle was also applied to sleeve cages, fig. (3). Although oral assimilation was more or less by-passed, the number of lever-presses still bore a direct relationship to meal size.

By using the same fluid diet exclusively throughout the work it was possible to avoid the difficulties of studying water intake at the same time. The only water the rats could obtain was that contained in the fluid diet they consumed. Water intake was, ipso facto, directly related to food intake.

Open cage

Unlike the specially constructed Skinner boxes described by Teitelbaum & Campbell (1958), the open cage was simply a modified rat cage as used in the laboratory. The dimensions were 10 x 8 x 10 in. high, with a hinged roof.

Modifications to the cage were as simple as possible. A hole, $2\frac{1}{2}$ x 1 1/4 in. high was cut in the mid region of the mesh of a side, and $1\frac{1}{2}$ in. from the bottom. Through the hole, a lever projected into the cage, the micro-switch system being attached to the wire mesh. The attachment was merely by four self-tapping screws which passed through the corners of the lever supporting plate, and the wire mesh, and screwed into two 1/8 in. aluminium strips inside the cage, fig. (56).

The feeding spout and food trough were attached to an adjacent side. A piece of indented steel sheet 1 1/4 x 1 1/4 in. with a right angle bend formed the trough, projecting from the side of the cage 1 in. from the floor. It was attached by 8 B.A. bolts sandwiching the wire mesh between the trough and a supporting plate outside.

The feeding spout was of 15 S.W.G. stainless steel tubing shaped with a double bend. The tip of the spout projected through the cage 1 in. above the trough. A wire grid, bolted to the wire mesh, protected the tip. The actuating lever was 2 1/4 in. above the floor, being at such a height that the animal could press it easily but not be likely to walk over it.

Sleeve cage

The sleeve cages were similar in design to those

described for rats by Morrison, Lin, Eckel, Van Itallie & Mayer (1958). Addition of a lever to each cage made it a type of Skinner box suitable for animals with permanently implanted fistulae.

For rats over 200g, the cage measured 10 x 2 1/4 x 2 1/4 in., fig. (4), but for smaller animals a cage of 10 x 2 x 2 in. was used. The frame of the cage was made of 1/4 in. galvanised wire mesh. A steel door 1 3/4 x 2 1/4in with ½ in. flanges on top and right hand sides, was hinged by its left side to the end of the cage. A wire hook on a steel spring kept the door closed against any pressure the animal exerted on it. The bottom of the door was edged with adhesive tape to prevent chafing of the animal's tail. On the front of the cage, a steel plate 3 x 3 in. was soldered to the wire. An aperture 1 3/4 in. square allowed the lever to project into the cage and the lever support plate was attached by 4 self-tapping screws to the front plate of the cage.

Two steel trestles, forming inverted Vs, supported the cage 2½ in. from either end of it. Two lugs cut at the apex of each trestle were soldered to the wire. Those trestles sat on an enamelled tray covered with absorbent paper.

Where an oral feeding tube was employed it was of

stainless steel 15 S.W.G. tubing held in place by a common laboratory clamp, fig. (3). The flexible polythene tubing connecting the fistula to the injecting syrings passed, along with the animal's tail, below the door of the cage. A double bend was formed in the polythene tubing by heating. It allowed the tube to follow the contours of the animal's back from its attachment to the fistula, until it projected from the rear of the cage.

The lever and switch assembly

The bar in the original Skinner box (Skinner, 1938) was a V-shaped wire projecting into the cage. The lever used in our apparatus was much more robust and was less likely to cause any trouble to the animal by entanglement. The levers used in the open cages and the sleeve cages were completely interchangeable.

The lever was mounted on a supporting steel plate and projected through a slot in the middle of it. It was hinged at a point 3/8 in. behind the supporting plate, on the end-plates of a piece of right angled steel with folded ends. The lugs of the lever were hinged by two 8 B.A. bolts tapped into the end-plates. The thread of the bolts was removed in the region supporting the lever lugs, thus allowing the lever to pivot freely. There was a non-abrasive lip on the lever, fig. (5). Four holes with clearance for the

self-tapping screws allowed the lever assembly to be locked firmly in position on a cage.

A "Burgess" changeover microswitch with an actuating spring lever was fitted directly to the lever supporting plate. The switch was adjusted so that the spring lever of the switch just maintained the lever in a horizontal position. The switch was clamped to the plate, end on, with a piece of insulated Paxolin packing inserted. A piece of angled aluminium bolted to the supporting plate was fitted to either side of the switch. Two bolts passed through the switch holes and sandwiched it in place between the aluminium plates.

Finally, a stop piece on the inside of the supporting plate prevented the lever from being depressed further than was necessary to throw the switch. The lever's return to a horizontal position depended entirely on the spring of the switch lever. 10g wt. on the tip of the lever was the minimum required to prevent the lever from returning. Return occurred every time, as long as no object was touching the lever.

The 3-core cable to the electrical relays was plugged into a miniature 3-pin socket close to the switch.

Cumulative pen recorder

The device used to make permanent records of each

experiment was a cumulative recorder. The records which it produced showed a cumulation of events with respect to time. The method by which this was achieved was to have a paper roll moving through the instrument at a constant rate and moving a pen across the axis of movement when an event was signalled. As commercial units are not available in this country, the recorder was designed by Dr. Morrison for this particular purpose.

The devices were all mounted within a brass sided stand, fig. (16). Two brass rollers of 1 in. diameter provided the track through which the paper passed. The paper supply lay within the stand and passing out at the back, passed over the writing platform on which it was kept flat by a brass plate 1/32 in. above the platform. The paper roll then passed over the rubber-coated driving roller and was firmly held down on it by the weight of the upper heavy 1 in. roller. The drive came from a mains powered Sangamo-Weston synchronous motor turning at 1 rev. per hr. The gearing was such that the paper was driven at 5/8 in. per hr.

The recording device was powered by a 20 V. electric motor which responded to small impulses. However, an internal clutch was fitted to prevent overrunning of the device by momentum at each impulse. A cam with six depressions was fitted to the drive of the motor and the cam

operated a microswitch controlling the motor power supply. The purpose of the cam was to ensure that whenever a brief impulse started the motor it would continue to run for at least 1/6 of a revolution before switching itself off. It was held on by the cam and switched off at each depression. The brief impulse from the 20 V supply through the time delay relay was always sufficient to start the motor, and ceased while the cam held the motor on.

A screwed brass rod was supported between the sides of the stand and was driven by the motor. On this screwed rod a carriage, supported by two polished steel rods could travel across the machine, fig. (7). The movement was simply produced by fitting a half-tube, threaded inside, to fit the rod thread and so be propelled forward from left to right so the rod revolved. On the moving carriage was fixed a ball-pointed pen which provided the record on the paper trace below.

To make the machine fully automatic and suitable for 24 hr recording, a fly-back mechanism for the pen carriage was installed and is shown in fig. (7). It consisted of a microswitch on the right hand upright triggered by the movement of the carriage against it. This was used to energise a solenoid with a pin engaging in the carriage as it approached the end of its traverse. Energising the solenoid raised the pin and carriage clear of the rod

thread and locked the carriage clear of the engaging thread of the rod. A typewriter carriage spring provided tension to return the carriage to the left hand side. There, a rubber stop released the lock and so the carriage thread re-engaged in the rod thread once more, ready for another traverse. To provide an accurate numerical record, a 24 V resettable counting relay was connected in parallel with the motor. 870 impulses were required for each traverse of the trace.

Time delay relay

One of the difficulties encountered from the first with the sleeve cage lever system was that of the rat pressing continuously on the lever, and the mains relay operating the process timer was overheated. Moreover, with that system rats would press continually during the period that the process timer was running anyway. Thus the association between lever-pressing and food delivery was confused.

To enable any press for a small minimum time to energise the relay system and to hold the process timer relay closed for long enough only to start it functioning, a relay with a time constant was installed. One other important function of this relay system was that by varying a resistance, the time for which the lever had to be released before it could be pressed again to advantage could be adjusted.

The 4.7K ohm resistance which was mainly used, gave a 1 sec delay period before the lever could be pressed again to close the circuit. The 10K ohm resistance gave a 2 sec delay. One set of the relay terminals was used to close the recorder circuit and another set closed the circuit controlling the mains relay, fig. (8).

The switch mechanism was efficient in operating all further relay systems. When pressed for a minimum short time the relay was thrown and held for adequate time to hold the mains relay and the recorder. The lever had then to be released for a minimum time before further pressing would operate the relays.

The mains relay, fig. (9), was installed in the circuit between the time delay relay and the process timer. It was a self-contained unit and the control terminals were connected across the time relay and the 240 V output terminals across the mains controlling circuit of the process timer.

Process timer

The process timer used in the circuit was a commercial R.F.T. Synchronous Process Timer manufactured by Messrs. Londex Ltd. It had a range of times from 0 to 60 sec with a scale reading $\frac{1}{2}$ sec intervals. By setting the pointer anywhere on the scale the timer would hold itself closed for that given time when energised. At the end of

that period it was self-resetting, but should the control circuit have remained closed, the process being controlled would cease. It could not be restarted until the timer reset. It was completely self-resetting but only when the control circuit was open.

The principle of its operation depended on the control mains circuit energising the relay which engaged the clutch of the Sangamo Weston synchronous motor with the time mechanism, fig. (10). This then started the process and also held its own mains relay closed. When the time expired, the process was switched off and the clutch was disengaged. The time mechanism then reset to its previous value.

After 6 months use, the timer relay developed a very noisy "chatter", and when investigated it was found that the 'slug' across the relay had melted off. The mains relay was then replaced and apart from the actuation at the beginning and end of the process, it remained silent in operation.

Uniselector pulse divider

In the sleeve cage system where two injection systems were used, a uniselector pulse divider was added to one circuit, fig. (1). Its purpose was to reduce the frequency of delivery of oral diet. While every lever-press resulted in delivery of intragastric food, the ratio of times that

oral food was delivered was varied. The range of these ratios was as follows - 1, 2, 4, 8, 12, 25, 50, ∞. This meant that for every time the oral injector was energised, the intragastric injector was energised the number of times shown on the dial. The technique proved quite useful in training, where the ratios were gradually moved from 1:1 to 25:1 or 50:1. Fig. (11) is a circuit diagram of the apparatus used.

Slow injection apparatus

The device used to deliver fluid diet to the animals in controlled amounts was the Continuous Slow Injector of Messrs. C.F. Palmer (London) Ltd. The slow speed of the injector provided a slow and constant rate of drive on the large syringes containing the diet. The rate of injection could be varied and the duration of the injection was controlled by the process timer. The rates of advance of the ram operating the syringe varied from 1 in. in 2 min to 1 in. in 32 min. Along with the bore of the syringe, the rate of advance determined the quantity of fluid diet delivered.

In 24 hr, a fairly viscous diet in volumes of up to 100 ml was often delivered. For these volumes large capacity syringes were used with a large delivery nozzle i.e. Luer fitting. Special adaptor taps were fitted to these

Luer syringes and the sockets of large serum needles were fixed to those adaptors by heavy rubber tubing.

The glass syringes used on the injectors were of either 50 ml or 100 ml capacity with the plain Luer fitting. To determine volumes at a given setting (speed of advance) and time (process timer setting) each type of syringe was calibrated - Table (14), Appendix II.

The all-glass 50 ml syringes were particularly susceptible to back leaks between piston and barrel and those surfaces had to be coated with a thin layer of Vaseline every time before use. Although the metal ringed-pistons of the large 100 ml syringes fitted much better, they were also coated with Vaseline before use. Only the larger syringes were used for intragastric injection to cope with the larger volumes involved. Polythene tubing connected the adaptor taps to the steel diet-delivery tubes.

Drinkometer

Since both water and food intake are dependent on one another where fluid diets are concerned it was thought advisable to find some means of recording the normal feeding pattern of rats where they are offered fluid diet by the normal water bottle method.

Graduated 50 ml cylinders were used as the containers and were fitted with a rubber bung through which a drinking

tube passed. A projecting platinum wire loop was laid as close to the lip of the tube as possible and the wire bound firmly to the tube with adhesive tape. The platinum loop was adjusted so that the tongue of the animal made contact between the loop and the diet it was licking from the tube. Another wire passed through the bung into the fluid. The two platinum wires were connected to a thyratron relay device controlling a drop recorder. Each lick by the animal then acted as a switch closing and caused an impulse to be passed to the recorder. The circuit was earthed to the cage and no unpleasant reaction was shown by the animal. It was found that the electrical impedance of the rat was too great to allow the animal to make the contact in the circuit as was described by Stellar & Hill (1952) since a different thyratron relay was used.

The records were made on a slow speed kymograph and were taken along with gastric contraction records from sleeve cage animals. The relationship between feeding by this method and the effects on gastric contractions could be recorded.

Recording of gastric contractions

In part of this investigation it was desirable to know the effects of various events on gastric contractions.

Morrison et al.(1958) described a system to record the contractions of the stomach of the rat. Rats were kept in sleeve cages similar to those already described. Latex rubber balloons, permanently implanted in the stomach were connected to a tambour system sensitive to volume changes. An attached lever produced the records. The balloons, polythene tubes and tambours were all filled with water to avoid the difficulties of calibrating an air filled system. Morrison et al.(1958), however, were aware that their system did not record pressure in a linear fashion on a paper trace. This was due to the resistance in the polythene tubes and the volume change in the tambour.

More satisfactory records were obtained on a long-paper kymograph by attaching a lever to a "Hydroflex" brass bellows which was connected by rigid tubing to the polythene tubing from the animal's stomach. A water manometer for calibration was connected by a 3-way tap to the tube system and a syringe was also fitted by another 3-way tap to allow adjustments of volume, and clearing of the tubes, fig. (12).

The rats had to be kept in sleeve cages. Originally two fistulae were inserted in each animal and one was used exclusively for recording. At the outset of these experiments latex balloons, lying inside the stomach, were attached to the recording fistulae. After one or two

attempts to record by this method it was discarded as the balloons perished in the stomach contents. In these cases it was found that an open-ended tube in the stomach proved an adequate means of recording. The later animals from which records were taken had only a single fistula implanted and a 3-way tap system allowed recording from the same outgoing polythene as was used for injection of fluid intragastrically.

The recording system was made of brass bellows and tubing and Pyrex glass tubing with tap systems. Glass tubing was connected by pressure rubber tubing and the serum needle and was also attached by that means. The system was filled with boiled water to exclude all air and was connected to the fistula by polythene tubing.

Clearing the tubing was achieved by connecting the reservoir syringe to the recording tube and applying slight positive pressure. Calibration was achieved by connecting the recording system to the graduated manometer.

Insulated room

The initial tests on fistulate and open cage animals were carried out in the open laboratory. As a result, the animals were subjected to changes in light, in temperature and to the general distractions of the laboratory. Weiss & Hurwitz (1959) found that reduction of stimulation

by sound etc. resulted in rats decreasing their intakes of food. For the more carefully controlled runs, the cage was moved into a partially insulated room designed for metabolic work. A thermostat mechanism connected to 2 x 100 W electric lamps maintained the temperature about 21°C when the environmental temperatures dropped. Air was circulated by an electric fan situated below the table on which the cage sat. Temperatures in the summer never rose above 24°C in the room. Even in winter, and especially at weekends when all departmental heating was off, the temperature never was allowed to fall below 21°C. A double glass window set in the fibre board walls allowed observation. A time switch controlled the internal lighting, switching on at 8.15 a.m. and 9.45 p.m. each day. The "Dexion" rack, fig. (13), with the electrical apparatus and recorder sat outside the room and connections with the cage and injector inside the room were by cables passing through a small hole in the wall of the room.

Stereotaxic instrument

Centres which exert neural control over feeding behaviour are known to lie in the hypothalamus. The experiments to determine the functions of these centres are usually done by ablation of one pair of these centres. The lateral area of the hypothalamus of the rat was destroyed and the effect produced was studied. The small dimensions of the rat brain demand a high degree of accuracy in placing the lesions and these lesions must be made with precision.

On occasion it was necessary to repeat the position of a lesion some time after the first operation. A rigid apparatus, with accurately graduated co-ordinates and fine controls was essential for such work. In the early straightforward stereotaxic instruments such as the Horsley-Clark (Clark, 1939), the head of the rat was fixed in a definite position and the surgical tool manipulated into place by sliding it along rods which allowed movement in three planes. The adjustment of such instruments is so coarse as to make repetition of a given position within reasonable limits (0.5 mm) impossible.

A much finer instrument is the Kreig-Johnson (Kreig, 1946) which allows accurate adjustment of the animal's head under a fixed electrode. One of the

difficulties with such a layout is that the electrode must always approach a point in the brain vertically, and, therefore, always through the same structures.

Stellar & Krause (1954) described a stereotaxic instrument specifically for use with the rat, which circumvented this problem. The headholder was moved by fine adjustment and the electrode was attached to a moveable arc in the antero-posterior axis of the rat. The arc could also be rotated round the antero-posterior axis and raised and lowered with respect to the headholder. Such an instrument allows any part of the brain to be approached from any angle in a hemisphere above the fixed points of the headholder. This condition is achieved by setting the headholder and arc system such that the co-ordinates of the point to be reached by the electrode lie in the centre of the sphere described by the arc holding the electrode.

The instrument used in all rats which were studied in this thesis was based on the design of Stellar & Krause. Readings could be taken to 0.1 mm. It was made by W. Dorward of Dundee to specifications drawn up by Dr. Morrison. In some ways our machine was an improvement upon the Stellar-Krause instrument while retaining all its virtues. The improvements were solely on the constructional side where

ballrace mountings and spring-loaded movements were incorporated where possible. They reduced irregularities in movement and avoided 'backlash'. All movements were provided with locking devices.

Fig. (14) is a general view of the instrument. Basically, it consists of a rigid base A on which are fixed a column B carrying the arc movements and electrode, and a lathe slide movement G and F on which the animal platform is mounted, K.

The lathe slides are graduated in cm and move in a horizontal plane, at right angles to one another. The lower slide moves rostro-caudally in a sagittal plane with respect to the animal, and the upper slide moves laterally. On the upper slide a rigid platform holder H is fixed and is topped by a removable paxolin animal platform K. This platform always reseats itself precisely on the top of the supports. On the platform itself is the headholder assembly consisting of adjustable earbars I and an incisor bit and clamp J. The centres of the tapered earcones and the top of the bit of the incisor clamp are of equal heights above the platform so that the auditory meati and the roof of the mouth are all in a horizontal plane when the animal is in position. A bar screws into place and locks the incisors between it and the bit. The clamp slides in a rostro-caudal plane and may be locked in position by finger

screws. The ear bars have graduations allowing centering of the head on the platform. Clutch systems are fitted to the screw on the earbars to prevent over-tightening with resulting distortion of the skull. Locking screws are again provided.

The whole platform assembly may thus be moved within the range of slide movements to any position in a horizontal plane to within 0.1 mm.

The assembly holding the electrode is composed of four movements. The column B on which the whole assembly sits has a screw adjustment making it possible to raise and lower the arc system. The arc assembly C and D can itself be revolved through 90^{o} in a transverse plane so that the electrode may be swung clear of the platform. The lateral movement C is calibrated in degrees and thus the arc may be tilted from the vertical in either direction. The arc itself carries graduations and its movement D enables it to be set so that the electrode moves from the vertical in a rostro-caudal plane. The fourth movement is that of the electrode holder itself. A ratchet operated clutch allows movement of the electrode in a downward direction only against a minimal resistance, fig. (15). The movement of the electrode is measured against a cm scale. All movements of B, C, D, E, are fitted with locking screws and there is also a locking device to set the arc precisely in the

rostro-caudal plane. The electrode holder carries a side screw to lock the removable electrode chucks in place. Stops are fitted to all movements in the machine so that over-running the available range of settings is obviated.

The screw movements of B and C and the rack and pinion of D are machined from brass. The shaft through C is fitted with ball races and movements B and D are fitted with spring loading devices to allow a positive movement. The arc itself is made of a Tufnol composition and the electrode holder has brass components.

The special electrodes (see below) are set in individual chucks, obtained from commercial pin tongs. The electrode end fitting into the chuck is carefully scraped for about 5 mm to remove all insulation and provide good electrical contact. There is a terminal point for the positive lead from the electrode supply on top of the electrode holder and the negative of the supply is connected to the incisor clamp.

Fitting of the animal's head between the earbars was facilitated by using Perspex earcones. Of exactly similar dimensions, these earcones were connected by a piano wire band which fitted into a groove in each. The taper of the earbars fitted into the concave matching taper in each earcone and the angle formed by the earcone taper was 135^{o}.

Electrode supply

The electrical circuit to supply the current to the electrodes is shown in fig. (16). A 30 V supply is connected across the input and with tissue impedance across the output, a constant current may be maintained by varying the resistance bridge. The positive output lead is connected to the electrode holder and the negative to the clamp on the incisor teeth of the rat.

A current of approximately 2mA for 15 sec was used to make lesions in the lateral hypothalamus. Electrical resistance changes inversely with the diameter of the metal tip of the electrode. To maintain constant current through a tissue of continuously varying impedance, the voltage fluctuates. No compensation for these changes could be made with the simple assembly used, which proved otherwise quite adequate.

Electrodes

In previous papers describing work with stereotaxic instruments, the current and time for which it is passed are usually given. Very seldom is there a size given for the electrode or a description of the material used. Stellar & Krause (1953) described a platinum electrode sheathed in a fine glass tube and bare for 0.25 mm at their tips. Teitelbaum & Campbell (1958), using a similar instrument,

gave the diameter of their electrodes as 0.25 mm. It may be assumed that their electrodes were similar in construction to that described by Stellar & Krause. It is not stated whether the diameter is that of the platinum wire or of the outside glass sheath.

Theoretically it would be desirable to make as little of an electrode track as possible, in the brain. It is essential to have a rigid electrode which is completely insulated except for the tip. It would seem then, that an electrode made of a pre-stressed metal, of as small a diameter as possible while still allowing it to remain rigid, would be most suitable. A thin coat of insulating varnish should coat the electrode except the very tip.

Our first electrodes were made of piano wire tensioned by the heat of an electric current and tempered by sudden cooling in water. These electrodes were dipped in "Hi-Meg" insulating varnish at least twice, drying between coats. From a study of the brains in which they were used, these electrodes were found to have leaked current along their length. Piano wire dipped in concentrated varnish did not take a uniform coat, there was only a beaded layer. There was no means of applying a thin even layer, and any flexion of the electrodes caused the varnish to flake off.

Piano wire dipped in a catalytically hardened

polyurethane lacquer was also covered by beads. However, it was found that the lacquer is quite suitable for application with an artists' fine spray. Two coats applied in this fashion gave complete insulation along the length of the electrode. Tests were made by running the electrodes through a saline bead in a wire loop and measuring the passage of current at any point. Voltages and currents were above those likely to be used in practice. The lacquer was quite resilient and did not shatter when the electrode was slightly flexed.

Table (16) presents a comparison of various electrodes tested for polarisation. Piano wire and Minalfa alloy resistance wire were each tested in two thicknesses. The nichrome wire tested was found less liable to polarise and was subsequently adopted for use. Minalfa was not chosen because of the possible electrolytic deposition of copper in the brain. The tests were made by immersing the electrode tips in a solution of casein in saline and passing current. The amount of casein precipitated round the tip seemed to bear some relation to the voltage applied. When polarisation occurred, the voltage across the electrode suddenly increased. The initial voltages were minimal when the electrode tips were newly cleaned.

Preparation of electrodes

Lengths of up to 6 in. of wire were stretched between electrical contacts exerting a pull on the wire. Current was passed until the wire glowed dull red and it was then plunged into cold water and the current disconnected. The resulting wire was straight and stressed. After cleaning with solvents, the wire was sprayed with polyurethane lacquer, forming two thin coats. The dried electrode wire was then cut into suitable lengths. By filing the tip straight across, a smooth surface resulted. The other end was then fitted into the small chuck of pin tongs and was ready for fixing into the electrode holder of the stereotaxic instrument. Tests for bad insulation were carried out on the coated wire before cutting.

Fistulae

The rats used in the sleeve cage system all had permanently implanted gastric fistulae. The simplest form of fistula is a fine plastic tube led into the stomach through the skin, muscle wall and stomach wall and anchored at all these points. Because of the obvious danger of infection, a tight seal must be formed by the stomach wall round the tube.

It was on this simple technique that the procedure described by Kohn (1951) was based. A plastic tube, flared at the stomach end was inserted through the stomach wall from a ventral incision and a purse string suture sealed it in place. The distal tube passed through the muscle wall and round to the base of the neck where it emerged dorsally, being sutured to the muscle. While Kohn presented evidence for the efficiency of the system over a short period, it was obvious that, for the long term and continuous injection work which we contemplated, this technique would require some amendments for use.

Morrison *et al*. (1958) used a modified Kohn fistula to which the gastric balloons were attached. To prevent the tube at the base of the neck from annoying the animal, they led the tubes out at the base of the tail and so adopted the sleeve cage system to prevent the animals from reaching the tube.

The original trials of fistula insertion were not too promising. As both recording from the stomach and injection into the stomach was required, two fistulae (one with a balloon) had to be inserted in each animal. To allow a much more permanent seal and avoid the danger of tube collapse a piece of stainless steel tubing, 15 S.W.G., was inserted inside the 0.25 cm outside diameter polythene tube for 0.5 cm. The end was flared to produce a flange. Once this was attached to the stomach wall and the purse-string suture pulled tight, a polythene washer was slid down the tube into place tight over the suture. Infection appeared in all early operations and tissue was repelled from the polythene tubing. It was then thought that the type of polythene used repelled the tissue and it was discarded. Permanent bends had been produced in the tubes by heating. They reduced the risk of the fistulae being pulled from the stomach by the tube tending to straighten.

To replace the polythene, bent silver tubes with nipples and silicone rubber washers replacedthe polythene tubing. Stainless steel tubing was forced through stab wounds in the skin. Fibrous tissue anchored the steel tubes firmly in place. Flexability of the tube system was achieved by linking the steel and silver tubes by silicone rubber, bound to the metal by silk suture to prevent leaks.

With this system, survival rate was greater but was still not sufficient to ensure satisfactory completion of the experimental sequence in each rat.

Ultimately, an open tube was used to record gastric contractions, since rubber balloons failed to survive immersion in the stomach contents. Only one fistula was implanted in the final technique. The infection which caused most of the trouble was in the subcutaneous tracks so it was decided to allow the fistula to come directly to the abdominal skin surface. Leakage from the stomach was the obvious source of infection due to gram-positive cocci and gram-negative bacilli. Every attempt was made to devise the most efficient seal.

The slotted head of a 0.14 in. diameter nylon screw was turned down to a nipple with a concave section. A hole 0.07 in. diameter was drilled through the screw and the central hole tapped to take stainless steel tube of 15 S.W.G. threaded 8 B.A. A nylon nut with surface to mate with the upper surface of the nipple was made to fit the screw, fig. (17). The length of the screw was cut to 7/16 in. and then a stainless steel tube with a right angle bend was threaded into the inner hole and heat sealed in place, after nylon cement had been used to fill in the thread as far as possible. The total length of the fistula was then 5/8 in.

and the nylon nut could be screwed up and down the shaft and taken over the bent steel tube without forcing it. This fistula was implanted in the stomach as described in Appendix I and locked by the nylon nut to the stomach wall, with nylon cloth washers to promote fibrosis. Further nuts and washers helped to anchor the fistula to the muscle wall and the steel tube passed through the skin by a stab wound to the exterior. The right angled steel tube pointed caudad and a polythene tube was then firmly attached.

Experimental animals

The rats finally used in these experiments were of an albino stock of Rattus norvegicus bred in this department. Males were used exclusively to avoid difficulties which might have occurred using females. The oestrous cycle causes many effects on the female rat and metabolism shows a fluctuation round the oestrous cycle. Food intake does not vary detectably (Morrison, 1955) but activity changes at oestrous (Brobeck, Wheatland & Strominger, 1947). The animals used in these earlier investigations on pregnancy (Morrison, 1956), growth (Cumming, 1958), and fasting (Cumming & Morrison, 1960) were females of the hooded Wistar strain. Originally we tried to use males of the hooded strain. However, the complete failure of the first series of hooded rats to survive implantation of the earlier types of fistula made us use albino male rats. Success was greater, whatever the reason, and we used the albino male rats for subsequent work. The hooded strain was never tested with improved fistulae so the fault may have been in our original technique.

Diets

Where the intragastric injection system was employed as a means of feeding animals, only a very fluid diet could be used. The diet had to be complete and one on which the rats could be maintained for a long time. Moreover, it had to be liquid enough to pass easily through small bore plastic tubing. Cows' milk is a fairly complete food but would have to be supplemented to maintain rats in health. A synthetic diet Metabolic Series 1 (M.S.1.) was used by Cumming (1958) to feed growing rats for metabolic investigation. This diet could maintain a normal rate of growth but was a powder not easily prepared in liquid form without considerable dilution. By changing the proportions of the constituents of diet M.S.1. the first liquid diet was prepared. Ingredients of this diet are given in Table (15). The diet was of a suitable viscosity and was prepared to provide 1 Cal per ml.

In a horizontal syringe the salts settled to the bottom and the fats came to the top. Separation occurred within 1 hr so that the formula was far from ideal for feeding over 24 hr. Subsequent modifications were made, Table (15) and one was finally adopted. This diet had sodium tauroglycocholate and cholesterol added as emulsifying agents. Sodium benzoate (Adolph, 1947)

restricted breakdown of the constituents by bacterial action.

The casein and other constituents were added to 200 ml of boiling water in a beaker and mixed into a paste. The casein was added first, and slowly, to prevent formation of lumps. Another 200 ml of distilled water was then added and the mixture stirred over a bunsen flame, noting the temperature. The paste had to be continually stirred otherwise it burned and stuck to the bottom of the beakers. When the temperature reached 90°C the mixture was poured into the container of a top drive macerator. The container had previously been warmed. The mixture was then homogenised for 15 min. It was continuously mixed while cooling from 80°C to 60°C as that was the temperature range for emulsifying. A temperature of 90°C was necessary to ensure complete solution of the casein. Penicillin et Sulphathiazol powder was added and the mixture diluted to 1 litre. It was allowed to cool while being agitated in a slow speed stirrer. Eventually it was stored in a refrigerator. When only removed for periods long enough to allow refilling of syringes, the diet would remain fresh enough for use after 10 days.

"Complan" is a commercially prepared soluble powder diet from Glaxo Laboratories. We were prepared to adopt it

for use if it did not settle out. The main constituents per 100 g were

13 g Protein : 16 g Fat : 46 g Carbohydrate

\+ sufficient vitamins and minerals for human requirements.

The powder dissolved fairly easily when diluted to provide 1 Cal/ml. However there was still a tendency for the layers to settle out. During the experiments the rats developed diarrhoea after 2 days of "Complan" feeding either intragastrically or orally. The diarrhoea continued until the animals had to be taken off experiment. It could be seen that the "Complan" was being voided in an almost undigested state.

When "Complan" given orally was replaced by the original fluid diet the diarrhoea disappeared and the rats recovered. The manufacturers claimed that they had experienced no trouble in feeding rats on "Complan" but from our own experience we decided it was unsuitable for further use.

METHODS

Lever cage systems

Discussion

In the investigation of feeding behaviour, lever-pressing activity as previously described has been used as an index of hunger. It is essential that feeding by lever-pressing should become as much an automatic response by the rat as any of its other daily activities. Although it was found that rats have a fairly high level of intelligence and can learn the lever-pressing technique in a short time, it was necessary to allow them to adapt to their new routine over at least 3 days. Luckily, from the point of view of the experiment, no rats showed any tendency to keep pressing the lever, with the minimum delay between presses, and then collecting the accumulated food. Even the most intelligent of all the rats which were studied failed to resist the temptation to devour the food as soon as it was delivered in another part of the cage.

There is one main assumption which has a bearing on the significance of deviation of results from the normal. It is that once the rat has been trained to press the lever to feed, it should thereafter continue to press the lever at such times as it desires to satiate its hunger. While there is every expectation that the rat will do so in the conditions to which it is accustomed, there is no guarantee

that it will continue when the conditions are changed either externally or internally.

When removed from the Skinner box for a short period and allowed to feed on cube diet and water, the rats showed little tendency to forget the actions required in the lever cage. The longer the period the animal was in the Skinner box the more rapidly it re-learned the technique. If the rats were off the lever system for more than 1 day they all re-learned at the same rate. After anaesthesia, rats were seen feeding before they had regained full control over their muscles. These instances support the assumption that, in general, performance on a lever system is not easily upset. There is no real proof that the results obtained can be used as conclusive evidence that appetite is absent or present in hypothalamic aphagic rats. A second lever was installed in the cage of one rat after training. It measured random lever pressing before and after a lesion operation. At no time did the second lever provide reward, and it could not be installed in a sleeve cage. Pressing of the second lever was minimal.

The reinforcement by oral diet of the intragastrically fed animals presented a problem in assessing a normal pattern over a period of many days. When the reinforcement became very infrequent there was a tendency for the lever-pressing responses to decay, not extinguish, over a three or

four day period. Thus, after a three day control period there was often a tendency for the responses to decrease without any external influence having been imposed on the rat. If, therefore, an operative procedure was superimposed on the natural decrease, then any change due to the operation would be difficult to assess. The changes in response which occurred naturally were so variable that it was impossible to predict their magnitude or direction. Only very large changes in feeding pattern could be detected but since the effect of the operation was almost an "all or nothing" response, discrimination between effects was not difficult.

While the problem of having to provide oral reinforcement was only partially solved, earlier investigations had shown that two or three meals could occur between two reinforcements. Although it was not wholly intragastric feeding, reinforcement orally once for each fifty intragastric rewards was frequently the closest we could approach it within practical limits. We decided to assume that for our purposes it represented intragastric feeding.

Reinforcement in the open cage presented a different problem. A reward was presented to the animal for every act of lever-pressing. The ratio of reward to action was 1 : 1 and so any slight defect in the delivery system would

have changed the ratio vastly. As would be expected where an animal is conditioned to respond to an external or internal stimulus for a reward, failure to receive a reward would cause extinction of responses. The animal would respond at a rate which increases, and then decreases exponentially. After extinction, if the reward is restored then the animal will respond until it reaches the previous normal rate (Skinner, 1938).

The original viscous diet went sour within 24 hr when left in the injector. Even after use of various bacteriostatic measures, 24 hr was the absolute maximum for the diet to remain suitably liquid in the syringe and tubes. If, for some reason, the delivery took place at very infrequent intervals the drop of diet, which inevitably hung from the spout, lost its water and solidified. The resulting stasis caused the diet to solidify in the metal and polythene tubes within a few hours if the blockage was not cleared. This overnight blockage was a frequent occurrence in the early days, but it was overcome, mainly by a revised routine.

The diet was originally changed every morning and the tubes checked for blockages during the day when the rat showed little activity. By evening the diet had been at laboratory temperature and had been fairly static for almost 8 hrs. There was therefore a fairly good chance of

blockage occurring before morning even though the lever-pressing activity increased.

By changing the syringe late in the afternoon and checking in the late evening, the risk of blockage by morning was greatly reduced. The chance of blockage occurring between morning and late afternoon was therefore increased but checks were fairly frequent. Feeding activity was usually so reduced during the day as to be little affected by blockages.

One of the procedures used to keep the feeding systems free from blockages inevitably affected the results. Although the volume delivered per press on any run was constant, the quantity of food delivered and the number of lever-presses did not always tally. A few presses of the lever were made on occasion by the operator to ensure that there was no blockage. More often, the injector was turned manually to force old diet through the tubes and no corresponding number of presses for that volume was recorded. The records of the volume of food delivered and the number of lever-presses are exact. In the circumstances they are independent of one another where daily absolute values are concerned.

Twenty four hours was nominally the length of each run. While the sleeve cage system was being checked and

changed daily, the animals were free to move around and groom themselves. The times taken for this procedure are by no means constant but they did not vary greatly. Changes and records were made at approximately 9.30 a.m. each morning.

Open cage routine

When an animal was set up in the open cage for experiment all the electrical and mechanical parts were checked. The changeover routine is described in Appendix I. Care was always taken in setting the syringe in the injector. The distance between the adaptor socket and the fully retracted injector ram was just sufficient for fitting a large syringe filled with 90 ml of diet. The syringe was slipped into place and the adaptor locked in its clamp with 2 finger screws. A check was made to ensure that the top of the adaptor was connecting the syringe and the polythene tubing. The ram was then pushed hard up against the syringe piston and the threaded section of the ram bearing was slipped into place. This engaged in the ram thread to allow it to advance. The lever was then pressed to initiate all the electrical events resulting in the injector working. By selecting a higher gear of the injector than was normally used, the tubing to the feeding spout was rapidly filled with diet.

Sleeve cage routine

The procedure just described was also used to prime the feeding tubes of the sleeve cage system. However, the intragastric tube required special attention and was usually checked for blockages with a nylon probe. Care was taken not to pass it too far into the stomach. The tubes were then filled with water and up to 1 ml flushed through to check patency. The diet tube could then be connected and primed as previously described for oral diet.

Any checks for blockages were usually made by advancing the injector ram by twisting by hand. If it were patent then diet would flow through. Otherwise the tubes were removed and cleared. No great pressure was ever exerted on the intragastric tubes.

Drinkometer routine

The graduated cylinder was filled with diet and the recording spout of the drinkometer pushed into place. The cylinder was then inverted with a finger over the tip of the spout. This prevented bubbles from forming an air lock in the spout. It was then clamped by a laboratory stand and clamp with the spout projecting into the cage. The electrical connections were made by fastening crocodile clips on the recorder leads to the wire leads from the spout.

Gastric contraction recorder

When gastric records were being taken, the writing point on the kymograph initially indicated 0 cm water pressure within the system. This was done by turning both 3-way taps to connect the reservoir syringe to the manometer and bellows. The levels were adjusted to zero and the manometer isolated from the system.

The reservoir syringe was then connected through the lower tap to the recording tube to prime it with water. The tube was connected to the cleared fistula and flushed through with 1 ml water. The taps were then turned to connect the recording tube directly to the bellows.

At various intervals the reservoir syringe was used to clear any small blockages from the recording tube at the fistula. Calibrations were obtained by changing the water levels in the manometer.

Training of rats

Some animals showed an ability to learn to press the lever to receive food with remarkable ease. All training began by using the operant conditioning technique. The "free operant" level of responses varied from animal to animal. Some pressed the lever frequently at random and soon associated the resulting rewards with this activity. Others did not press the lever often at random and so did

not become aware of the relationship between lever-pressing and the frequency of rewards. The range of times taken by the rats to learn the technique varied from 35 min to 72 hrs. When there was no response after 4 hrs, various inducements were made to speed up the learning process.

Sometimes a trained animal was put into the cage with the novice and left for 24 hrs to train it. On occasion this did not work, the trained animal pressing the lever for the untrained animal to gain the benefit. In this case the usual incentives were eventually offered to the untrained animal on its own.

a) Open cage. By waving a thin strip of card in front of the lever a hungry rat would usually react by leaping at it and in the process falling on the lever. The card was immediately dragged round the bars of the cage to the feeding spout. The rat followed the card and had the delivered diet brought to its attention. After repetition of this procedure, the animal would usually show signs of appreciating the sequence of events. Thus it eventually learned the acts required to produce its food reward. Once the technique was learned, no further trouble was encountered with any of the animals.

b) Sleeve cage. The feeding spout of the lever cage

was very close to the animal's head as it lay with the lever in front of it. Again, by using card and moving from the region of the lever to the spout the association between the lever and the food was soon learned by the animal.

Operation to make a gastric fistula

The procedure for inserting fistulae in the stomach was initially as simple as possible. The technique first used was similar to that of Kohn's (1951) method as modified by Morrison et al.(1958). All anchoring of the tube system to the animal was done by silk sutures round the tubing and secured through either stomach wall, abdominal wall or skin. No great antiseptic precautions were taken and the animals were given penicillin post-operatively.

After all the first series of rats developed infections, the operations were performed under more sterile conditions and sometimes pre-operative dosage with antibiotics was tried. While the design and construction of the fistulae themselves were changing, the methods used in implanting them were also changing.

A full description of the final operation is given in Appendix I and fig. (18) shows the stages in that operation. A brief outline of the first operation and the major modifications at intermediate stages are also listed in Appendix I.

Operation for hypothalamic lesions

The production of hypothalamic lesions for these studies was done by inserting an insulated electrode from the stereotaxic instrument described into the brain at the estimated locus of the lateral area. The lesion was produced by passing a direct current using the electrode as anode. This is a much more practicable method than either using radioactive electrodes (Settlage & Bogumill, 1955) or a high-energy proton beam (Leksell, Larsson, Andersson, Rexed, Sourander & Mair, 1960).

To allow for the effects of the basic operative procedures themselves, control operations were carried out. As the full operation consists of basically a) anaesthetising, b) passing the electrode through the brain and c) inflicting the lesion, controls for the earlier parts of the operation were carried out. Anaesthesia was produced by giving the rat 0.05 ml Nembutal and placing it in a desiccator with a pad of cotton-wool soaked in ether. Thereafter ether anaesthesia was maintained for 10-15 min by keeping a beaker with ether pad close to the animal's nostrils. The rats remained unconscious for 30 - 60 min and even when they recovered consciousness activity was reduced for some hours.

The effect of passing the electrode was then investigated 2 days later. The procedure for this sham

operation is identical to the full operation except that no current is passed. As was shown by Morrison & Mayer (1957b), the sham operation could induce aphagia and adipsia for one day or so before recovery took place.

While the series of experiments continued, some difficulty in producing adipsia and aphagia was encountered. To establish the co-ordinates required and also the current, voltage and time, a series of lesion operations was completed on Rats of series B and D and the effect on water intake was observed. As aphagia from lateral hypothalamic lesions is always accompanied by adipsia (Morrison & Mayer, 1957a), the effect on water intake is a clear criterion for success of the operation. The brains of these animals were studied histologically to relate physiological response to destruction of anatomical regions.

Histological preparation

Where the physiological effect of lesions of the lateral hypothalamus was studied, a check was always made to see that the lesion was situated in the correct anatomical region.

When the animals were no longer of use from the point of view of records, or if they died, or if the adipsia and aphagia were confirmed, the brains were removed. Rats were killed by an overdose of ether and the cranium exposed. The bones of the top, back and sides region were removed with bone cutters and the brain carefully reflected forward after severing the cord. At the same time the cranial nerves on the floor of the skull were divided and eventually the optic nerves cut well forward. The brain was then removed and fixed in 10% neutral formalin for 1 week. The brain was then trimmed down to the region required, embedded in paraffin & serial sections at 5μ were cut. These were then mounted and stained with Haemalum and Eosin.

Calibration of injectors

Before injection of diet directly into the stomach could be undertaken, the volumes to be injected had to be considered. The volumes were not too critical, as long as the rat could control the total volume of a meal. The rate at which the diet was delivered was important to prevent it entering too quickly.

The injector was supplied with a range of gears which controlled the rate of advance of the ram and therefore the rate of delivery from the syringe. The process timer controlling the injector had also to be set to a time which allowed a suitable volume to be delivered at the best rate of delivery in the circumstances.

To calibrate the timer and injector system, a series of volumes delivered at a variety of times and also speeds was required. A syringe of the type being investigated for use was filled with diet (or water) and connected to a measuring cylinder. Volumes from either 20 or 50 presses at each time setting were taken in triplicate. Agreement within 1% was required for the readings to be accepted. The readings were noted from the volumes delivered into the cylinder.

The calibrations for each type of syringe used, and for two injectors are shown in Appendix I. The 100 ml glass barrelled metal piston syringes were normally used

because of the convenient large capacity. From the calibration figures the volumes delivered at a given speed at a given time could be seen at a glance. The importance of these figures in the oral feeding experiments is mainly in giving an idea of the volume delivered for each lever press.

Calibration of cumulative recorder

Because of the difficulty in attaching a convenient writing point there is no record of time on the cumulative recorder trace. The only record is that of the time marked on the trace at change-over and other similar events. The lack of a time trace is not really important because of the large intervals involved in such short lengths of paper.

In analysis of the traces, however, some unit of time must be used in assessing meal size. For this purpose a calibration of 15 min intervals on a trace moving at the standard rate was taken. A 15 min timer was made of a 4-sprocket cam closing electrical contacts, mounted on a synchronous motor rotating at 1 rev/hr. Closing the contacts served to energise a 5 sec process timer connected to a relay. Closing of the relay energised the driving motor of the recording pen movement. The result was a movement of the pen for 5 sec in every 15 min on the trace. By using dividers set to this distance, the 15 min interval could be used as a standard time in analysing the trace.

General plan of the experiments

Partitioning of feeding behaviour into psychical and mechanistic elements has been possible by recording motivated lever-pressing activity and also the ensuing calorie intake. A Skinner box system provided the results over 24 hr periods.

The experiments which were carried out fall into two natural divisions i.e. using rats feeding by mouth, and rats feeding intragastrically. As an incidental accompaniment to these experiments, subsidiary investigations were often conducted along with them.

Rats - numbered Series A in the records

This, the main experiment, was devised to obtain records of rats feeding in a Skinner box normally, and then after lesions of the lateral hypothalamus which produced aphagia. Control periods to partition the effects of anaesthesia and of passing the electrode through the lateral hypothalamic area were also included. Visual observation of the amounts of diet ingested was correlated with the recorded lever-pressing activity associated with appetite.

Rats - fistula series (No prefix letter in the records)

A parallel experiment involving rats which fed through gastric fistulae were also carried out. By comparing the two systems it can be seen that the only theoretical

difference is in the mode of ingestion of the same diet. Any troubles which might appear in an oral ingestion mechanism have been by-passed in the rats with fistulae.

Certain other difficulties have, however, appeared. Successful implantation of the fistulae, followed by a period of completely intragastric feeding was required. A period of recording after the lesions were placed, had then to follow. Rats with implanted fistulae were trained to use the lever mechanism to feed. Subsequently, investigations were made as to the possibility of maintaining such animals for a number of days on intragastric feeding.

When available, rats with fistulae and feeding intragastrically, had lesions placed in the lateral hypothalamus. The effect of aphagia upon their performance was recorded. Tests for the completeness of the aphagia had to be carried out periodically.

The relationship of gastric contractions to the intake of food and also to lever-pressing was investigated in rats with implanted fistulae. A drinkometer provided means of recording oral ingestion while the lever system provided a means of obtaining patterns of appetite.

Rats - Series B and D in the records

To obtain a precise location for the lesions, which were to produce aphagia, experiments were conducted on a large number of animals in contrast to the studies on

individual animals conducted in the preceding experiments. The physiological response of these animals was compared to the location of the site in the brain. Histological examination of the affected region of the hypothalamus provided a check for the stereotaxic co-ordinates used. The co-ordinates found to be most reliable in producing aphagia and adipsia were established in these experiments as a result of studies on about thirty rats in all. These co-ordinates were subsequently adopted in the later experiments.

During the course of these experiments, observations upon behaviour relating to feeding were made when possible.

Details of experimental design

1. Series A (open cage)

Recordings from the open cage Skinner box were made with the cage in the isolated room described on p.(70). Where the rats were taken from a training system or laboratory cage, one day was allowed for equilibration to the new environment. For all experimental runs subsequent on Rat 1A a three-day control period then ensued. Next, the effect of anaesthesia was noted for one day and the day following anaesthesia allowed for recovery.

At this point a sham operation was performed and recording was continued until recovery was noted i.e. after an interval of 4 to 5 days. Sometimes the rats were removed for a time to recover completely. If the feeding patterns returned to normal within a day or two of the sham operation, the animals were often kept on the open cage system until at least 4 days of constant performance resulted. A minimum of 3 days control period was allowed for all animals which had been removed from the Skinner box for a period.

Following the second control period lateral hypothalamic lesions were made and recording continued until the animal was sacrificed for examination of the brain. During the post operative period, a number of days of aphagia and adipsia occurred first. The term "recovery period" refers to the last 3 days prior to the animal being

killed; time of killing was decided on, either because recovery was complete for 3 days, or because the rats were moribund. The sequence of periods may be summarised thus - 3 days control period; 1 day anaesthesia; 1 day recovery; 1 day sham operation; 2 days recovery; 3 days after lesion operation; 1 "recovering" day; 3 days "recovery" period.

This plan has provided daily values for each period. For statistical analysis the three days prior to anaesthesia have been used as the "control" period. Only the 3 post-operative days following infliction of lesions are regarded as demonstrating the effect of the lesion operation. The fourth day before death has also been included and is referred to as the "recovering" day. The last three days are called the recovery period.

The variation in values of lever-pressing and calorie intake between animals and between days has been estimated for the aforementioned periods. The methods of analysis used, Analysis of Variance and t test, are those detailed in standard texts (e.g. Fisher, 1946). The Analysis of Variance is valid over the period of days tested, for the 5 rats. A source of variance in food intake due to change in body weight of the animals may be disregarded. The weights remained constant during the periods that feeding took place. Comparison between treatments and control period

was by comparing the mean values of these periods by a *t* test. The conventional 0.05 level of probability of the significance of differences has been adopted. The interpolation of probabilities was derived from the tables of Fisher & Yates (1948).

Excepting the first run (Rat 1A), all the experimental results were susceptible to this treatment.

2. Fistula series (Training period)

The object of the first investigation using the sleeve cage mechanism was to show that rats could be maintained on an autoinjection system. By decreasing oral food-reward and eventually allowing the rats to feed almost wholly intragastrically, it was hoped to provide evidence of the success of the system. Irrespective of the length of time normally taken to reach this stage, (normally 2 - 3 weeks) recording was continued subsequently until a minimum of 5 days intragastric feeding had occurred.

3. Fistula series (effect of operation)

Because of the time required to obtain results from rats with fistulae, no control sham operations were normally performed. When an animal had been feeding for at least 2 days intragastrically, the lesions were placed and recording continued. Breaks occurred in the ensuing period as the animals were removed to test whether the aphagia and adipsia still occurred.

It was eventually decided to overcome the break in recording by keeping the rats in an open cage during the days when aphagia was being looked for. The tests were carried out at 3-day intervals. By this means, a gradual recovery could be discovered by observing whether the animal fed orally. Only one rat was studied in this way.

4. Fistula series (recording of gastric contraction)

Records of gastric contractions of many rats were taken during a period of feeding intragastrically. Others were recorded from while feeding by drinkometer. In some cases records before and after the lesion operation have been obtained.

Presentation of data

The raw data were obtained mainly from the cumulative record traces. As each day's trace is 15 in. long it is impossible to reproduce copies for each and every day of running. Where reference to any particular pattern is made, a photograph of the original trace is included. All the daily values extracted from the traces are listed *in extenso* in Appendix III.

One meal has been defined as a period of lever-pressing activity to supply not less than 1 ml of diet i.e. 15 lever-presses on the open cage system, separated by at least 15 min from subsequent feeding activity. From the traces, daily values for numbers of meals, calorie intake and lever-pressing totals were obtained. Body weights were also recorded and are included along with the other three variables in each day's values.

During the complete series of experimental runs, the change from British Summer Time to G.M.T. and *vice versa* occurred on more than one occasion while an animal was on record. Unless there appeared to be undue activity in the hours preceeding changeover, the extra or lost hour was disregarded.

RESULTS

Open cage experiments - series A rats

Complete results from experiments on thirteen male rats were obtained from the records. With the exceptions of Rats 2A and 3A from which recordings were not taken, the results of Rats 1A and 4A to 15A are presented in Table (17). For the following reasons, certain results have been omitted from the prime calculation.

i) Rats 6A and 14A diedduring the sham operation and Rat 11A died during the operation of producing the lesions.

ii) Histological examination, figs. (39) & (40), showed the lesions of Rats 7A and 8A to be in areas other than the correct lateral area. Graphs of these experiments are shown in figs. (19) & (20).

iii) Immediately after the operation to Rat 4A, it was found that there was a short-circuit in the electrical supply and therefore it was possible that no current might have passed at the electrode.

iv) Rat 5A was able to drink fluid diet after its first operation. Even though this rat was operated on successfully on a second occasion, it suffered double operative trauma.

The remaining 6 rats therefore provided the results on which conclusions could be based. Unfortunately the

experiment of Rat 1A was not of the same format as subsequent ones and could not provide data for statistical analysis. Graphs of the results of the six experiments have been prepared, figs. (21 - 26), showing calorie intake, lever-pressing response, body weight, and the number of meals for each day of the experiment.

Calorie intake and lever-pressing

Apart from the diagrams prepared for each experiment, two tables (6), (7), contain the values of calorie intake and lever-pressing which were used for statistical analysis. From the tables (6), (7), the results shown in Tables (8 -13) were calculated.

From Table (8) it can be seen that there was no significant difference between animals in either calorie intake or lever-pressing during the first 8 days tested. There is a highly significant difference between days, however, ($P < 0.01$ in both cases), which indicates that significant changes were taking place in that period.

While the graphs demonstrate these variations in both calorie intake and lever-pressing in successive days, it is in fact during the periods of treatment that significant changes are to be looked for. Consequently ad hoc statistical comparisons have been made between means of treatments and recovery periods, and the mean of the control

period. From these t tests, Tables (12), (13), the significance of changes due to treatment were assessed.

The analysis of variance on Table (9) shows that, within the control period, there is no significant variation from day to day in calorie intake or lever-pressing. The variance in lever-pressing between animals is, however, on the borderline of significance.

From Tables (12), (13), it can be seen that values on the day of anaesthesia for both lever-pressing and calorie intake are significantly lower than those of the control period (cal $P < 0.01$; lever $P < 0.02$). On the following day (5 of experiment) there was no detectable difference. The sham operation caused a significant drop for one day in calorie intake ($P < 0.001$) and in lever-pressing ($P < 0.001$). Recovery of these activities on the following two days (7,8), was complete, as indicated by a return to the control levels.

When the values of calorie intake and lever-pressing for the day of anaesthesia (4) are compared with those for the day of the sham operation (6), no significant difference is found ($0.3 < P < 0.4$). When the individual graphs are inspected, however, it can be seen that there is a greater variation of response from animal to animal following the sham operation. In Rat 10A, for instance, both calorie intake and lever-pressing fell almost to zero levels after the sham operation, but not so markedly after anaesthesia.

Following the production of lateral lesions in the hypothalamus, all calorie intakes for three days remained at zero. On the "recovering" day, (day 12 in analyses, day 4 after the lesions), there was no improvement. For these 4 days no statistical analysis is either possible or necessary. Despite the absence of food intake, the animals continued to press the lever and those records have been analysed statistically. Although no food was eaten, lever-pressing was significantly above zero for the first three post-operative days but significantly below control values. When compared with the three post-operative days, the lever-pressing for the subsequent "recovering" day shows a significant increase ($P < 0.01$). There is no difference between the animals, however, ($P > 0.2$), Table (10).

During the recovery period (days 5 - 7 after the operation), lever-pressing was statistically identical to that of the control period although calorie intake was still depressed. Despite the mean lever-pressing value being back to normal levels, a study of individual variation within the groups shows that some animals had recovered less completely than others. Thus the analysis of variance, Table (11), for the combined data of control period and recovery period with respect to lever-pressing, shows that a difference between animals can be detected ($P < 0.05$). As the control period values showed no such difference, it can be assumed

that it is within the recovery period that this variability has arisen. Comparison of the individual recovery days with the control period in Table (11) shows that none of the recovery days differs from the control period significantly Nevertheless, the means of days 14 and 15 are significantly different ($P < 0.02$), which shows that significant changes are occurring from day to day within the recovery period.

From Table (6) it is seen that calorie intakes for two rats during the recovery period remained at zero and these have been excluded from the analysis of variance to maintain a random distribution of samples. As only those values for Rats 10A, 13A, 15A have been analysed statistically the results obtained cannot be regarded as being from a random sample. A comparison of these 3 rats was made between values for the individual recovery days and the control period. Only the values for day 13 differed significantly from the control period ($P < 0.001$), Table (12). The difference between day 15 and the control period was bordering on significance. There was no significant difference between days 14 and 15 for calorie intake. A difference between animals can again be detected ($P < 0.05$).

These statistical findings are summarised in graphic form in Tables (2), (3) which show the mean changes which occurred in calorie intake and lever pressing throughout

Table (2).

LEVER-PRESSING DURING OPERATIVE PROCEDURES

Rats 9A,10A,12A,13A,15A.

	N	$\bar{x}$	S.E.	Difference from Control.
Control	15	1051.9	49.0	
Anaesthetic	5	614.2	118.3	significant
Recovery	5	1230.6	136.8	
Sham operation	5	438.4	144.2	significant
Recovery	10	982.3	94.5	
Lateral lesions	15	19.5	11.5	significant
Recovering day	5	107.0	19.8	significant
Recovery	15	926.2	82.6	

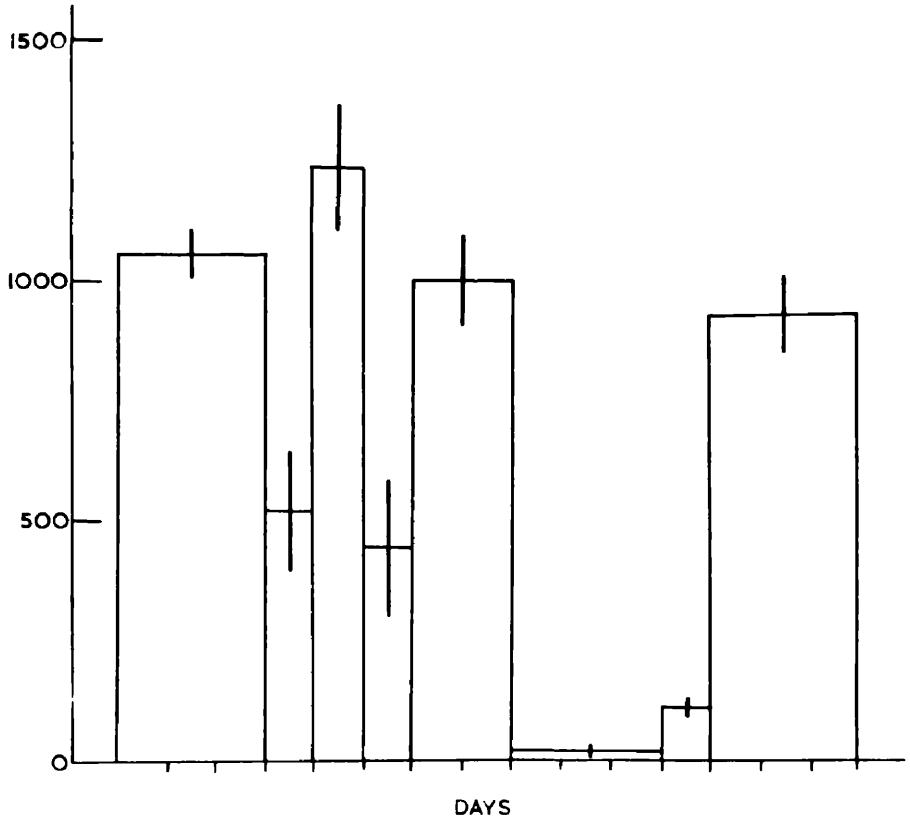
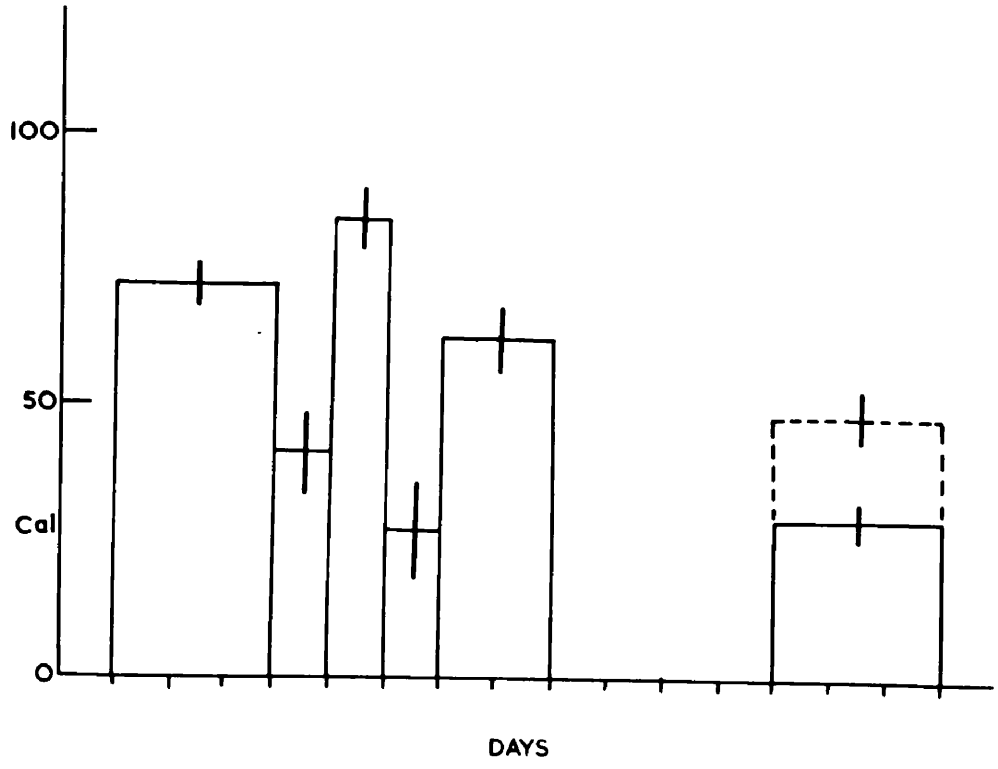


Table (3).

CALORIE INTAKE DURING OPERATIVE PROCEDURES

Rats 9A,10A,12A,13A,15A.

	N	$\bar{x}$	S.E.	Difference from Control.
Control	15	72.3	4.1	
Anaesthetic	5	41.6	6.6	significant
Recovery	5	84.0	6.1	
Sham operation	5	27.2	9.0	significant
Recovery	10	61.8	5.8	
Lateral lesions	15	0		significant
Recovering day	5	0		significant
Recovery	15	28.7	3.5	significant
	(9	48.0	4.5	significant)



the experiments. The significant increase in lever-pressing on the "recovering" day (12) can be seen, and is not accompanied by any increase in calorie intake. During the recovery period (days 13 - 15) mean calorie intake for either the non-random 3 rats, or all 5, does not attain the range of the control level. During this period, the lever-pressing recovery values do not significantly differ from the control mean.

A simple system of scoring based on the crude mean values of treatment totals allows a more direct comparison between calorie intake and lever-pressing changes.

Table (4).

Comparison of treatments

Scoring related to crude means of results from 5 animals.

	Calorie Intake	Lever-pressing
Control	100	100
Anaesthetic	60	60
Sham operation	40	45
Lesion operation	0	0
'Recovering' day	0	10
Recovery Period	40	90

During the control period and pre-lesion treatments a direct relationship between calorie intake and lever-pressing can be seen. During the "recovering" and "recovery" periods the scores for lever-pressing and for calorie intake differ markedly.

The data for Rat 1A, which were excluded because they were not of the same form as those of the other experiments, are shown in fig. (21). There is a marked similarity in post-operative response to the other 5 experiments. The result of Rat 1A may be taken to provide support for those experiments upon which the analysis were made.

Although discounted from the final analyses because histological examination failed to confirm the success of the operation, the results of Rats 7A and 8A are interesting. While both sets of lesions to Rat 8A failed to produce any effect, fig. (8), the sham operation produced an almost total aphagia for one day. While no such effect was observed with a sham operation to Rat 7A, fig. (9), the lesions themselves produced 2 days of aphagia, with a spontaneous recovery of eating and lever-pressing on the third day. In none of the other rats with lesions did the recovery occur so soon after the operation, so that the delayed recovery in those cases may be attributed to the lesion itself.

An attempt to correlate random lever-pressing activity

(providing no reward) with the food-directed lever-pressing was made with Rat 4A. The second lever provided a count of the random presses before and after treatment. Table (5) shows the variation in 4 pre-sham operation days and the 4 following days. The random responses are small compared with food rewarded presses. They do not change markedly after the operation, when intake of food had decreased. It may be inferred that little change in random pressing activity resulted from the sham operation, although a wide scatter of values has occurred within the 8 days.

Table (5)

Rat 4A. Random v food-directed lever-pressing

					Sham operation			
Days	1	2	3	4	5	6	7	8
Random	1	14	2	8	2	4	9	47
Food Directed	1169	1322	1063	831	204	365	994	840

The effects of dehydration and inanition both increased during the post-operative period. During the early post-operative days, however, only the trauma and effects of the lesions could cause the changed response. Recovery took place, and while it was doing so the dehydration and inanition decreased. It would appear unlikely that the recovery values could be a reflection of changes in the animal due to deprivation.

Body weight

The daily body weights of all six rats remained constant throughout the experiments prior to the lesions being made. Rats 10A and 15A both showed complete aphagia and adipsia for 1 day after the sham operations. In both cases a drop in body weight occurred but soon increased to normal. The decreased food, and consequently water, intakes could have been responsible for these reductions of almost 20 g in each case.

In all experiments, however, the success of implanting lesions could be measured to a large extent by the steady decrease in body weight which resulted. The effect was due both to starvation and dehydration although a noticeable antidiuretic effect appeared 2 days after placement of lesions. Dehydration, nevertheless, occurred, as a minimal amount of water was lost in order to excrete waste products. Activity was usually reduced in the first few post-operative days but there was still sufficient energy expenditure to account for a large proportion of the tissues utilised. Even the minimal metabolism compatible with life results in a continual loss of weight by any animal.

Gut fill is the weight of food material, both digestible and undigestible, which is present at any one time in the gut of a fed animal. It is usually quite a constant value. If this food material is removed from the gut, either

by absorption or as faeces, then the gut fill will decrease until the gut is empty if food is not ingested. There was little residue from the synthetic diet to be voided in the faeces. Consequently, the gut fill lost on the first day of fasting can be assumed to be almost totally utilisable as nutrient. When the fluid diet was being used, only a negligible amount of faeces was voided. It was made up mainly of mucus and biliary residues, and there was very little change in the amount excreted post-operatively.

Recovery was also reflected in the change in body weights. In all cases where the ability to consume diet recovered, the body weights either rose or levelled off. The lowest body weights recorded for Rats 9A and 12A showed a decrease of 35% and 33% respectively from pre-operative levels. Both rats were moribund terminally so that a 33% decrease in body weight of a starved rat may indicate the level of the condition which becomes non-reversible.

Meal numbers

By "meal" is meant not less than 15 lever-presses isolated in time by 15 min from other lever-pressing activity. The reason for this choice is given on p. (113). The meals recorded throughout each experiment are an indication as to whether the "static" mechanism controlling food intake remains functional after the lesions produce

aphagia. Although the traces provided by the cumulative recorder were such as to allow both measurement of daily meal numbers and of their sizes, only the numbers have been utilised. The ratio of calorie intake to number of meals would have provided the average meal size. As this would not be an accurate indication of individual feeding pattern during the recovery period when the lever-pressing pattern was changing from day to day, it is of little value in the present investigation. Meal numbers simply signify periods during which the rats were motivated to press the lever by the urge to eat. They do not indicate active ingestion, which might not always accompany motivation to feed. Where continuous lever-pressing occurred it was plotted as one meal (one beginning and end of a meal) and marked with the letter C on the graph.

No very constant pattern is obvious in the control periods. There is little correlation between meal numbers and calorie intake for any control day. This implies that there is no constant size of meal for any given rat. It is noticeable, however, that, in the control period prior to the placement of the lesions, neither the calorie intakes nor the numbers of meals fluctuate over a wide range. Following the operation, as lever-pressing fell to zero so, of course, did the number of meals. Meal frequency returned to the normal range for the control period during the recovery phase.

This definite feeding pattern is some indication of the relationship of lever-pressing to appetite. While not actually eating, the rats still pressed the lever during the recovery period. It was in a pattern closely resembling that which appeared while eating occurred. This provides some support for the hypothesis that the lever-pressing is an indication of an appetitive motivation.

During the recovery period, meal patterns have appeared almost as frequently as in the control period, in 4 of the 6 runs. In one other (Rat 10A) continuous lever-pressing occurred for 3 days before eating took place. In the remaining Rat 13A, the picture is obscured by the fact that both meal patterns and eating recurred within the same 24 hr period. From the trace, however, it was seen that lever-pressing in meal patterns occurred some 12 hours before the rat was seen to lickup diet. Although not clear enough to support the other 4 results numerically, these last two cases do not act as evidence to the contrary.

A diurnal variation in meal patterns was quite noticeable in the control periods, the number of meals increasing during the night, fig. (58). After recovery no clear variation within a 24 hr period was observed.

Sleeve cage experiments - fistula rats

a. Training to feed intragastrically

The records from the first series of rats with a gastric fistula confined in sleeve cages were carried on the kymograph recording apparatus before a cumulative recorder was available. The information obtained is thus limited and the results are considered mainly as records of the development and trials of the system. The later results may be considered as the definitive series of data and consequently the presentation of results below is essentially confined to Rats 18 - 51. Since some of this later series died before useful records could be obtained, only the data from the experiments on Rats 18, 24 and 43 being trained to feed intragastrically, and from Rats 31, 43, 46 and 51 after lateral lesions, are presented in graphic form, figs. (27 - 34).

The final data are presented to provide information about a) the adjustment in feeding during the preliminary period of training to the apparatus, and b) the response in feeding following brain damage. In each period we shall consider total calorie intake, lever-pressing activity, body weight change and the number of meals taken per day.

Complete records of a training period are presented for Rats 18, 24 and 43, in Table (18). Rat 43 was taken through the training procedure twice and so two records

were obtained. As the occurrence of events during the training period are not directly comparable in time in any of the 4 records, they are represented as separate graphs and no attempt has been made to correlate the various events. Reference will be made to the graph of each record and where comparisons are made, they will be between isolated events.

Calorie intakes during the training period were made up of both oral and intragastric totals, oral intake diminishing as the training to feed intragastrically progressed. Although the proportion of the intake made up of oral food varies throughout the training period, only the total intake is recorded.

Reinforcement with oral diet was provided by a fluid diet made by diluting a volume of the standard diet with the same volume of water. Consequently, even when the volume of oral diet was large, the intragastric calorie intake always exceeded the oral intake. Both intakes have been grouped together for purposes of recording calorie intake, but during the purely intragastric feeding period which occurs at the end of the training period, the daily calorie total is a true record of intragastric intake. In three instances the rats were fed at the rate of 0.14 ml per lever-press i.e. speed 16 of the injector. In the second run of Rat 43 the intragastric injection rate was 0.25 ml per lever-press i.e. speed 8 of the injector.

Calorie Intake

Calorie intake did not remain steady at any time during the four training periods, nor during intragastric feeding periods. Rat 18 showed an initial fall and then a gradual increase during the training period, fig. (27). There was an immediate fall while feeding purely intragastrically and then a slow increase in food intake began. On the 17th day of the run, "Complan" diet was substituted for the laboratory preparation. As discussed under Diet (p. 88) the "Complan" proved unsuitable and the animal almost stopped pressing on the 21st day and was taken off.

Rat 24 was removed for 2 days shortly after the training run began. No great fluctuations took place during training although a great deal of variation is obvious during the intragastric period, fig. (28). On the first run of Rat 43, a remarkably steady intake was soon achieved and this state was maintained during intragastric feeding, fig. (29). On the second run of Rat 43, fig. (30), fluctuations in calorie intake appeared, but after intragastric feeding began, intake remained much steadier, although falling off a little towards the end of the run. Only in the case of Rat 24 did the intragastric feeding intake ever fall below the minimum recorded on the mixed oral and intragastric feeding. The last day of feeding on "Complan" diet by Rat 18 is excepted because the animals

health was affected.

The comparison between the means of the periods of training and of intragastric feeding shows a decrease, although not marked in the runs of Rat 43.

Rat	Cals/day means	
	Training	Intragastric feeding
18	54.2	33.9
24	71.2	58.5
43 i)	59.6	56.6
ii)	57.5	48.6

Lever-pressing response

The lever-pressing response of Rat 18 showed great variation during the training period on oral + intragastric food, and fell off markedly at the changeover to purely intragastric feeding. Thereafter it remained low until the last day of "Complan" feeding when it fell almost to zero. A rhythmical effect was shown by Rat 24 when lever-pressing. After intragastric feeding alone this wave form continued but at a lower level. The response of Rat 43 on the first run did not stabilise but increased during the intragastric period. On the second run, the responses remained fairly steady but decreased slightly after intragastric feeding alone began.

From the shapes of the graphs it would appear that lever-pressing varied in all 4 cases to quite a large degree.

It must be remembered, however, that injector speeds were increased at various points in the training routine. As an increase in speed doubled the rate of injection, so was the volume of diet delivered doubled. The changes in response imposed by changing the speeds have not been allowed for in comparing daily values. Calorie intake, however, is a true biological variant and can be regarded as valid irrespective of the method of feeding. Lever-pressing is dependent upon the rate of reinforcement which provides the calorie intake.

Body weight

Body weights did not fluctuate greatly from day to day. It is noticeable that there was a continued decrease throughout the training and intragastric feeding periods. Although gradual, this decrease is quite distinct in all 4 animals.

There is a lack of daily weight records for Rat 18 from 14.5.59 to 20.5.59. This was because of a change in the routine so that the rat was not long enough out of its sleeve cage to be weighed. Where difficulty was experienced in changeover, the animals were sometimes not weighed. The absence of one day's record is overlooked as the difference in weight between consecutive days is normally quite small.

Meal numbers

The meal pattern and number of meals per day varied

a great deal. The number of meals eaten daily is represented graphically for all four records, figs. (27-30). The means of the number of meals per day for the four runs is given below, showing a drop of almost 50% in each case after changing to purely intragastric feeding.

Rat	Mean no. of meals	
	Training	Intragastric
18	10	4
24	10	5
43 i)	10	6
ii)	5	3

A sample of the meal pattern of the early training period of Rat 43 is compared with a meal pattern of the same rat feeding wholly intragastrically, fig. (59). These samples were chosen at random and any variation in the pattern can be seen.

The rats have been able to maintain an intake of food to allow them almost to balance energy expenditure. The gradual decrease in body weight signifies that rats feeding solely by intragastric fistula do not regulate energy balance as effectively as rats feeding normally. The urge to eat can provide almost all the food for the necessary calories, however. The patterns of meals change in the process.

Sleeve cage experiments - fistula rats
b. Effect of lateral lesions on feeding

The animals used to investigate the effect of lateral lesions on intragastric feeding, (Rats 31, 43, 46, 51), are too few in number for any statistical analysis to be made. The results are themselves clear enough cut to allow some conclusions to be drawn from them, if only from their consistency.

It has been discussed before, that at a rate of feeding reinforcement orally once in fifty intragastric rewards, the animal should be for all practical purposes feeding intragastrically. In those experiments the lesions were made when each animal had been feeding for one or two days at this low reinforcement rate. No control periods for anaesthetic effect and sham operation were run as the time available for conducting experiments is short when fistula rats are used.

The effects of the lesions on calorie intake; lever-pressing; body weight; number of meals will again be dealt with separately. Graphs of the four daily values for each animal have been prepared, figs. (30 - 33). Except for one day after the lesions were placed, no records were made from Rat 46 after the operation.

Confirmation of adipsia and aphagia

Simply by recording water intake in an open laboratory cage for one day, adipsia could be detected when present.

Rat 46 was not adipsic when tested before the post-operative recordings were taken. On the 3rd and 6th post-operative days respectively, Rats 31 and 43 took no water. On the first post-operative day, Rat 51 was confirmed to be aphagic by open-cage-Skinner-box performance. Three days later, another day in open cage confirmed that aphagia still persisted. After a further 3 days the animal was now eating. From the second post-operative day onwards, lever-pressing continued in both open and sleeve cages. These findings confirm that although unable to ingest food or water orally if the animals had an urge to eat they could continue to feed intragastrically by lever-pressing activity.

Calorie intake

Quite a large difference in the intake of food is noticeable between animals for the two days before the lesion implanting operation. After the operation no animal failed to take food intragastrically.

Three out of the 4 animals were, however, aphagic for the last part of the recovery period. As has been just described, Rat 46 was not adipsic and aphagic, either after the sham or the full operation. After the full operation,

in fact, the calorie intake was almost equal to that on the day before the sham operation. The appearance of the brain showed that the lesions were not correctly placed and so the animal was not adipsic or aphagic because of that.

In the post-operative days that they were recorded in a sleeve cage, both Rat 31 and Rat 43 continued to press the lever and food intake remained close to control levels. Both were aphagic at that time. Rat 51 also fed quite effectively each day it was in a sleeve cage feeding intragastrically. On the first and fourth post-operative days it was aphagic. By the seventh day ingestion of food by oral route was normal.

Lever-pressing response

There were great fluctuations in lever-pressing after lesions were made in the brain of Rat 43. A value of 2180 was recorded on the fourth post-operative day and then lever-pressing returned to normal before the rat became moribund. An average daily value was 300. After the operation, lever-pressing by Rat 31 remained constant for 2 days that records were taken. The post-operative responses of Rat 51 returned to normal within 3 days in the open cage. On the fifth day, a sudden increase in lever-pressing to 2400 (as against an average of 400) took place but returned to normal the following day. Lever-pressing

responses for the first day in the open cage were zero, but on the fourth and seventh post-operative days they were within normal limits. Thus, in spite of the operative interference causing aphagia, the lever-pressing procedure for obtaining the food survived the operation.

Body weight

Because Rat 46 was hypersensitive post-operatively, it was difficult to weigh and no records are available for its body weight during that period. The one post-operative weight recorded for Rat 31 shows no marked decrease from control levels.

The weight of Rat 43 fell slowly after the lesion operation, and then markedly on the day of increased food intake. As there was an increase in water intake, diuresis may have caused the sudden loss in body weight. There was a recovery of weight (presumably by hydration) on the following day, before a slow decrease occurred.

Number of meals

Both Rats 31 and 46 exhibited "continuous" feeding patterns after lateral lesions were placed in the hypothalamus, figs. (31), (33). Although a "continuous" pattern of food intake during the first post-operative day was shown by Rat 43, a daily meal pattern reappeared subsequently. On the day of increased lever-pressing by Rat 43, the number of

meals rose to 12, which was the highest number recorded for that rat. When Rat 51 showed a marked increase in lever-pressing response on the fifth day after the operation, there was no correspondingly large number of meals.

After the first day of post-operative aphagia, Rat 51 showed little difference in numbers of meals between open cage and sleeve cage performance.

To summarise, it is apparent that those three animals which had lesions causing aphagia and adipsia were able to obtain food after the operation as effectively as before it. By circumventing the necessity for oral ingestion of food, it has been possible to overcome the aphagia caused by lesions in the lateral hypothalamus. The mechanisms which are sensitive to changes in energy balance and serve to maintain equilibrium have not been impaired.

Studies of brain damage

Detailed studies were made of serial sections of the hypothalamus from each animal in which lesions had been placed. To facilitate identification of the parts of the rat hypothalamus, an unoperated control rat was killed. From its brain, sections were prepared and compared with the description of the hypothalamic nuclei and fibre tracts of the rat described by Kreig (1928). When an attempt was made to correlate the structures seen with the descriptions from Kreig and more recent work, it was found that some discrepancies occurred in nomenclature. An atlas of stereotaxic co-ordinates for the rat hypothalamus (de Groot, 1959) makes use of a terminology uniform with recent usage. In the descriptions which follow, names of hypothalamic nuclei will be based on de Groot's terminology.

To produce aphagia and adipsia, lesions have to destroy bilaterally parts of the lateral hypothalamic areas. These areas occur within the coronal section which includes the ventromedial nucleus. The lesions have to be placed between the levels of the supra optic nucleus rostrally, and the dorsomedial nucleus caudally, fig. (36a). Lateral area cells in fig. (37b) lie lateral to and below the fornix. Lesions were thus aimed at the area between the fornix and the pyramid. The areas affected by lesions at previously chosen stereotaxic co-ordinates, Table (19), were checked by

studying the brains of rats of Series B, D.

Series B and D

To find a size of lesion sufficient to cause a persistent aphagia and adipsia, but not large enough to be lethal, lesions were made in the brains of Rats B11 - B16. The lesions resulting from 15 sec of current flow were generally larger than those from 8 sec. However, no difference in physiological response was shown if the animals survived the operation. Rat B16 died and a haemorrhage was found under one lesion. Only a unilateral lesion was present in Rat B13 which showed no aphagia. The other four rats had all exhibited aphagia and adipsia. In each case the lesions were placed in the lateral areas of the hypothalamus, destroying cells of the type described by Kreig as belonging to the lateral nucleus.

Experience in placing the lesions was gained with Rats B18, B20, B22, D01, D02, D03, D05 and D07, with definite success. When the brains were examined the lesions were all found to be located in the lateral areas on both sides. B17 and B21 died shortly after their operations. On removing the brains, large blood clots could be seen under the hypothalamic region of both rats. Sections subsequently showed that great areas of subthalamic and lateral cortical areas were destroyed by the haemorrhage.

A similarity in both anatomical placement of the lesions and the resulting physiological response was found when using the one set of stereotaxic co-ordinates. It appeared feasible to rely on these pre-chosen co-ordinates for subsequent experiments.

Series A

Each brain of the rats of Series A was examined in detail to determine the validity of experimental results. The serial sections were studied to note the changes observed between the levels of the supra-optic nucleus and the dorsomedial nucleus, fig. (36a). A protocol record of such notes as were made for the brain of Rat 9A is given in Appendix IV. The notes below have been made to draw attention to the damage caused by each lesion. Photographs, figs. (38 - 45), at the middle level of the ventromedial nucleus have been included for those animals whose results are shown graphically. Scatter in the size of lesion is shown in fig. (35). The terms medium, large, and small, have been used below to describe the areas of destruction. These terms are intended to refer to sizes relative to those of other lesions.

Rat 1A - Although no large lesion appeared, the tissue of the lateral area was torn on the right side. This torn tissue occurred in the area which was intended for

destruction and extended rostro-caudally for the thickness of the ventromedial nucleus. The left lesion occurred in the correct area but was found almost as far forward as the optic chiasma. Damaged tissue appeared in the left pyramid.

Rat 4A - The right lesion was rather low but in the correct rostro-caudal and lateral planes. The left lesion destroyed the pyramid and appeared to be too lateral to cause much damage in the lateral area.

Rat 5A - A small right lesion and a large left one both occurred at a hypothalamic level caudad to the third ventricle. The right was bounded from below by the optic tract. At the level of the dorsomedial nucleus there was an area of the left pyramid which had degenerated. That area was localised to a small length of the pyramidal fibre tract.

Rat 7A - The right lesion was of medium size but was caudad to the ventromedial nucleus and rather low. The lesion was diffuse and damaged the pyramid but it did not affect sufficient of the lateral area to be called successful. The left lesion was centred at the correct level although it spread far back. The lesion was large and again the tip of the pyramid was damaged.

Rat 8A - On the right side a large lesion was present but only occurred caudad to the ventromedial nucleus. The location was under the pyramid, extending into the lateral

cortex. The lateral area was virtually undamaged. The left lesion, however, was in the lateral area level with the ventromedial nucleus and damaged the optic tract.

Rat 9A - On the right side a very large lesion extended high above the pyramid but affected the lateral cells. Both the pyramid and optic tract were damaged. A large left lesion lay in the correct region.

Rat 10A - A medium sized lesion on the right side was centred too far caudally. The shape was such that it extended forward into the lateral area at the ventromedial level and damaged the pyramid. A very large area of damage at the correct level surrounded the left lesion. The damage covered the lateral area and the optic tract was affected.

Rat 12A - A medium sized lesion on the right stretched back from the ventromedial level to the mid dorsomedial level. It lay close to the optic tract and destroyed lateral area cells. The left pyramid was in the centre of the lateral area and part of the pyramid was damaged.

Rat 13A - A medium sized lesion on the right passed through the pyramid into the centre of the lateral area. The left lesion also was of medium size but stretched forward as far as the supra-optic level. Damage was extensive in the lateral area.

Rat 15A - The right lesion hit both pyramid and optic

tract rather laterally. It was large enough to destroy lateral area cells, however. On the left, a large lesion occurred in the centre of the lateral area.

Fistula series

Rat 31 - Although no discrete lesions were visible, vertical lines of leucocytes showed that a track had been made just caudad to the level of the supra-optic nucleus. On the left side the lateral cortex showed signs of tissue breakdown. Absence of lesions suggests that very little current may have passed through the brain tissue.

Rat 43 - The right side of the hypothalamus was in such a state that the tissue would not section. The rat died many hours before the brain was fixed and only torn shreds of tissue could be seen on the right side. The left lesion extended to the lateral cortex and damaged the optic tract. Both paraventricular nucleus and ventromedial nucleus were present at that level.

Rat 45 - Just above the optic tract at the level of the ventromedial nucleus, a large lesion occurred on the right side. The left lesion was in a similar position on the other side. Both lateral areas were damaged.

Rat 46 - A large right lesion occurred below the fornix at the level of the ventromedial nucleus. It appeared to lie too medially to cause much damage to lateral cells. The left lesion did occur in the lateral area at the correct level.

Rat 51 - The right lesion was fairly large and extended into the lateral cortex. Lateral cells were affected. On the other hand, the left lesion was rather high, level with the pyramid and was mainly lying rostral to the ventromedial nucleus. It was large enough to extend into the lateral area.

General appearance

The appearance of the brains with lesions has been very constant. In all cases, the lesions have been made at the same stereotaxic position relative to the bregmata of the individual animals. In one or two cases one or other of the lesions has been misplaced in a rostro-caudal plane. By using the zero setting in the sagittal horizontal plane of the stereotaxic instrument as central, Table (19), a very high degree of symmetry in arrangement of the lesions has been possible. Some of the electrode tracks show a small degree of deflection into the lateral area at the base of the brain. This appears to be due to the electrode tip being deflected medially by the natural boundary of the optic tract. The pyramid does not seem to have caused any deflection. In most cases the electrode passed completely through part of the pyramid near its tip.

Where, in a rat, the lateral cortex has been damaged, there was no apparent difference in behaviour from any other

rat with lesions. Every rat in which haemorrhage occurred during the passage of the electrode died. Complete disruption of brain tissue took place in these cases.

The lesions themselves were constant in appearance. No cellular elements could be seen in the centre of coagulation apart from some red blood corpuscles. At later stages, leucocytes began to invade the area. The fringes of the lesions were most often separated from the central portion by a space, presumably a contraction artefact. Outwith the lesion fringe, tissues were usually of normal appearance. With the exception of Rat 5A, which has been discussed, no changes in fibre tracts etc. were seen in a hypothalamus in which lesions were made.

The median forebrain bundle was always affected by the lesions which destroyed the lateral area. However, no definite changes, apart from the lesions, rostral to or caudal to the lateral area were noticeable.

The size of the lesions

It was found, while testing electrodes, that even within one type of electrode the size of the lesions varied after a standard flow of current. The standard current and time was 2mA for 15 sec and despite these constant figures the area covered by lesion differed from one brain to the next.

During tests with the thin and thick electrodes,

voltages were noted and found to vary. The variation was kept to a minimum if electrode tips were carefully cleaned immediately before use. From that time on, all currents, voltages and times of electrical flow were recorded, Table (19), when lesions were being made. In Rats B11, 12, 13, 17 and 18 current was passed for 15 sec but only for 10 sec in Rats B14, 15, 16, 20, 21 and 22. Current was maintained as close to 2mA as possible and the voltage noted each time.

A close histological examination of the brains was made to determine the extent of the lesion. The sections judged to have the maximum cross sectional area of lesion were then selected. The maximum cross sectional area was chosen as the most convenient means of estimating lesion size. It is not known just how the maximum area may be related to the actual volume or shape of the lesion.

The areas of each lesion were drawn on thin tracing paper at 70x magnification upon a camera microscope screen. Each area was then carefully cut out leaving a thin lamina of the area of the lesion. By assuming that the tracing paper was of an even thickness, the area of each shape was related to its weight. Three control squares, representing 1 mm square magnified 70 times, were weighed; each shape was then weighed individually. The weights were compared with those of the control areas and so the areas of the shapes were calculated.

The areas of the lesions have been plotted against the

energy dissipated at the electrode tip, (mA x V x sec), in fig. (35). No linear relationship can be shown as a large scatter of plots has occurred. However, it cannot be concluded that the size of lesion is not related to the energy applied to brain tissue. It may simply be that the maximum cross section is not a good criterion of total size of lesion. The rostro-caudal depth varies from one lesion to the next. If there was a convenient method of calculating the volume of the lesion from serial sections then a truer correlation could be made. The scatter of values is certainly too wide to allow predetermination of a lesion size by the energy applied to the electrode tip.

Gastric contractions

Records of normal gastric contractions were obtained from rats with two fistulae inserted. Recordings were taken direct from one fistula without a balloon attached. During a period in which the rat was satiated, changes in pressure occurred in the stomach as shown in fig. (47). The basic tone of the stomach changed and superimposed upon it, larger pressure fluctuations appeared at intervals. These larger excursions were of short duration and could well indicate waves of contraction which passed down the stomach wall.

The records obtained from fasted rats in figs. (48), (50), & (54), showed that 'hunger' contractions were occurring. Those contractions were greater in amplitude than normal contractions, and of increased frequency during a burst of activity. The periods of increased activity occurred in a rhythmic pattern at approximately 20 min intervals. While the rats were being allowed to feed, no pattern similar to the 'hunger' contractions ever appeared.

When the rats were allowed to feed intragastrically they did not lever-press at times which corresponded to any regular gastric activity. Where intragastric feeding took place as definite meals i.e. responses were not scattered at random, there was no correlation with gastric contractions, figs. (47) & (49). The study was carried out to find any association between gastric contractions and food intake in

a meal pattern. Thus, in the first rats with implanted fistulae, the rather random lever-pressing did not provide any valid results.

During intragastric feeding, lever-pressing and an increase in contraction size and frequency appeared to coincide. After careful measurement from corresponding points, it was found that the gastric activity seemed to result from the food being injected into the stomach, as shown in fig. (52). The feeding was not consequent to any changes in contraction pattern.

When the rats were being fasted, large 'hunger' contractions appeared and occurred rhythmically. When ingestion of food took place either orally or intragastrically, those 'hunger' contractions disappeared as shown in figs. (48a) & (50). Stomach tone increased, presumably due to distension by the food. The distension was gradually reduced over a 15 to 20 min period. The rats were allowed to feed orally by drinkometer on the same diet as was injected intragastrically. By this means it was hoped to show whether there was any connection between gastric contractions and feeding. A difference between oral and intragastric feeding might then have been noticed.

When diet was drunk at random, feeding and gastric contractions in fig. (53) were not related. Following 'hunger' contractions, of course, ingestion of the diet caused

the contractions to disappear in figs. (48a), (50b) & (54). Ingestion of food orally was not effective in reducing the intermittent contractions of the non-fasted rat's stomach. That finding, supported the possibility that the large excursions represented waves of gastric contraction.

Figs. (51) & (53) show that the drinkometer was operated by the rats in a very definite meal pattern. The frequency of meals was slightly different from that during intragastric injection. From the gastric contraction patterns where feeding is taking place, no association with feeding can be deduced for either intragastric or oral feeding.

Behaviour of the rats

a) In Sleeve Cages

Confinement in a sleeve cage did not produce any long term effects upon the ability of a rat to feed in a meal pattern. The initial restrictions imposed upon the rats appeared to annoy them for a few hours, but without exception they quickly became accustomed to their new environment.

Apart from cleaning themselves while free for a period each day, the rats had to restrict their grooming to the head and forelimbs. A plug of yellow material accumulated under the prepuce of some of the rats kept in sleeve cages. As the rats often failed to do so themselves, the plug had to be removed daily. The secretion appeared to come from the preputial glands and was only found in those rats restricted in sleeve cages.

A definite pattern of grooming, best described as 'face-washing' occurred regularly after meals. It may be that this behaviour is analagous to that described by Monnier & Tissot (1958) in the rabbit, and elicited by stimulation of the hippocampus. As 'face-washing' was easily observed in rats with fistulae a correlation between this behaviour, injection of food and fasting gastric contractions was noted in fig. (46,a). A definite connection was found between injection of diet and 'face-washing' and is described in full

in Appendix V.

While kept in sleeve cages rats moved back to the rear of the cage to defaecate and urinate. The animals also tended to sleep in the rear part of the cage and moved forward to feed by pressing the lever. After a meal had been injected intragastrically the rats usually slept. The active periods were not always spent feeding, although random lever-pressing followed what was obviously 'exploratory' behaviour. The animals explored the forward part of the cage, investigating the retaining rods, feeding tube, lever etc.

Following injection of diet in known volumes into the stomach, Rat 45 showed a definite response and chewed at the glass retaining rod across the sleeve cage, as recorded in Appendix V. Many other rats chewed at the wire mesh of their cages immediately after meals. That behaviour appears to be a displacement activity evoked by satiety when no mastication or licking has taken place.

b) <u>After lateral hypothalamic lesions</u>

Although previous reports have described hyper-sensitivity of rats following lateral hypothalamic lesions it was not very obvious in the present experimental series. After the operation, some care had to be taken in handling the animals but they struggled no more than normal rats when

firmly gripped. Prodding, however, could usually produce an exaggerated response.

For the first few days of adipsia and aphagia, the rats tended to be less active. Activity gradually increased, especially if increased interest was shown in the food which resulted from lever-pressing. In rats of series A (open cage) failure to recover the ability to eat sometimes occurred. In such cases, activity decreased after 10 days and the rats became moribund. If and when that occurred the rats were killed. The moribund condition of Rat 9A is reflected upon its last day's activity in fig. (22) and it was subsequently killed and the brain removed.

A peculiar posture was seen to occur only in rats which had had hypothalamic lesions made. While quiescent, these rats adopted the crouch position seen in fig. (55). The hunched back remained obvious when the animals moved. Even after functional recovery from the lesions these rats often retained the crouch posture.

Recordings of gastric contractions, fig. (46,b), were taken from Rat 45 after lateral hypothalamic lesions were made. The lesions appear to have no effect on fasting gastric contractions, which were inhibited by injection of 5 ml of diet. No 'face-washing' behaviour resulted, however. It is in direct contrast to the readily repeatable response

shown in fig. (46,a) by the same animal before the operation.

When rats were kept in laboratory cages after successful placement of lateral hypothalamic lesions, a type of food directed activity was seen after 5 - 6 days. After a similar time post-operatively, rats of series A began to respond by lever-pressing and they showed interest in the food delivered. Aphagia continued, but was not due to complete disinterest in the food.

At the stage of recovery just described, rats given cube diet, often appeared to eat it. When close observation was made, however, it was found that rats chewed the cubes. The crumbled cube was not swallowed, but simply fell to the floor. When fluid diet was available to aphagic rats, they seemed aware of it, but no food was ingested, fig. (56).

c) Rat 5A post-operatively

Lateral hypothalamic lesions were not successful in producing adipsia and aphagia in Rat 5A. Ten days after the operation the rat was feeding and lever-pressing normally. Quite suddenly feeding ceased and lever-pressing became a random activity. The rat moved very unsteadily in tight circles round the cage. Some ten days later the rat was lever-pressing and eating normally but ran round the cage continually, and in a clockwise direction only.

Another ten days later, lateral lesions were successfully placed in the hypothalamus and the animal became

aphagic and adipsic, but resumed lever-pressing before it was killed. Brain sections show that on the left side, the pyramidal tract had a large area in which fibres had degenerated, fig. (57). The region was at the level of the ventromedial nucleus and was well defined rostrally and caudally. Only one lesion was correctly placed, and apart from the pyramidal fibre degeneration, the whole brain appeared normal at that level.

It is possible that the delayed effect was due to the fibre tracts at the region of the first lesions being damaged. Subsequent degeneration may have caused the 'space' to appear in the pyramidal tract, causing interruption in transmission along a whole fibre system. No obvious explanation for the change in behaviour of the rat can otherwise be made. The latency of the adipsia and aphagia which appeared 10 days after the first operation is difficult to explain.

DISCUSSION

The conclusions which can be drawn from data depend to some extent on the reliance which can be placed on the techniques involved. The success of the present study depends on the accuracy with which lesions were placed in the lateral hypothalamus of specially trained rats. The lesions not only produced adipsia and aphagia, but a quite localised region of the lateral hypothalamus was destroyed on each occasion when aphagia was produced. As the present investigation was concerned with the action of these lesions upon brain function it was very important to have them accurately placed. Only 23% of the operations were unsuccessful, although some rats died from respiratory failure or haemorrhage. No previous series of experiments involving the same operation seems to have had a similar rate of success.

Patterns of feeding behaviour

Reproducible results were obtained from rats operating the Skinner box system. It must be appreciated that general behaviour does not directly affect the operant conditioning of rats to lever-press when there is an urge to eat. Even when behavioural changes occur after a brain operation, the strength of lever-pressing response will still depend upon the motivation to eat. That is assuming, of course, that skeletal motor activity is not impaired. Direct

observation of behaviour of the rats has been an invaluable check upon that.

Unoperated rats did not have any difficulty in adapting to the method of obtaining food by pressing the lever. In general, the calorie intake was so constant as to allow them to maintain fairly constant body weights. It is not difficult to envisage that the same mechanism operates to maintain an energy balance.

It cannot be claimed that the patterns shown by rats feeding on fluid diet were typical of feeding on laboratory cube diet. Teitelbaum & Campbell (1958) found that the daily number of meals on a fluid diet differed from that on a solid diet. The present results, however, are closer to their values for a solid, rather than a liquid diet. Valves fluctuate from day to day and represent a true biological variation. Nevertheless, meals occur rhythmically and represent a motivation to feed. Although its nature is still unknown, the signal indicating a "hunger" state within the body arises very regularly. The concept of a "static" mechanism (Kennedy, 1950; Mayer, 1955; Brobeck, 1955; Morrison, 1959), controlling feeding as an on-off switch is well supported in this work.

Gastric contractions related to feeding

Although small intragastric pressure changes occur continuously, only larger pressure changes (5-15cm H_2O) are

due to true waves of contraction. It is noteworthy that rats in the present experiments did not begin meals precisely after an increase in gastric contractions. Appetite does not appear to result from localised sensations in the stomach. Indeed, the amplitude of contraction often increased after a meal; the rats were then presumably satiated.

On the other hand, after a fast, gastric "hunger" contractions appear. This has long been known and is associated with a sensation of increased appetite. These large, rhythmic waves of contraction disappear after a meal. It is then assumed that the animal is satiated. During this period of "satiety" in the rats studied in this thesis, contractions are similar to those which occur when the animals feed normally. Thus, present findings contradict the idea of using the picture of gastric activity to indicate an internal state of either "hunger" or "satiety". Morrison, Lin, Eckel, Van Itallie & Mayer (1958), and Mayer & Sudsaneh (1959), showed that increased carbohydrates, adrenalin, glucagon, and insulin, in the bloodstream inhibited "hunger" contractions in normal, aphagic and hyperphagic rats. Whatever the inhibitory action, ingestion of food can cause internal changes sufficient to inhibit large gastric contractions.

The injection of food by intragastric fistula is as

effective as oral ingestion in inhibiting "hunger" contractions. Meals are completed and the rats appear satiated. It therefore appears unlikely that a small oral component is necessary to cause satiation. Feeding to the level of satiation may not be sufficient to maintain energy balance. The studies on rats feeding intragastrically show that there is a loss of body weight by that method of feeding.

Present findings are in agreement with the concept that the lateral hypothalamus does not directly influence gastric contractions (Mayer & Sudsaneh, 1959). Results show that even a rat known to have damage in the lateral hypothalamus still showed normal gastric contractions although the associated behaviour was absent. Despite the state of deprivation which occurs when "hunger" contractions appear, aphagia persists after lateral hypothalamic lesions in the rat. It is important to discover whether an appetite drive still exists under those circumstances.

Aphagia after lateral lesions

The general picture of oral feeding after operation shown in this thesis is in complete agreement with previously published work. After an operation to place lesions in the lateral hypothalamus, rats became aphagic. Some recovered after a minimum of 4 days while others would have starved to death were they not killed. There can be no doubt that the

aphagia results from destruction of a precise area of hypothalamus. It is to be presumed that the sham operation will result in complete aphagia for one day only in those cases in which the electrode caused trauma of an area of the lateral hypothalamus. Although anaesthesia and the operative procedures for making a brain lesion were both followed by a decreased intake of food, they may not act in the same way as the brain lesions. Anaesthesia caused the rats to be inactive for 15 - 25% of the 24 hrs available for consumption of food. However, there is no indication of how much longer activity may have been reduced. Decreased intake may simply reflect the reduced activity during part of the day on which treatment occurred. Apart from the cases of aphagia previously discussed, the sham operation had a similar effect to that of anaesthesia. The amounts of anaesthetic administered alone, and during the operations were identical. Where the lateral area of the hypothalamus was not damaged bilaterally, the sham operation procedure alone appeared to have only a negligible effect on food intake.

As reported by previous workers, (Anand & Brobeck, 1951; Montemurro & Stevenson, 1955-56; Morrison & Mayer, 1957a), lesions in the lateral area of the hypothalamus of the rat produce aphagia and adipsia. In some rats the aphagia was complete and they never ate after the operation. Some rats

in the present work did recover after at least 4 days of aphagia. Recovery from aphagic to normal levels of feeding did not take place overnight, but rose slowly to within the control range after 3 days of partial feeding. Other rats were still gradually increasing their intakes at that time. Even in these instances of temporary aphagia the lesions caused a much greater change in feeding than the sham operations which caused only a transient hypophagia.

Morrison & Mayer (1957a) found that, where "escape" from adipsia took place, water intake increased over 1 - 4 days. Where water was withheld from sham-operated animals, complete aphagia continued until the water was restored, even though the rats would only have been aphagic for 1 day with water available. Recovery was complete (85 - 130% of control levels) within the first day, which indicates that as soon as water was replaced the intake of food took place at a normal rate. The present findings indicate that a functional recovery was taking place gradually, and that it was not due to deprivation of food or water. The reason for recovery is still unknown. It may, as Mayer & Morrison (1958) have proposed, be due to mildly damaged peripheral parts of the lateral area of the hypothalamus taking over control.

The results of the open cage experiments make one point very clear. Every rat eventually began to lever-press

after lesions were made in the lateral hypothalamus. Lever-pressing occurred before ingestion of food was resumed by those rats which "escaped" from the aphagia. Even more important is the fact that even those rats which remained aphagic until death, began lever-pressing. The resumption of lever-pressing at control frequency did not take place in one day, but was gradual, like the recovery from aphagia.

But what of the lever-pressing in the immediate post-operative period? It was reduced almost to zero and was probably a random, non-food-directed response. Only the lesions could have been responsible for the decreased response rate, either directly or indirectly. General activity was not affected to such an extent that it would cause lever-pressing to stop. Only a reduced motivation to eat could have been the cause. The aphagia did not result from the lack of lever-pressing. Indeed, all previous work has shown that hypothalamic aphagic animals will not eat any food which is offered to them.

The production of lesions by electrical co-agulation is bound to cause a great deal of trauma to otherwise undamaged regions of the brain. The 4-day period of aphagia and reduced lever-pressing may indicate the minimum time for the brain mechanisms to recover from the operation. Lever-pressing, although resumed prior to the recovery from

aphagia, increased at a rate similar to the increase in rate of ingestion.

Survival of the urge to eat

The previous work which has been done using a Skinner box system demonstrates that lever-pressing activity and eating occur in sequence. The experimental results of this thesis confirm all that has gone before, but one point which might well be overlooked has been brought to light. Before discussing that point it is important to clarify the steps in which feeding occurs. The rats may be said to press the lever to obtain food. It is more accurate to say that they press the lever when there is an urge to eat. Experience has shown them that having done so, they can fulfil the consummatory act with the food which appears. Two steps in the behaviour of feeding immediately become obvious. First is the response to a motivation of appetite i.e. the lever is pressed. Food appears as reinforcement for the initial response and acts as the second stimulus. Consumption of the food is the response to that stimulus. Each stage is required in operant conditioning. As has been mentioned before (p.45) a reflex behavioural response occurs in a predictable fashion. A reflex arc can be fatigued to reduce its predictability. The reflex arc, of course, can be damaged as well as fatigued.

Looking at the two stages of feeding behaviour just mentioned, we can see that the second i.e. ingestion reflex, is subject to damage or fatigue. We can also see that failure of either of the stages of behaviour causes a break in the whole chain of events and the consummation will not take place. Bearing this in mind, let us look at the results obtained from rats recovering from lateral lesions. The mean values from open cage experiments show that on the "recovering" day, after the operation, all rats remained aphagic. An increase in frequency of lever-pressing, significantly different from the post-operative period, occurred on the "recovering" day. Obviously, the first step in the behaviour of feeding was taking place. The second step had not occurred i.e. the rats did not ingest any of the food which resulted from lever-pressing. In the subsequent three "recovery" days, lever-pressing approached the frequency of the control period. Two rats remained aphagic but three were able to eat the food, although in amounts which still differed significantly from control values.

During the "recovering" and "recovery" periods, the second, consummatory, stage of feeding was either absent or impaired. The motivated act of lever-pressing occurred throughout those periods at a reduced frequency on the "recovering" day. Aphagia was caused only by the failure of the rats to ingest food. The rats were thus

motivated to eat, but could not complete the consummatory act. After the minimum 4 days of aphagia, the hunger state persisted in all rats. It is not illogical to propose that the sensation of appetite gives the rats an urge to eat, and so result in lever-pressing. Whatever is the blood-borne signal of hunger, heat or metabolite, the hypothalamic centres appear to be receptive to it.

Intragastric feeding after lateral lesions

After lateral lesions were made in the hypothalamus, rats in sleeve cages continued to press their levers. Aphagia and adipsia existed for a few of the days while the animals in fact fed through the gastric fistulae. The amounts of food which were ingested by this method prevented the loss in body weight which appeared during aphagia in open cage rats. If the intake of food is regarded as part of the mechanism for maintaining an input of energy to balance output then it appears to function well in aphagic fistula rats. The difference between those rats and the open cage rats was that ingestion, the second stage of feeding, was completed by a mechanical process. The failure which causes aphagia then appears to be in the skeletal mechanisms of food ingestion of rats feeding orally. Studies of behaviour of the rats may disclose a clue to the activity which is lacking.

Nature of aphagia

All the rats which were lever-pressing, but aphagic,

were active and so could reach the food which was available. Some even appeared interested in the food but none could lick it. It might be proposed that when supplied only cube diet, aphagic rats could not masticate it, but chewing and biting were often seen in aphagic rats during the present studies. Adipsia could be due to the inability of the animals to lick fluid. In the experiments in this thesis only fluid diet was delivered and the aphagic rats were unable to ingest it. When the fluid diet was placed in their mouths the rats did not swallow it. Some could, however, chew cube diet quite efficiently. They did not swallow it either.

The fact that aphagic rats could chew solid cube diet, but not swallow the pieces supports the previous finding. Some rats may have been unable to masticate or lick food. It appears that all were unable to transfer food from the oral cavity to the oesophagus.

A criticism may be pointed at the method of recording from rats during a period of severe fasting and water deprivation. There is no doubt that the effects of dehydration and inanition both increased during the post-operative period. In the first days, however, only the operative trauma and the lesions could result in any behavioural changes. While still in a deprived state, the rats began to lever-press. Although the activity may have been due to deprivation and fluctuations in ionic balance,

the aphagic fistula rats continued to lever-press while suffering no lack of fluid or nutrient. The results presented in this thesis show that as the rats recovered from aphagia, lever-pressing became more frequent. Recovery of feeding meant that dehydration and inanition were decreasing. Thus, it appears likely that non-reversal of activity signifies that it was not originally caused by internal inbalances in the rats. Aphagic rats which were fed water by stomach tube did not all "escape" although the rate of "escape" was greater than that for rats not given water (Morrison & Mayer, 1957a).

The rats in a moribund condition might be taken as examples of animals suffering from nutritional deficiency and electrolyte inbalance. Those which had to be killed had lost 35% of their post-operative body weight. Rixon & Stevenson (1957) found that chronically starved rats survived until they had lost 42% of their initial body weights. It may be assumed that, when moribund, the rats were still capable of living for a few days i.e. until they lost another 7% of their initial weights. The depressed activity appears to result from their chronically deprived states.

On the few days immediately after the operation aphagia could have been caused by either a lack of motivation or a failure to eat, as neither were present. On the days that rats were motivated to lever-press they were often

aphagic. The neural mechanisms sensitive to the "hunger" signal appear to have been functioning efficiently then. If, as has been proposed, the satiety centres inhibit the hunger centres (Morrison, 1959), then failure of the feeding centre to detect hunger does not necessarily imply that the satiety centre is active. The lack of behavioural response i.e. grooming, in the aphagic fistula rat when fed appears to show an absence of satiety. Satiation and grooming behaviour may have been correlated with one another before the lesion operation. Thus, satiation may not occur after the aphagic rat is fed, since no grooming is seen. The actual inhibition of the lateral hunger centre may give rise to the conscious sensation of satiety.

Integration

Andersson, Jewell & Larsson (1958) reported that stimulation of the "drinking" area of the satiated conscious goat produced an increased urge to drink. So strong was this urge that the goats would even lick distasteful solutions. As that was no "normal" urge to drink, either a complete lack of satiety sensations, or a direct motor action, appear to be the alternative causes. Andersson et al., however, concluded that stimulation gave rise to a sensation of thirst. Brobeck (1955) suggested that damage to the feeding centre allowed the satiety centre to act unopposed in aphagic rats. The present results tend to

oppose Brobeck's views. Although they remain aphagic, the rats do not appear to be satiated, when judged on "face-washing" and motivation to eat. Anand & Brobeck (1951) stated quite definitely that the animals with lateral lesions seemed to have lost the urge to eat. Their view implies that the sensory function of the feeding centre was destroyed. Bruce & Kennedy (1951) were of the opinion that lateral lesions caused anorexia, again the result of a defective sensory inflow to the lateral area of aphagic rats.

When satiated rats were stimulated in the lateral area of the hypothalamus, they began to eat (Miller, 1960). A definite food-seeking response was seen only during the period of stimulation. Miller found that satiated rats in a Skinner box system began to lever-press when stimulated. Those results indicate that it is the urge to eat which is brought about by stimulation, rather than reflex activity involving ingestion. Stimulation of the lateral area superior and lateral to the fornix elicited eating in satiated rats (Miller, 1960). That area was not extensively damaged by lesions in the present experiments. It may be that the more ventral aspects of the lateral area are more concerned with motor effects. There is no information to suggest that while one part of the lateral area has a sensory function, another region may not have a motor function.

Stimulation of these "sensory feeding areas" in

satiated hypothalamic aphagic rats might produce very interesting results. Would it be found that sensory and motor mechanisms are integrated in the lateral hypothalamic area? The variation in effects resulting from placement of lesions could be accounted for by damage which affects different parts of the lateral area to varying degrees.

Morrison & Mayer (1957a) and Mayer & Sudsaneh (1959) suggest that lesions in the lateral hypothalamus produce aphagia by interfering with the motor outflow rather than with the sensory inflow. The higher rate of "escape" of aphagic rats fed water by stomach tube as compared with that of rats without water, led Morrison & Mayer (1957a) to their conclusions. They propose that the enforced ingestion was sufficient to re-establish the motor pathways which had been damaged by the lesions. Mayer & Sudsaneh (1959) formed their opinion simply from the fact that gastric contractions still respond to metabolic changes after the lateral hypothalamus is damaged.

To date, the results presented in this thesis are the strongest support for the concept that lesions damage the motor route. Although on the days immediately after the operations the urge to eat is absent, it would appear that inability to eat would in any case have caused aphagia. It is on the "recovering" day and also during the "recovery"

period that damage to the motor pathways can be envisaged. Since the animals showed the desire to eat but could not, there is not much doubt that the neural mechanism controlling ingestive activity was damaged.

It would be virtually impossible to detect the actual nervous pathways and centres involved. The results from the fistula rats indicate that a mechanism of ingestion, such as mastication or deglutition, is involved. Feeding intragastrically, the fistula rats can by-pass the neural block. The observations on aphagic rats which could bite and chew cube diet appears to dispose of mastication as the mechanism damaged. All aphagic rats, however, had difficulty in swallowing. That reflex may well be the one which was blocked.

The reflex nature of oesophageal peristalsis was observed by Meltzer (1899). The complete reflex activity of swallowing was studied by Miller & Sherrington (1918). They found that fluids placed in the mouths of decerebrate cats could elicit swallowing much more readily than could solid food. Although oils produced little effect, alcohol and other fluids were very easily swallowed. Oil and alcohol together formed a strong stimulus. These results of Miller & Sherrington might explain intake of water being recovered before intake of food by rats with lateral lesions. Indeed, Teitelbaum & Stellar (1954) found that there was a

definite order in which certain foodstuffs were accepted during recovery, e.g. sweet fluid chocolate, milk, water, and eventually cube diet. It is not inconceivable that since the foodstuffs caused different degrees of stimulation they could elicit the reflex at different thresholds of recovery.

As Brobeck (1960b) has said, it is possible that the lateral area facilitates feeding reflexes. Damage to either hypothalamus or reticular formation can result in loss of motor ability in many systems (Magoun, 1950). Stimulation of various areas has produced mastication and licking. Stimulation of the vagus (Forssberg & Larsson, 1954), amygdala (Wood, 1958; Gastaud, 1952), olfactory tubercle (Kaada, 1951), have all caused a swallowing reflex. Bazett & Penfield (1922) made cats decerebrate at such a level as to destroy temperature control although they were still able to swallow. Cortical activity is not essential in the complex activity of feeding (Penfield, 1958). The parts of the brain involved in eliciting feeding behaviour are obviously very diffuse. No speculation as to the route taken by the reflex arc, or the level at which hypothalamic influence is exerted can therefore validly be made. The rats have been judged to be unable to ingest food. The use of the term aphagia in describing the condition studied would appear to be more precise than anorexia, which implies a loss of the urge to eat.

SUMMARY

1. The literature relevant to neural mechanisms which control feeding is reviewed and discussed. Related aspects of alimentation are also considered. A description is given of behavioural terms and methods used in the present work.

2. A detailed description is given of the design and construction of an apparatus to permit the recording of patterns of feeding in the rat over 24 hr periods.

3. An operative procedure for permanent implantation of gastric fistulae in the rat has been developed and is described. The training of rats to feed by auto-injection intragastrically through this fistula was undertaken and is described.

4. The use of a stereotaxic instrument in producing hypothalamic lesions with great precision is fully described.

These techniques have been used to study the effects of lesions in the lateral hypothalamus on feeding patterns of two groups of rats - a) feeding orally, b) feeding intragastrically.

5. Results are presented of daily calorie intake, lever-pressing, body weight, and meal numbers from 13 rats feeding orally, before and after an operation for the placing of hypothalamic lesions. Results, illustrated by diagrams, for 5 rats in which lateral hypothalamic lesions were successfully made, are analysed statistically.

A mean decrease of 40% in calorie intake and lever-pressing activity occurs after anaesthesia and a 50% decrease after a sham operation. However, bilateral lesions in the lateral hypothalamus result in complete aphagia and adipsia for 3 days. Lever-pressing activity is minimal over the same period. Thus the effects of the brain lesion differ in severity from those of the sham operation markedly. Later on, during recovery from the operation, lever-pressing activity increased significantly above the immediate post-operative level. Nevertheless, the rats remained aphagic, two until death. The remaining three rats began to eat during the last three days of the experiment. Lever-pressing in all 5 animals was not significantly different from the control period during the final 3 days. Histological examination showed that damage occurred bilaterally in the lateral areas of the hypothalamus of all 5 rats. In all animals who did not develop aphagia, a failure to place the lesion in the lateral area of the hypothalamus was demonstrated by histological examination.

6. Rats were successfully trained to feed for a few days by auto-injection through a gastric fistula. Training records from 5 such rats are presented and the course of feeding on 4 of these subjected to lateral lesions of the hypothalamus did not alter the intragastric feeding from

the actual period of operation onwards. Three of those rats remained unable to ingest food orally for a few days post-operatively. In one of these rats the pattern of post-operative feeding differed from that before the operation. In that case, however, there was little damage to the lateral area of one side of the hypothalamus.

Intragastric ingestion of food was as effective as orally ingested food in causing gastric "hunger" contractions to disappear. Sequences of grooming behaviour occurred regularly after manual injection of diet into the stomach. This behaviour did not occur in a rat which had a lateral lesion in each side of the hypothalamus.

7. The implications of these results are discussed. It is suggested that the aphagia produced by lateral lesions of the hypothalamus (so-called "feeding centre") represents damage to the neural pathways controlling swallowing. This is consonant with the observation made in this thesis that whereas appropriate lesions of the hypothalamus lead to complete aphagia, the animal's 'urge to eat' continues to be manifested. This is evidenced by the return of lever-pressing activity even while oral ingestion of food remains completely absent.

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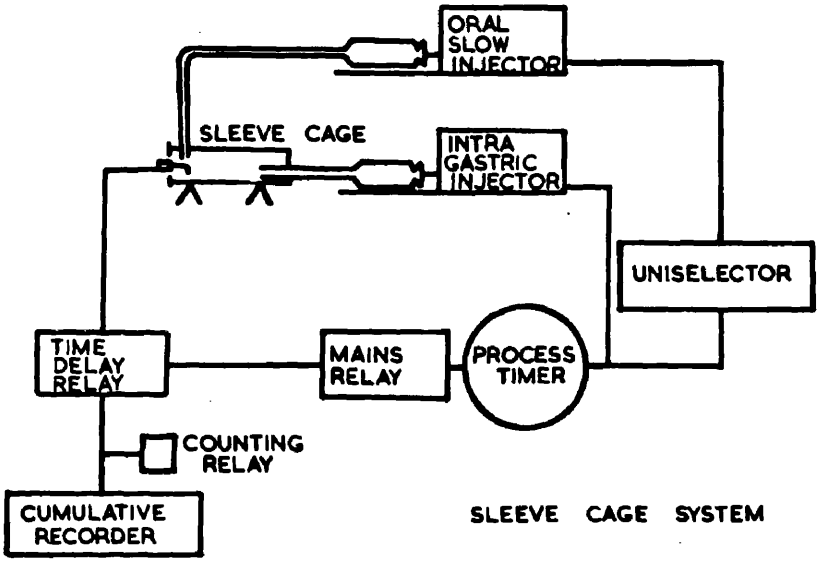
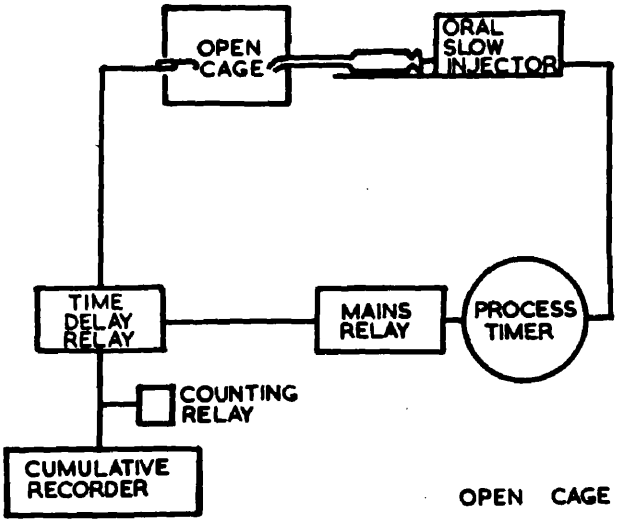


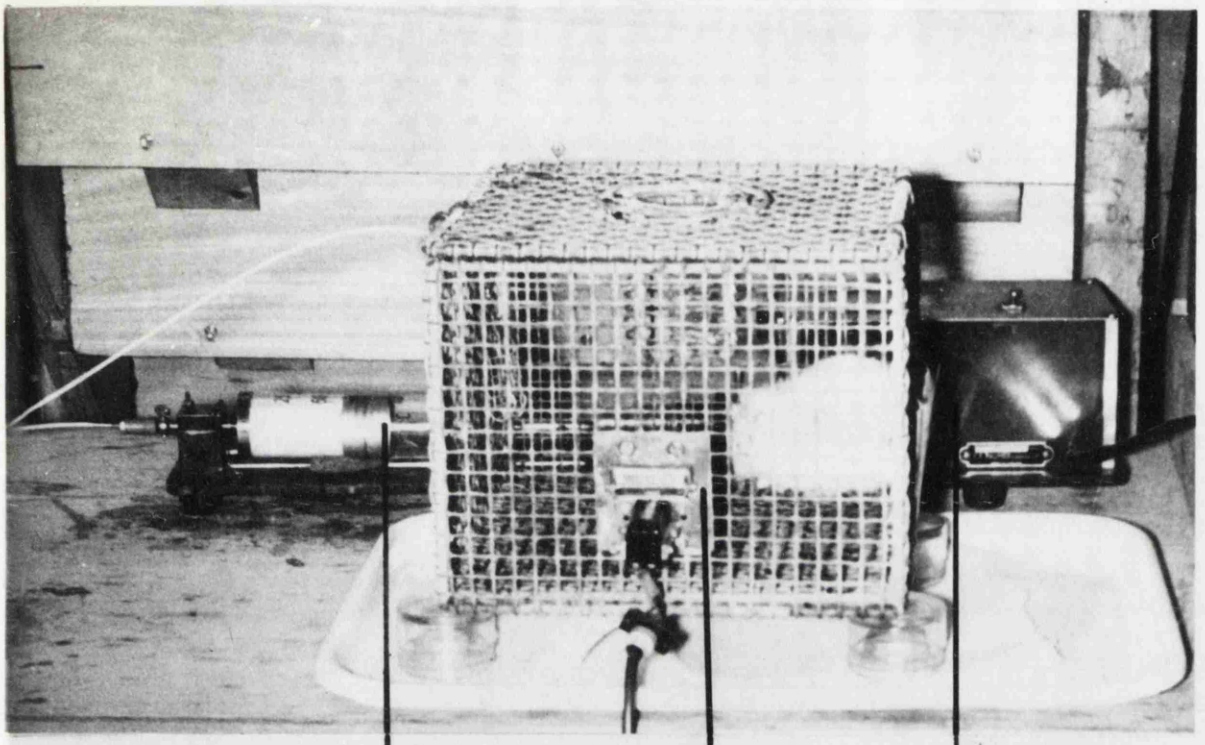
SLEEVE CAGE SYSTEM

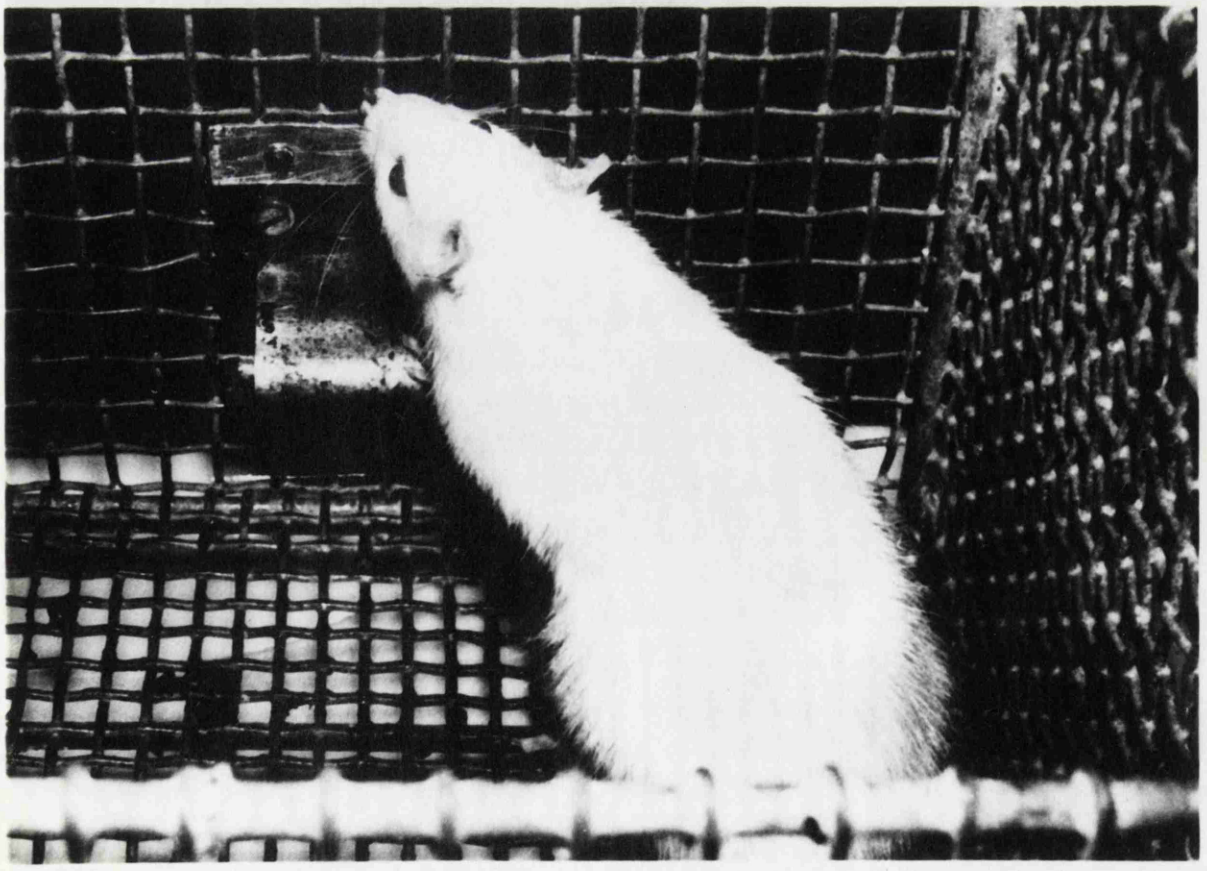


OPEN CAGE SYSTEM

DIAGRAMMATIC LAYOUT OF APPARATUS

Fig. (1.)



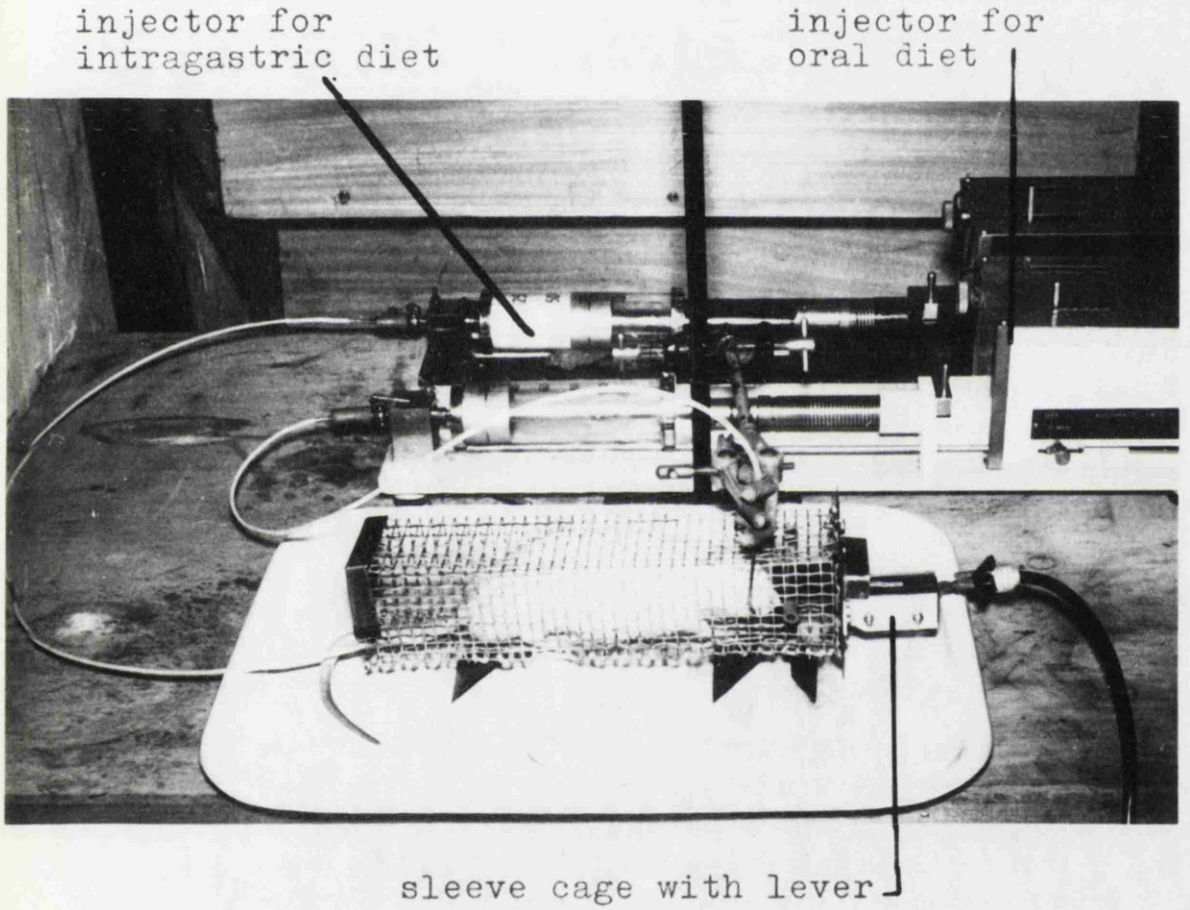


Rat in Open Cage Feeding by Lever-pressing,

a) method of providing fluid diet, and

b) rat pressing cage lever to obtain food.

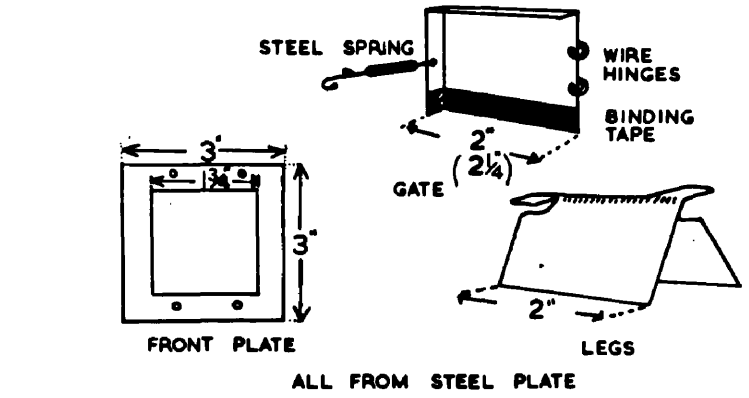
Fig. (2.)



Rat in a Sleeve Cage Feeding Orally and Intragastrically on Fluid Diet By Lever-pressing.

Fig. (3.)

SLEEVE CAGE DIMENSIONS



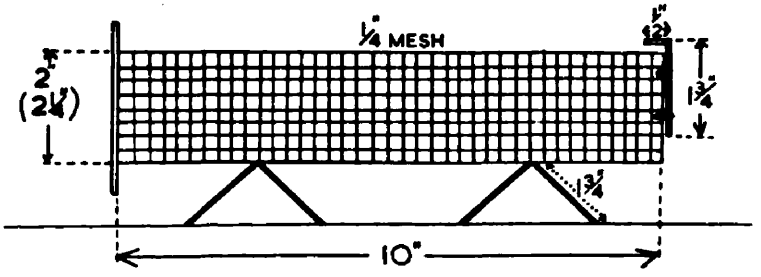
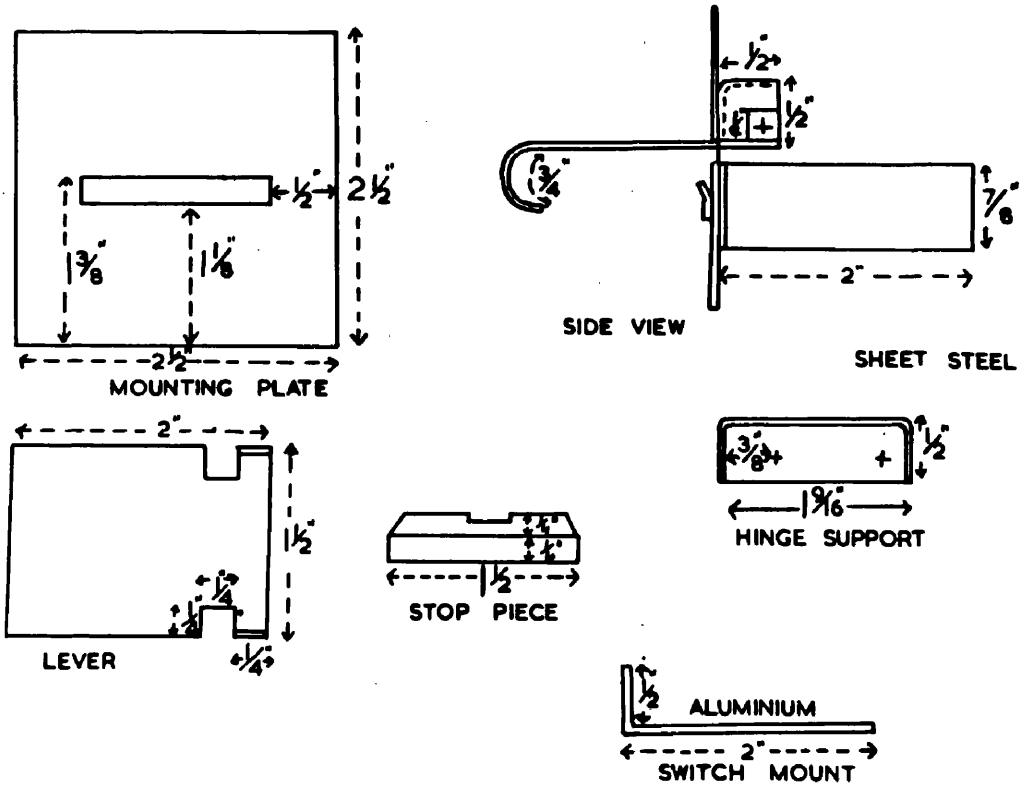
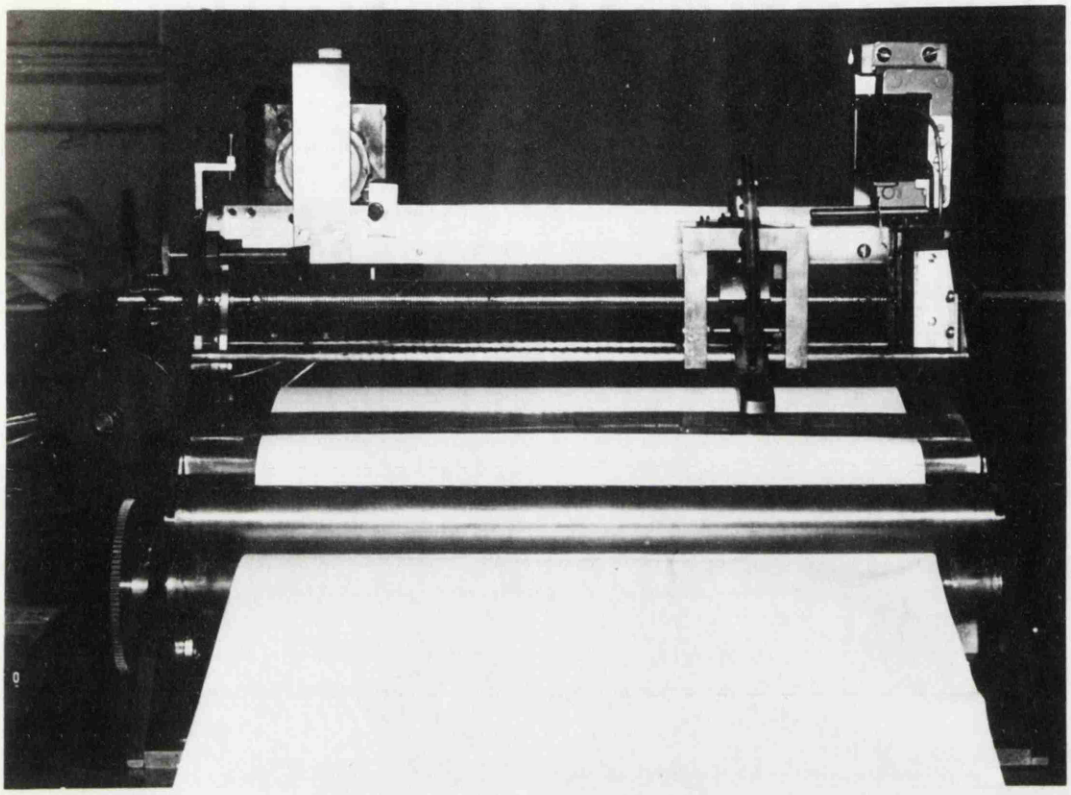


Fig. (4.)



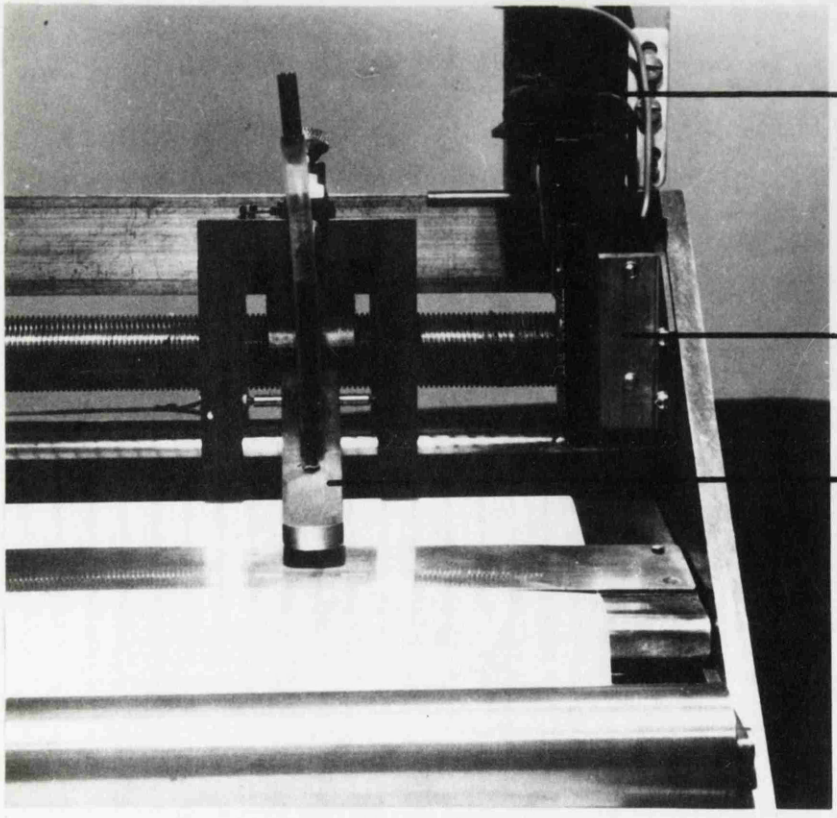
LEVER DIMENSIONS

Fig. (5.)



Cumulative Pen Recorder for Recording Individual Electrical Events over a 24hr Period.

Fig. (6.)



Close-up View of the Automatic Reset Mechanism.

Fig. (7.)

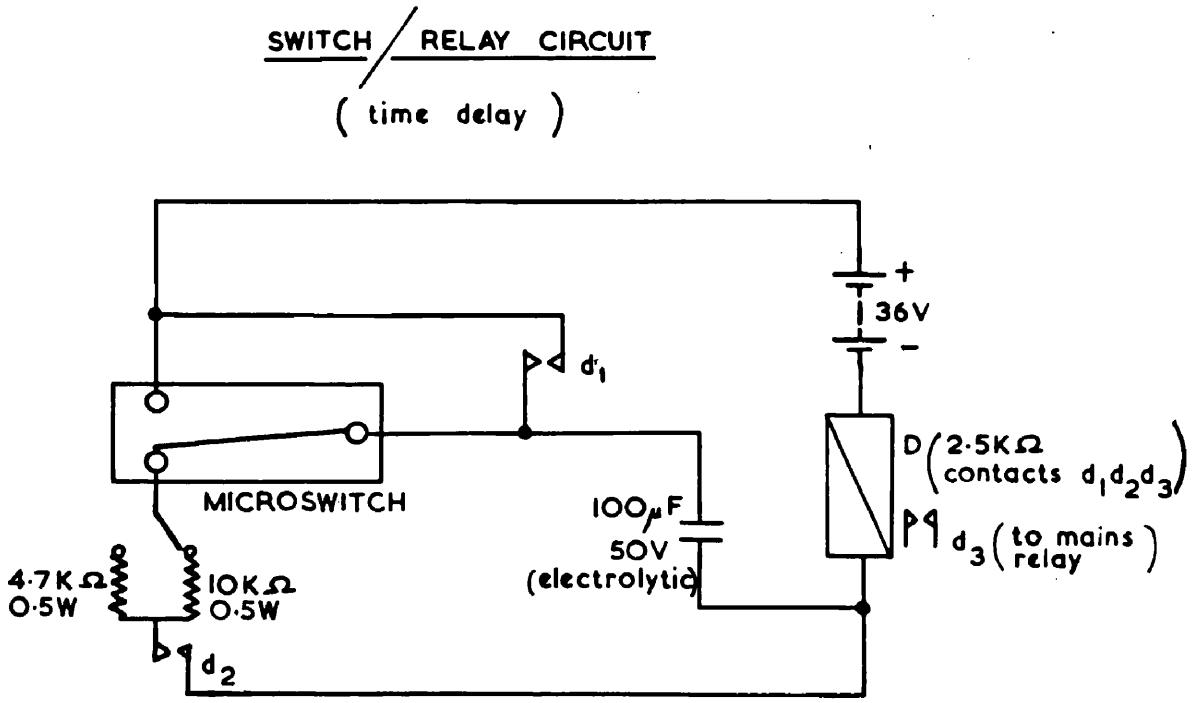

SWITCH / RELAY CIRCUIT
(time delay)
+
36V
-
d_1
MICROSWITCH
100μF
50V
(electrolytic)
D (2·5KΩ contacts $d_1 d_2 d_3$)
d_3 (to mains relay)
4·7KΩ
0·5W
10KΩ
0·5W
d_2

Fig. (8.)

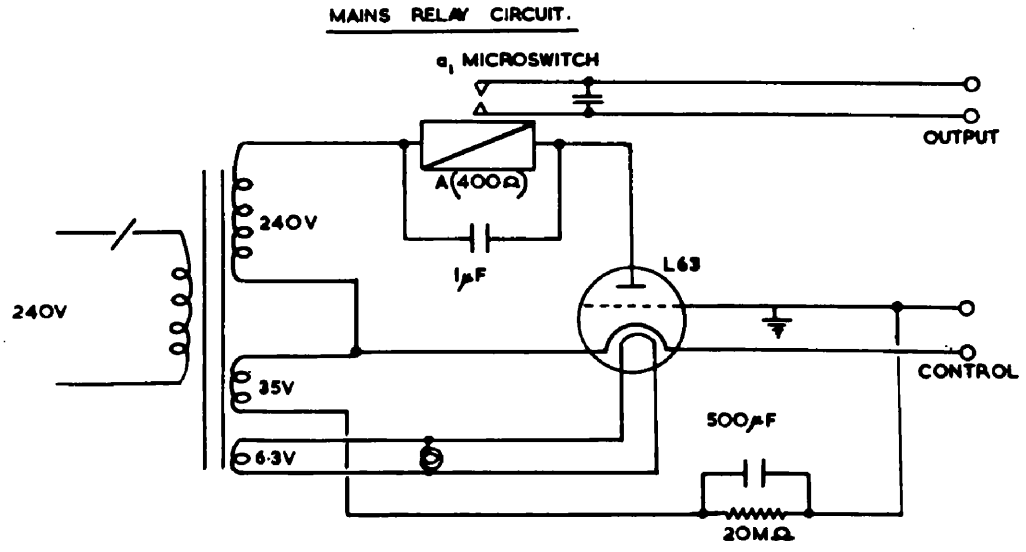
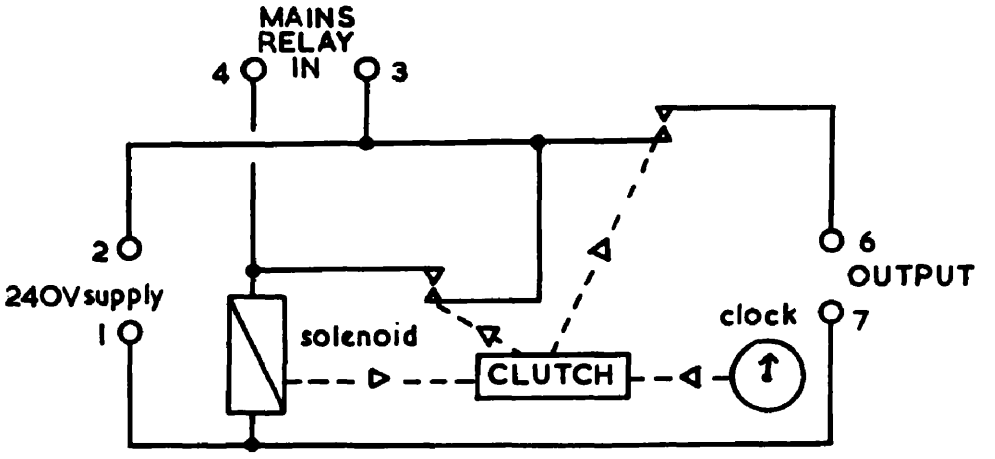
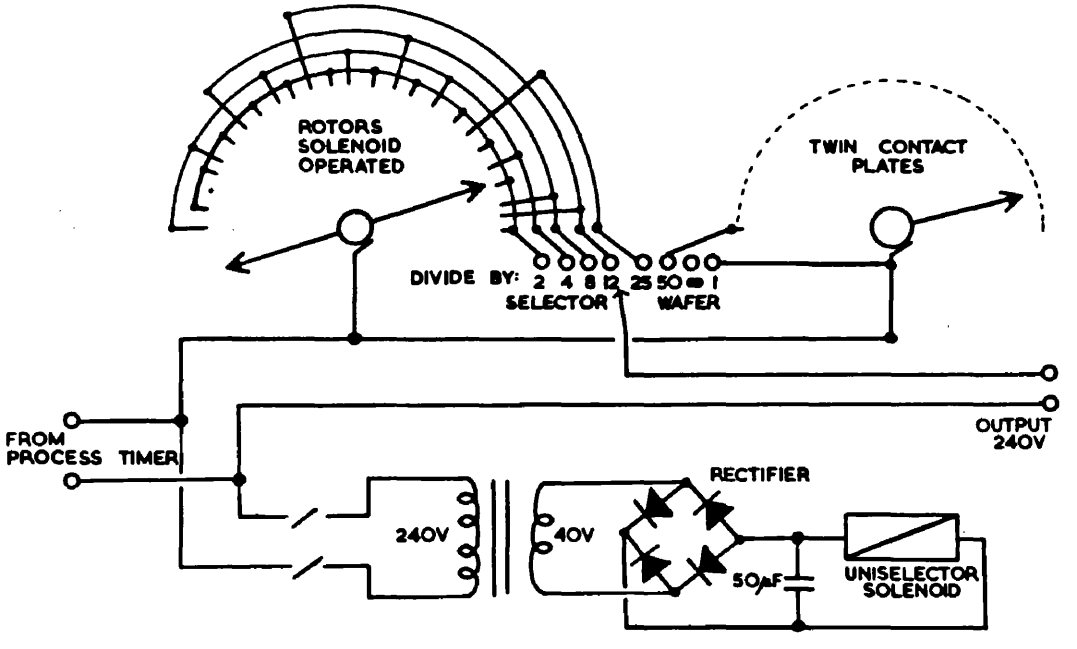

MAINS RELAY CIRCUIT.
a_1 MICROSWITCH
OUTPUT
A(400Ω)
240V
1μF
L63
240V
35V
6·3V
CONTROL
500μF
20MΩ

Fig. (9.)



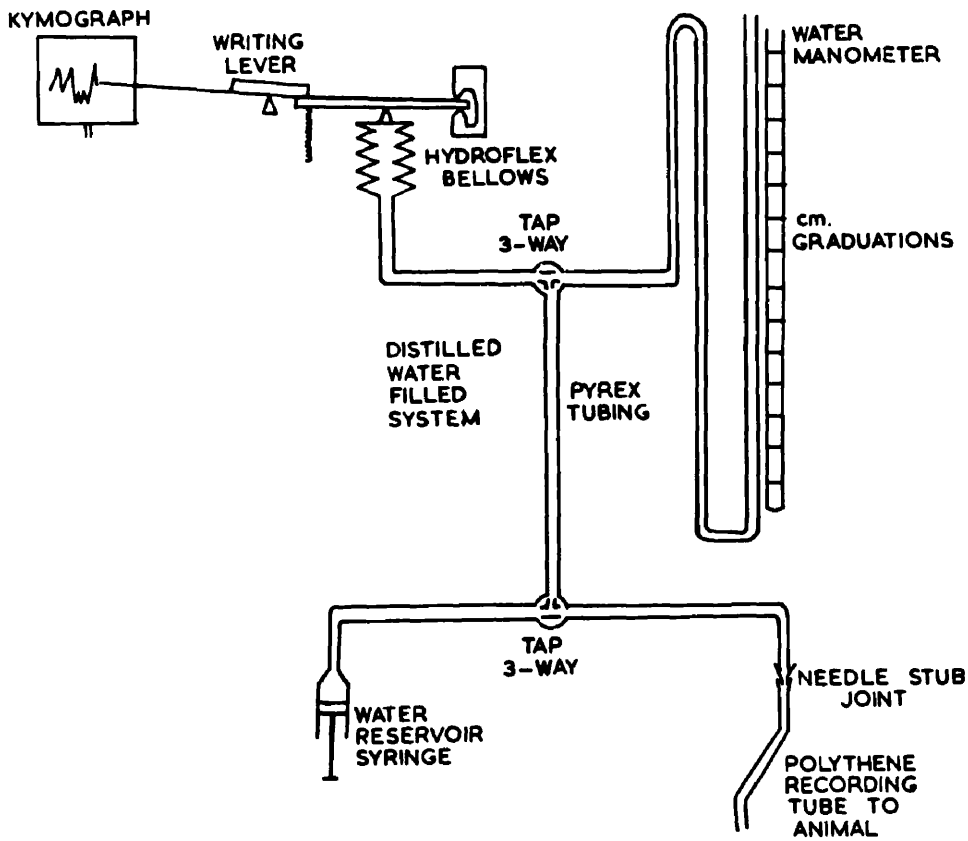
PROCESS TIMER CIRCUIT

Fig. (10.)



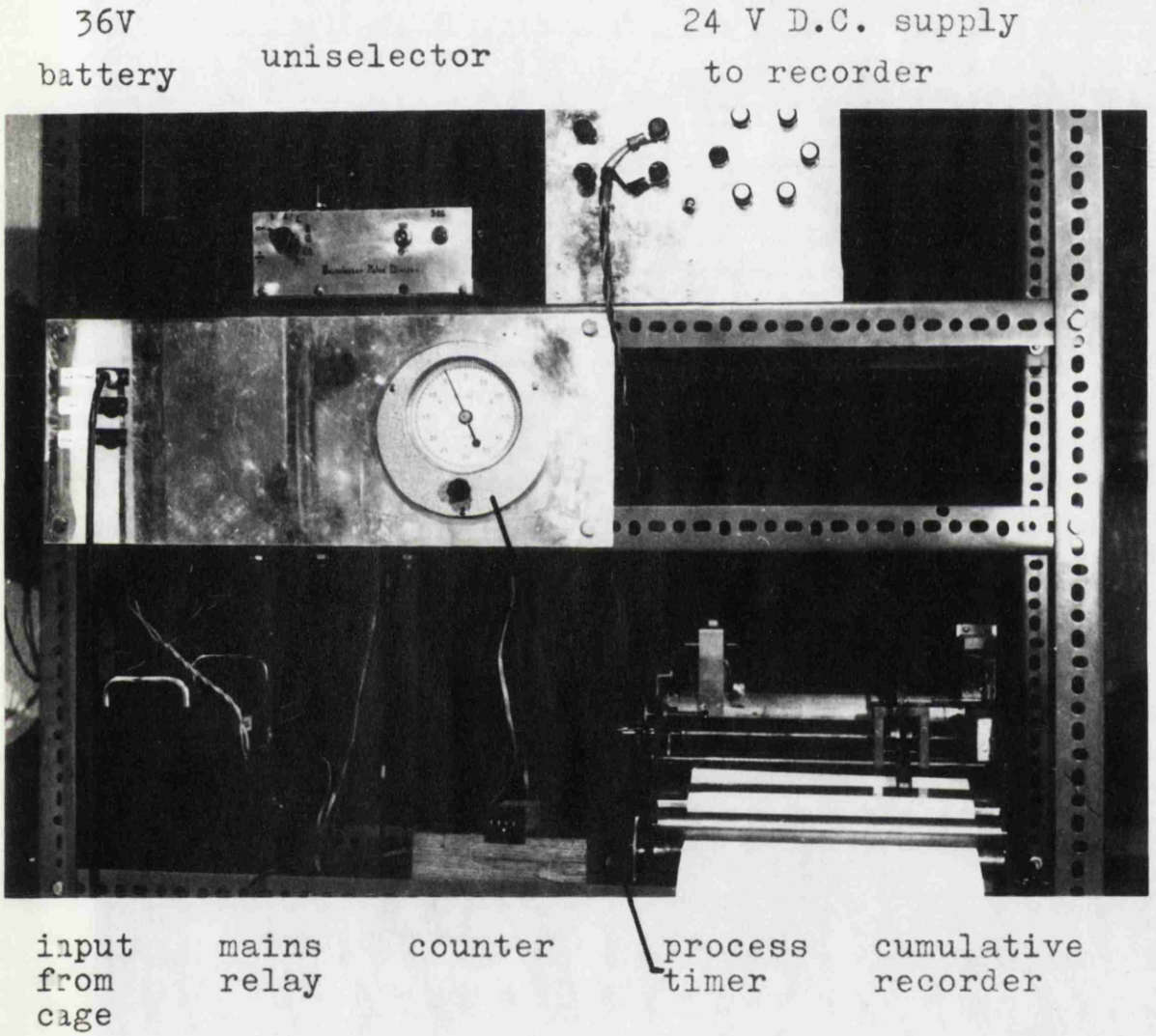
UNISELECTOR PULSE DIVIDER CIRCUIT

Fig. (11.)



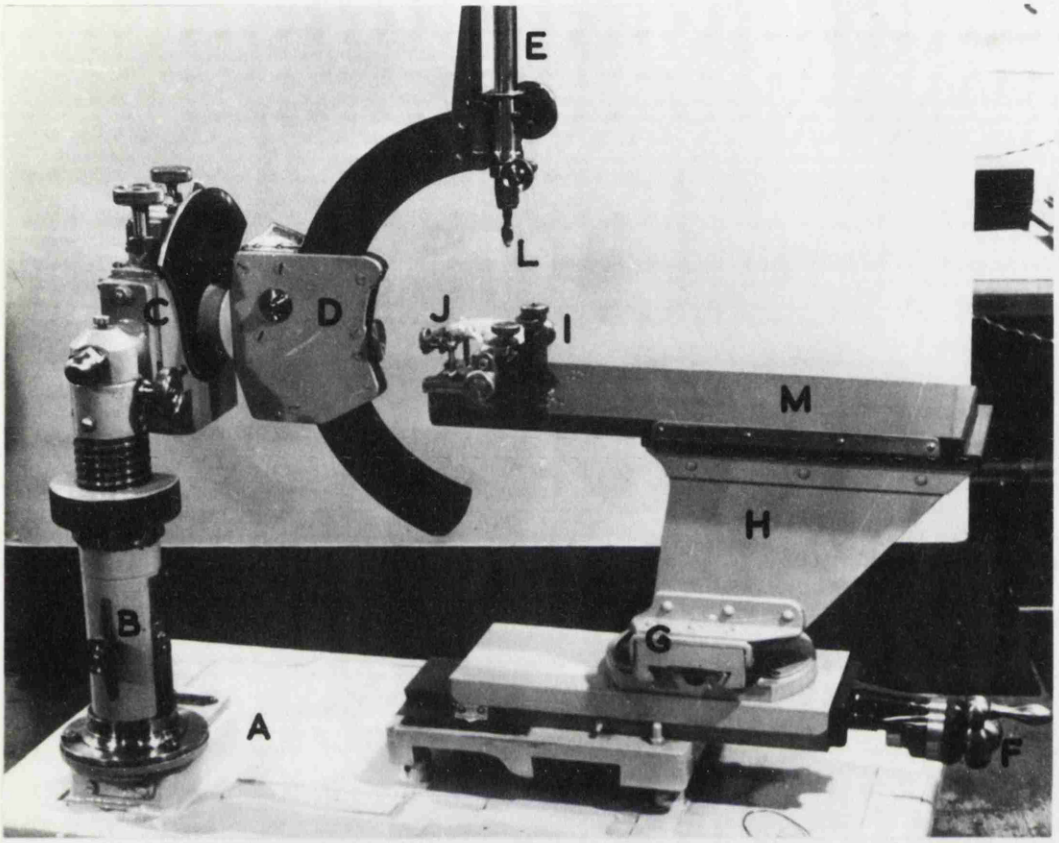
GASTRIC RECORDING SYSTEM

Fig. (12.)

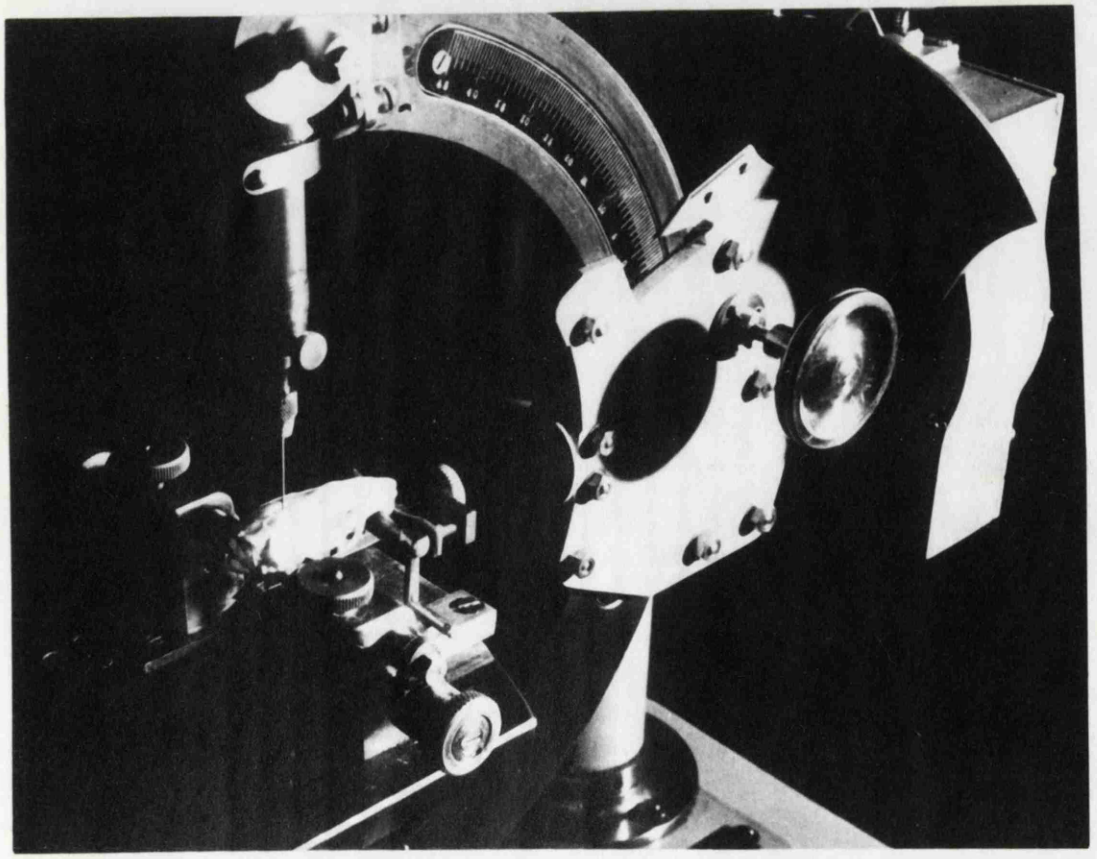


Rack Outside the Experimental Room Carrying the Recording and Controlling Devices for the Skinner Box System.

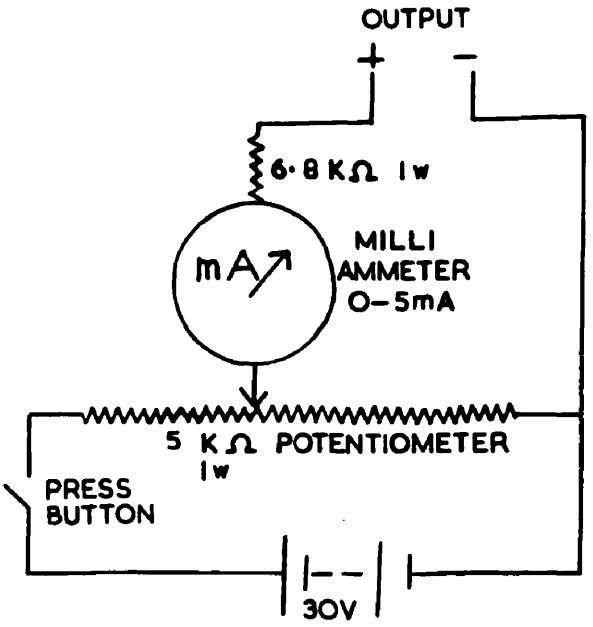
Fig. (13.)



General View of the Stereotaxic Instrument.
Fig. (14.)

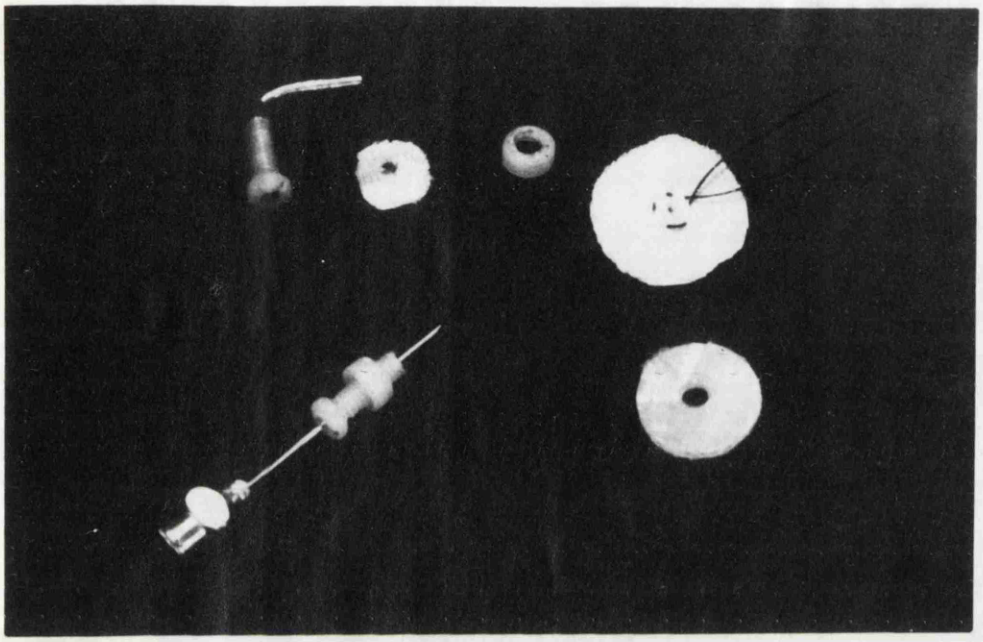


Electrode Above the Skull in Position for Vertical Entry.
Fig. (15.)



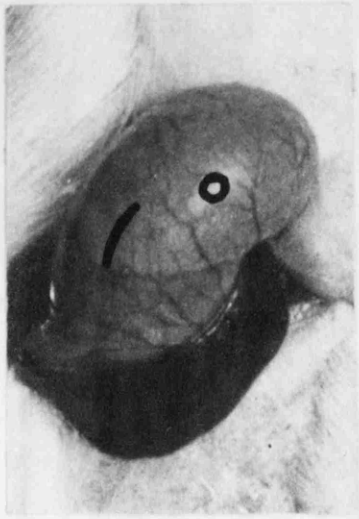
ELECTRODE SUPPLY CIRCUIT

Fig. (16)



a) Components of the fistula for permanent implantation in the stomach of a rat. The basic nylon screw is on the lower left.

(Actual Size)



b) Position of incision and insertion of the nylon tube in the stratified squamous epithelial layer of the stomach.

Fig. (17).

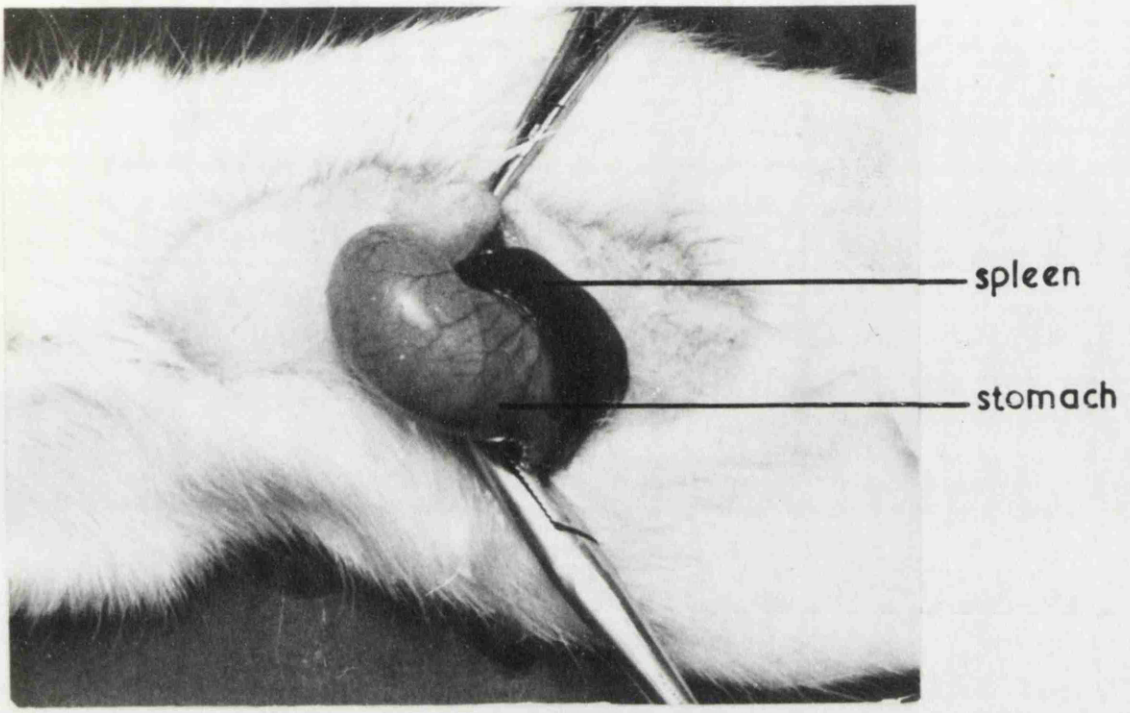


Fig. (18,a). Stomach of rat displayed – left lateral aspect.

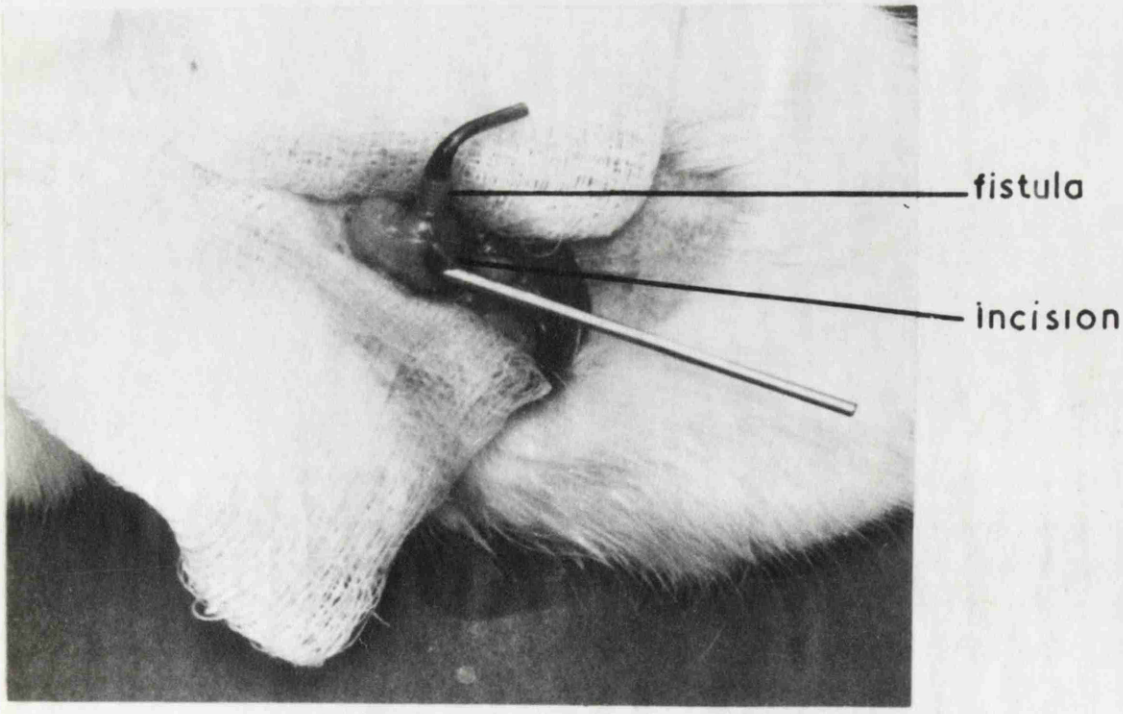


Fig. (18,b). Nylon fistula emerging through stab wound.

Fig. (18, c). Fistula sealed through stomach wall.

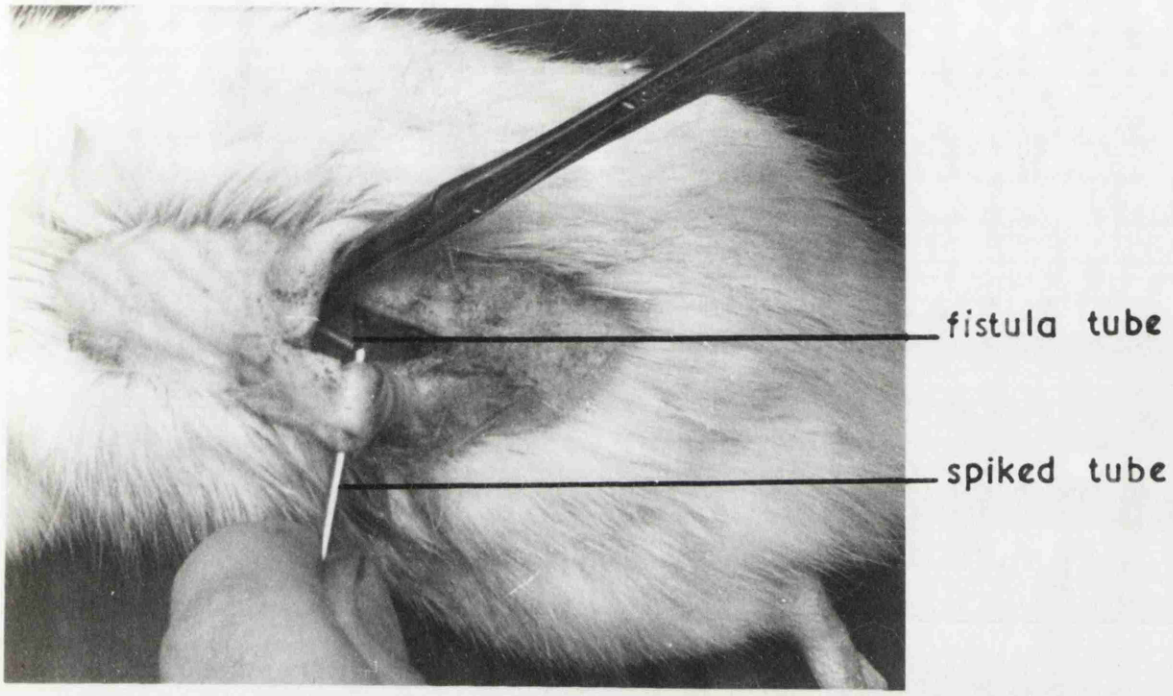


Fig. (18,f). Method of passing fistula tube through skin.

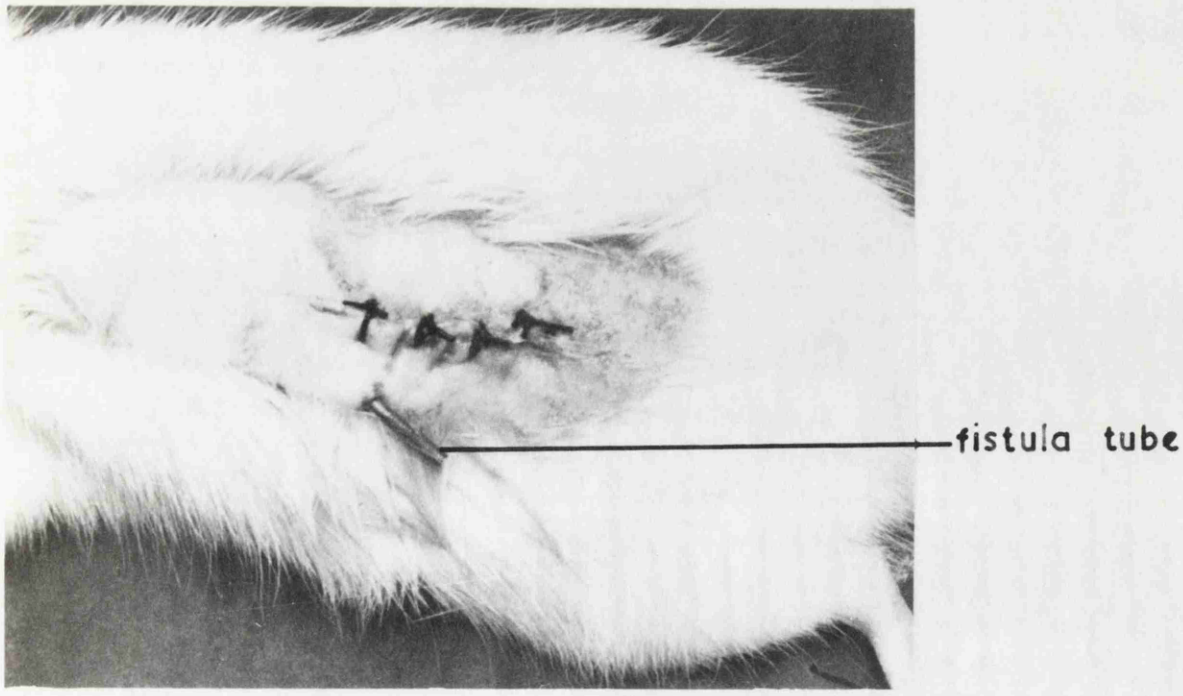
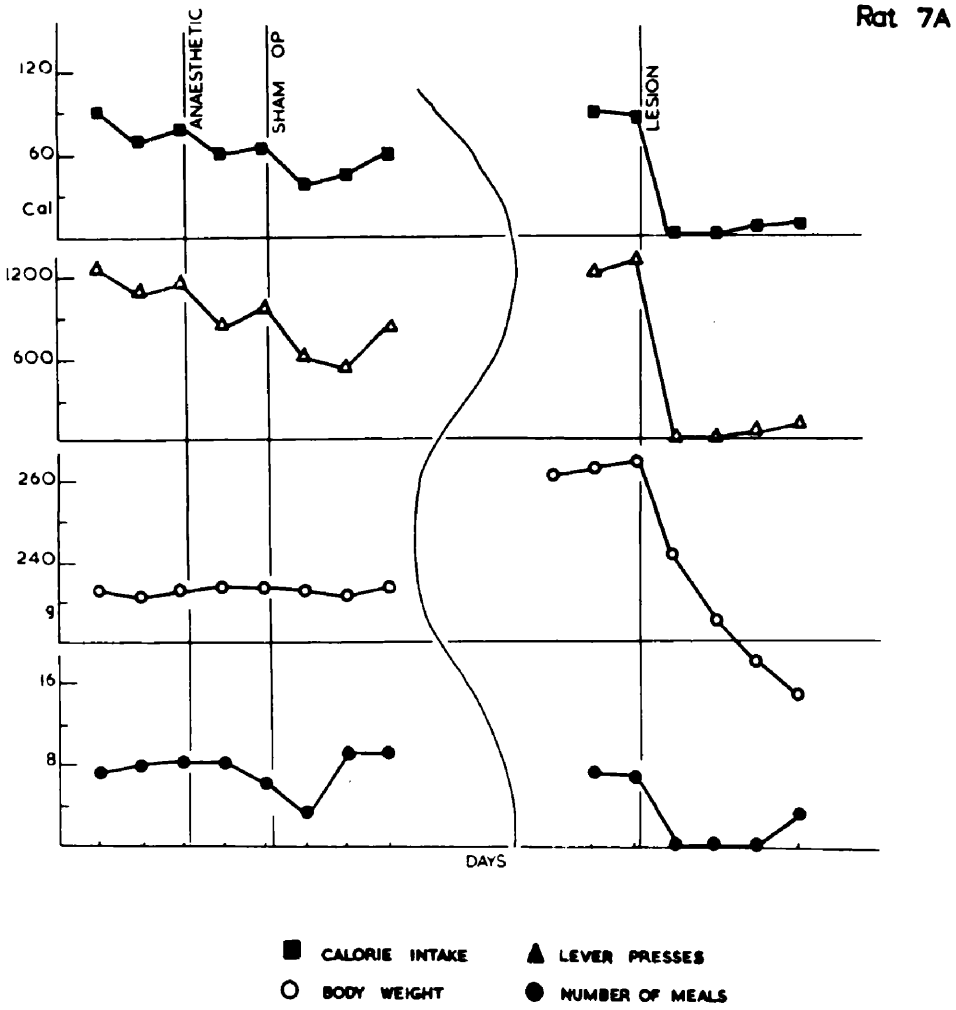
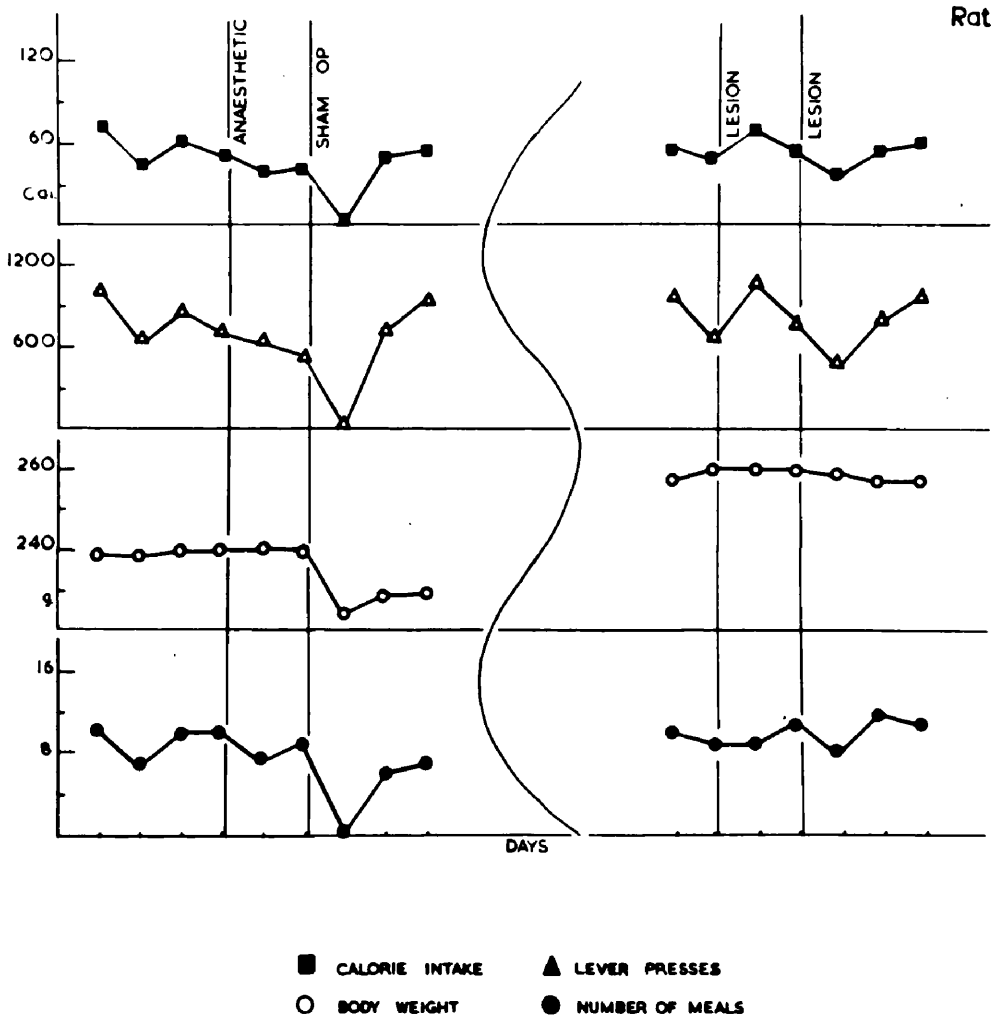


Fig. (18,g). Skin sutured. Operation complete.



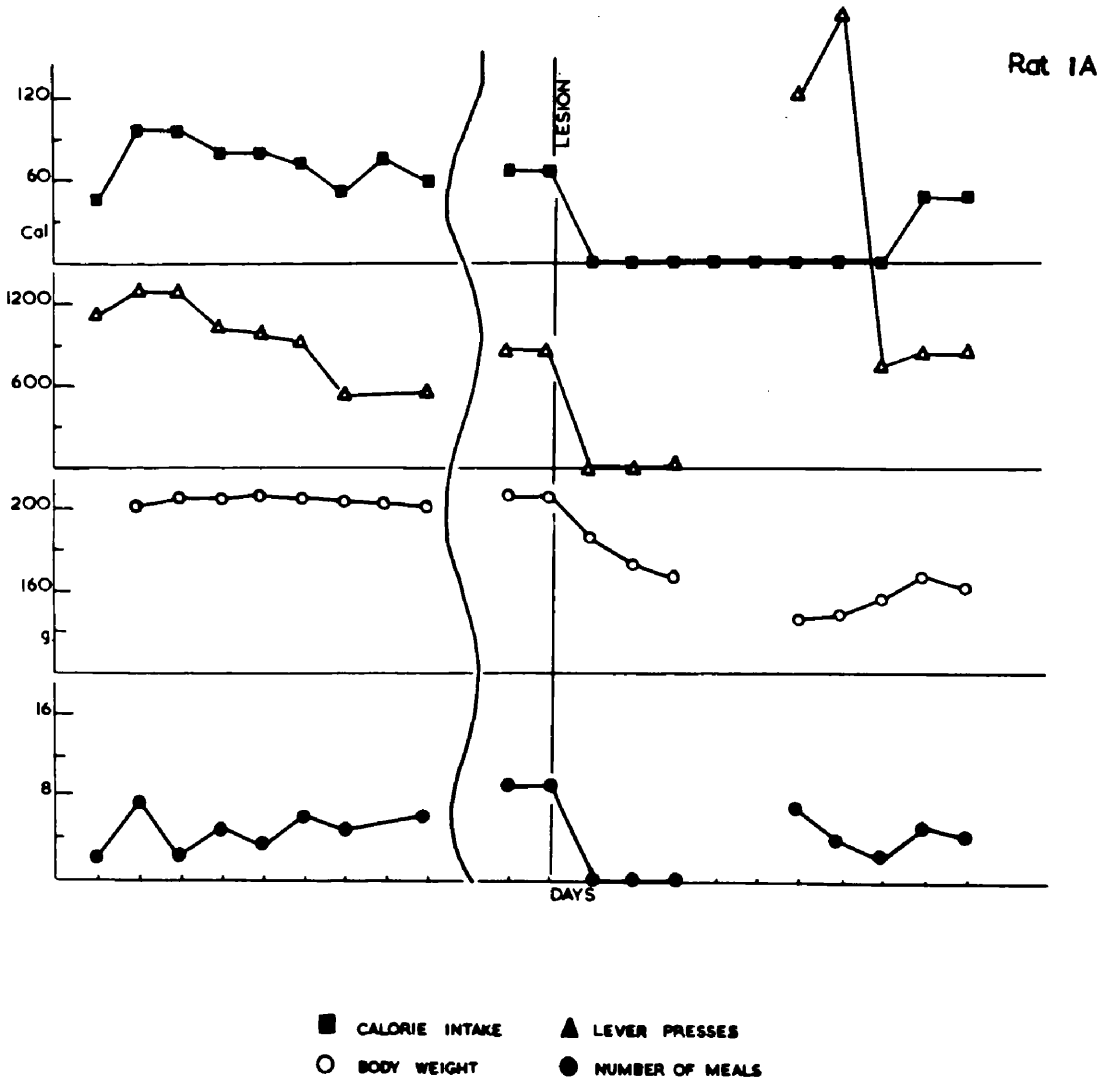
Effect of Operative Procedures on Oral Feeding of Rat7A. Recording from Open Cage Lever System.

Fig. (19).



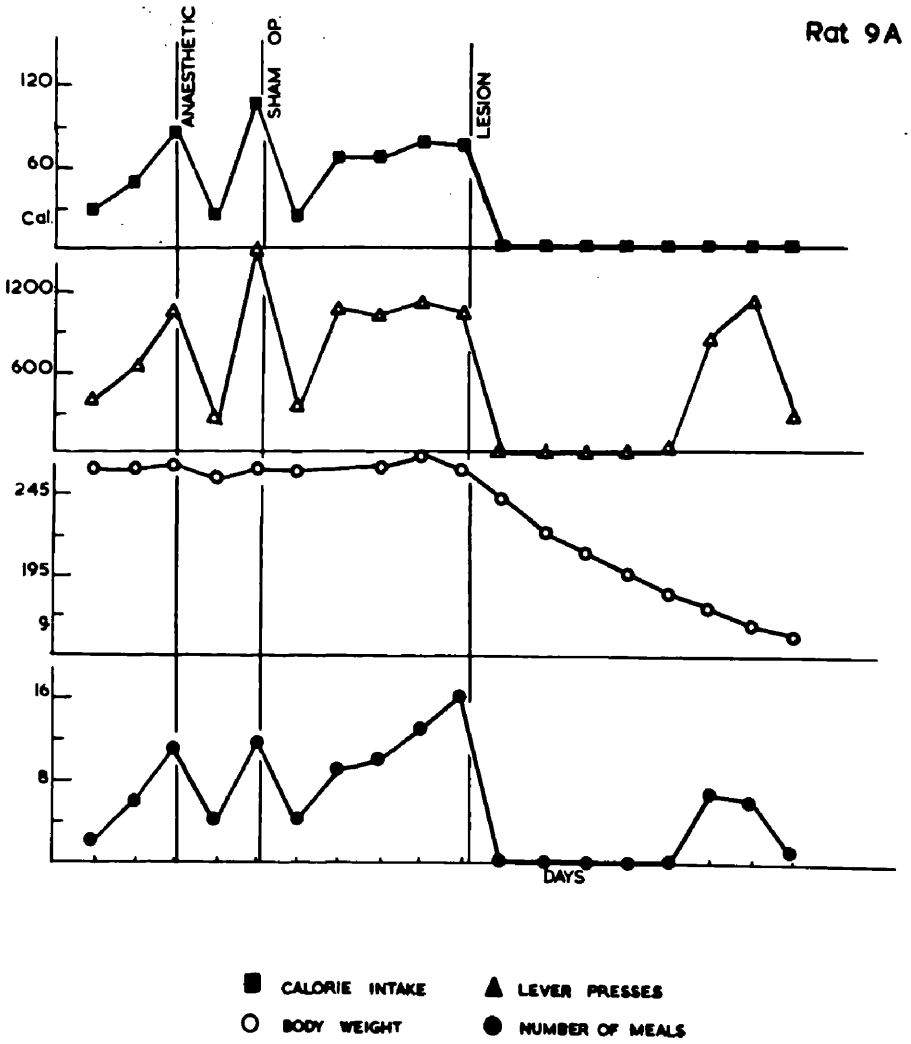
Effect of Operative Procedures on Oral Feeding of Rat8A. Recording from Open Cage Lever System.

Fig. (20).



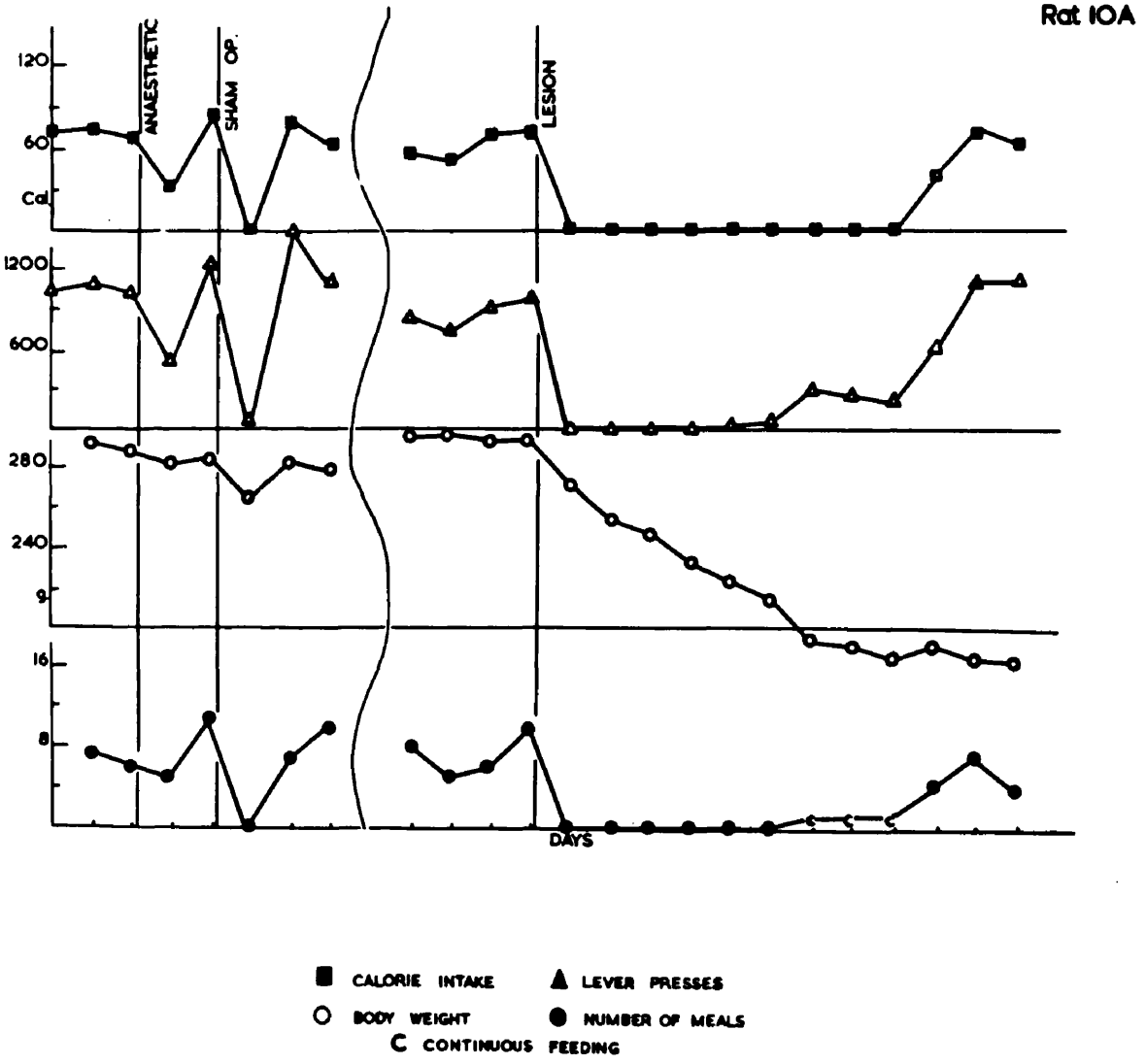
Effect of Operative Procedures on Oral Feeding of RatlA. Recording from Open Cage Lever System.

Fig. (21).



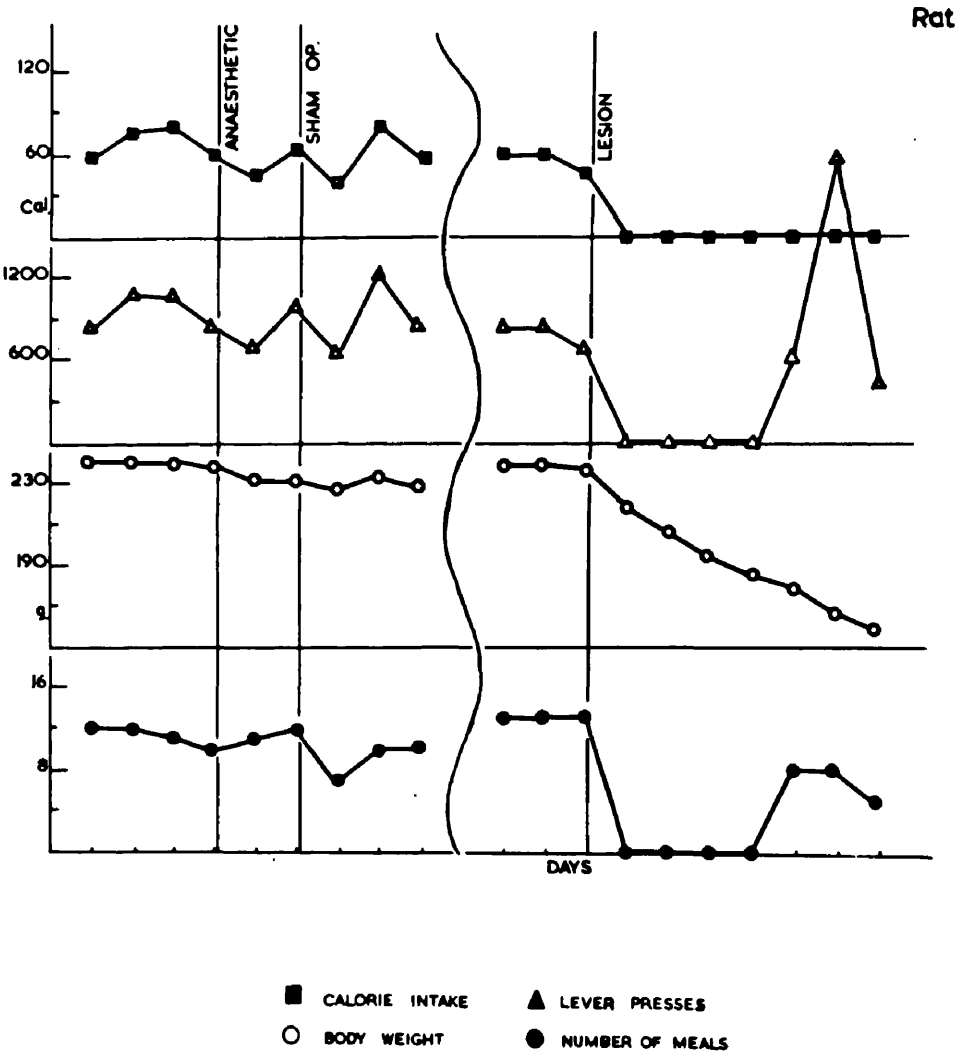
Effect of Operative Procedures on Oral Feeding of Rat9A. Recording from Open Cage Lever System.

Fig. (22).



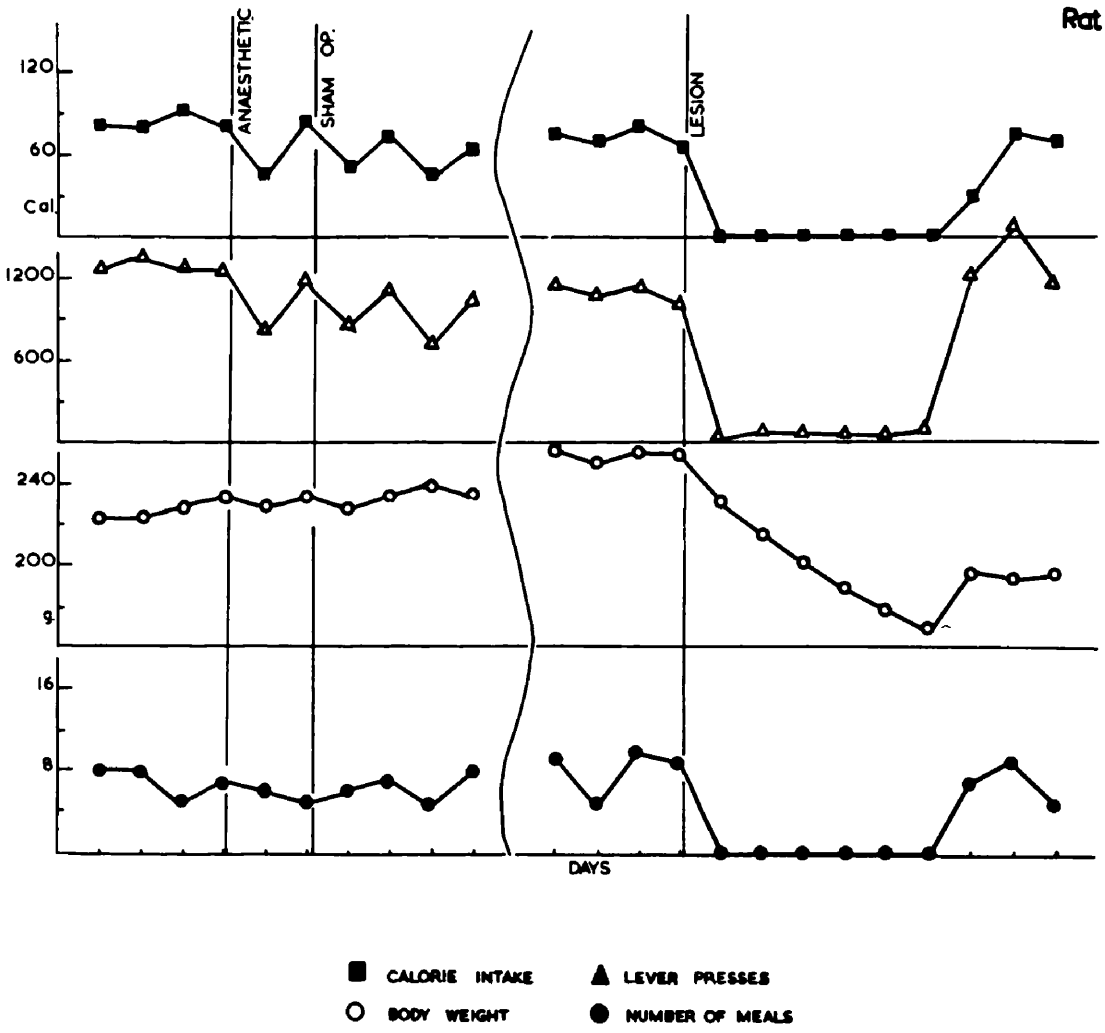
Effect of Operative Procedures on Oral Feeding of Rat 10A. Recording from Open Cage Lever System.

Fig. (23).



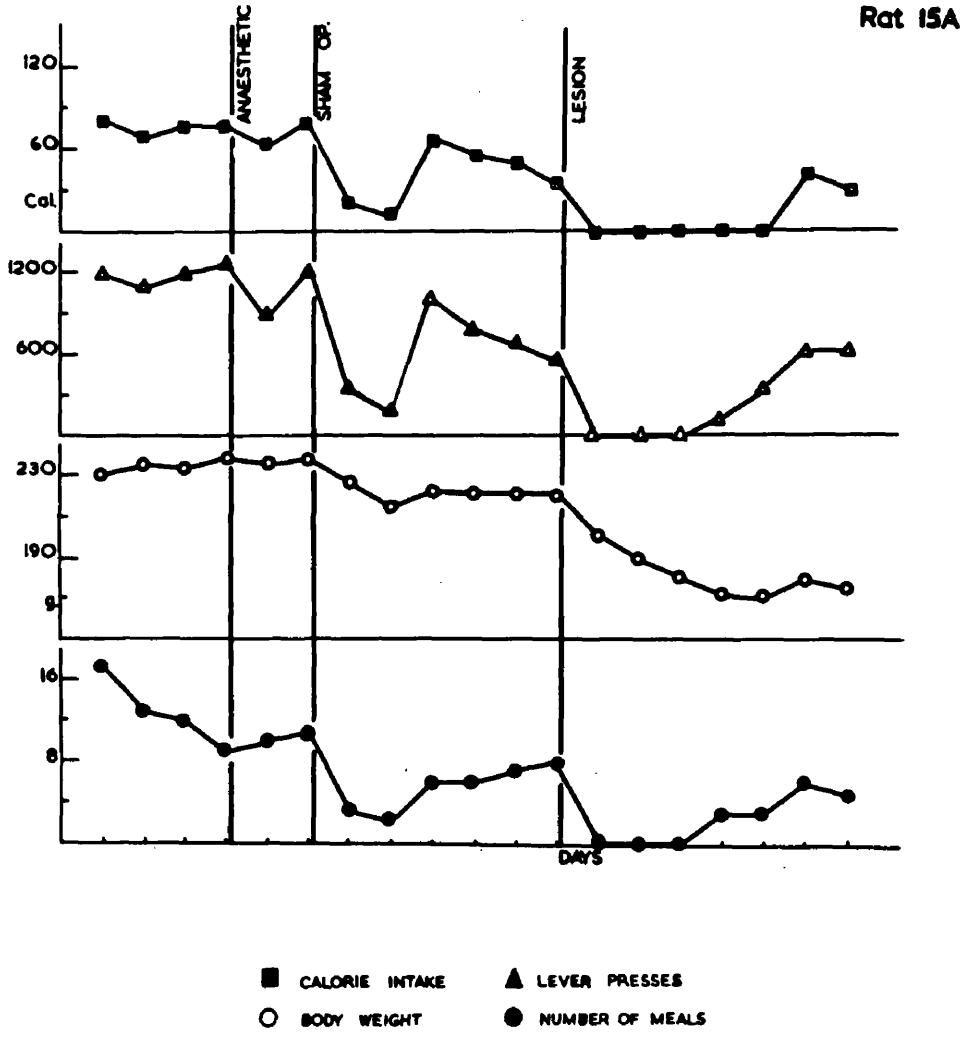
Effect of Operative Procedures on Oral Feeding of Rat12A.Recording from Open Cage Lever System.

Fig. (24).



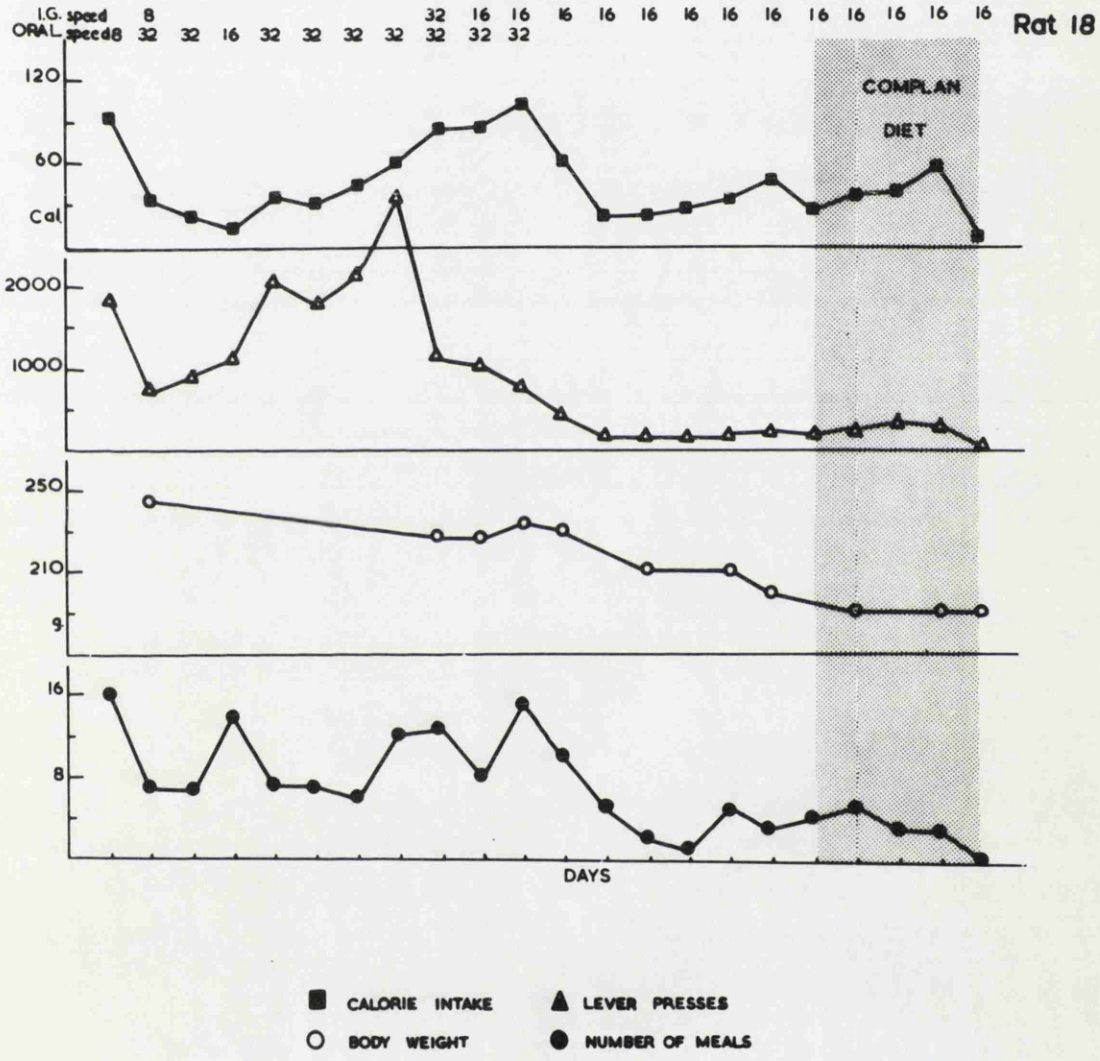
Effect of Operative Procedures on Oral Feeding of Rat13A. Recording from Open Cage Lever System.

Fig. (25).



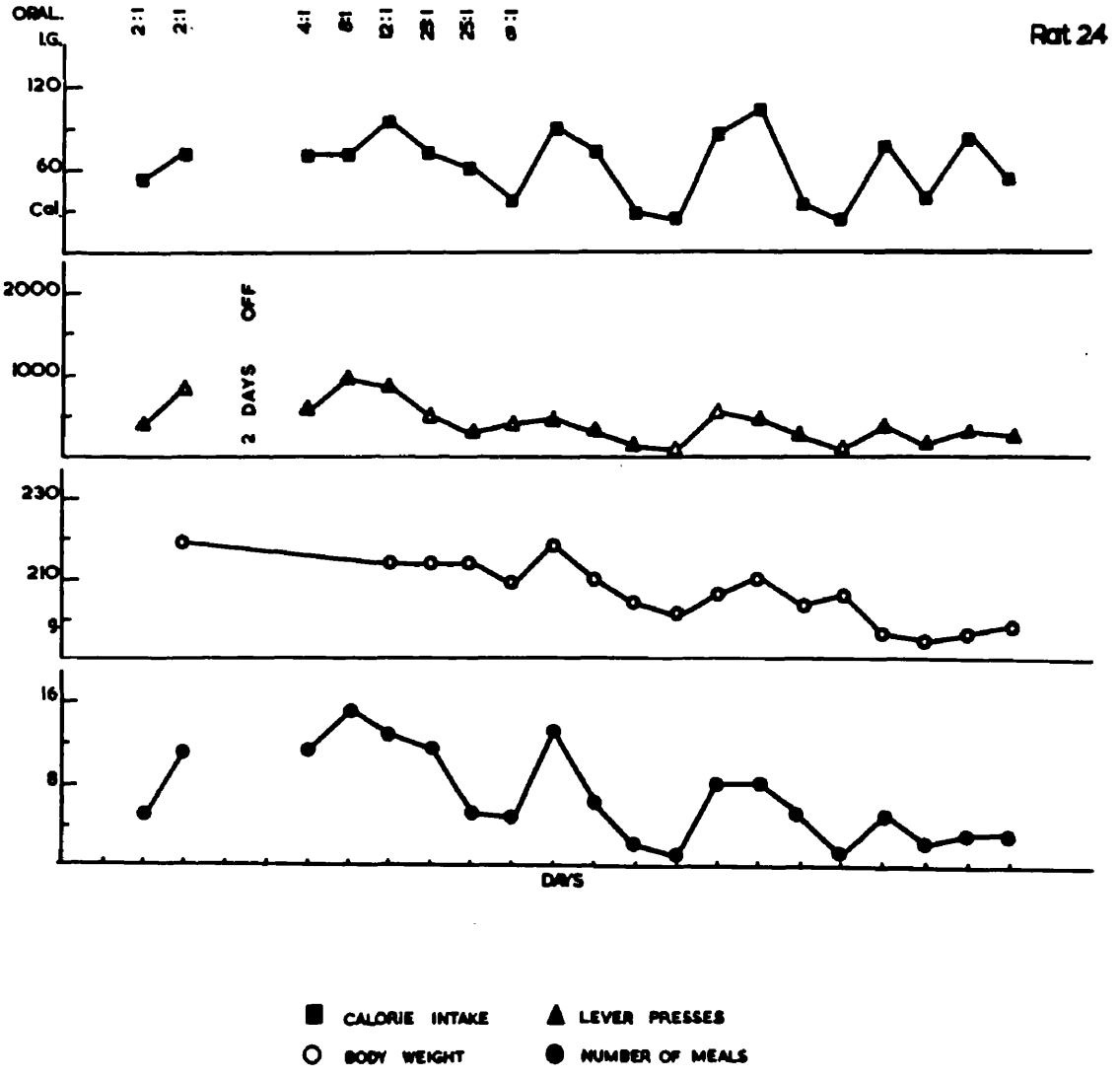
Effect of Operative Procedures on Oral Feeding of Rat15A. Recording from Open Cage Lever System.

Fig. (26).



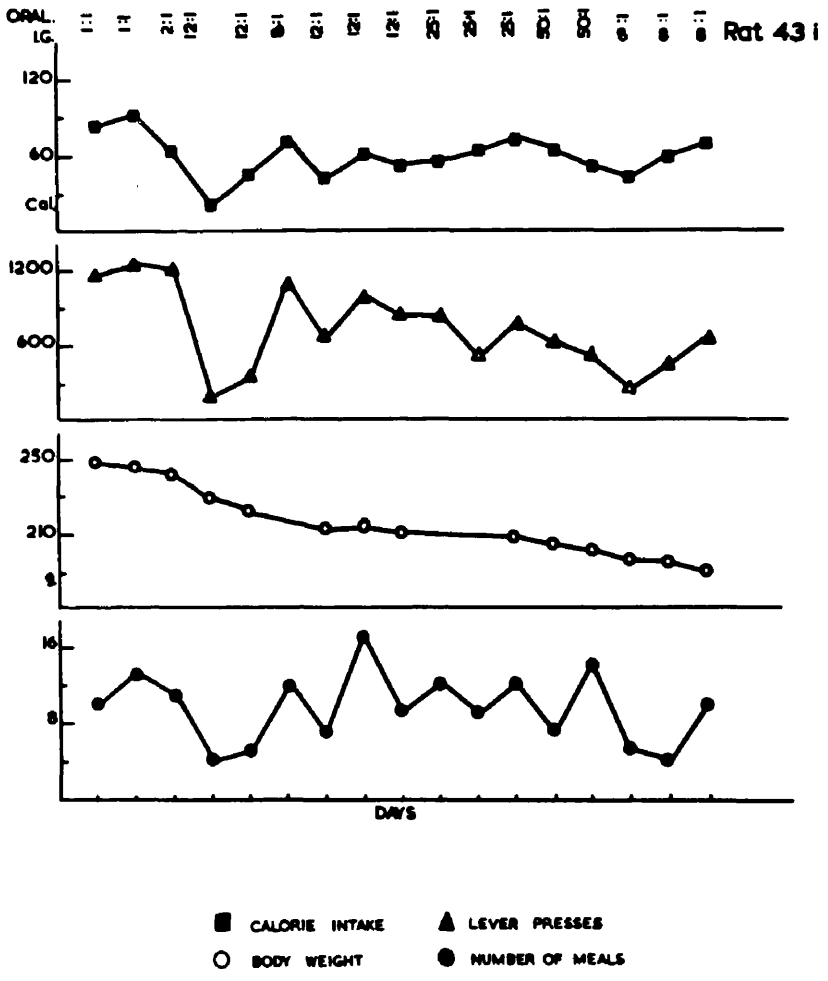
Experimental Period of Rat18 during Changeover from Oral to Purely Intragastric Feeding.

Fig. (27).



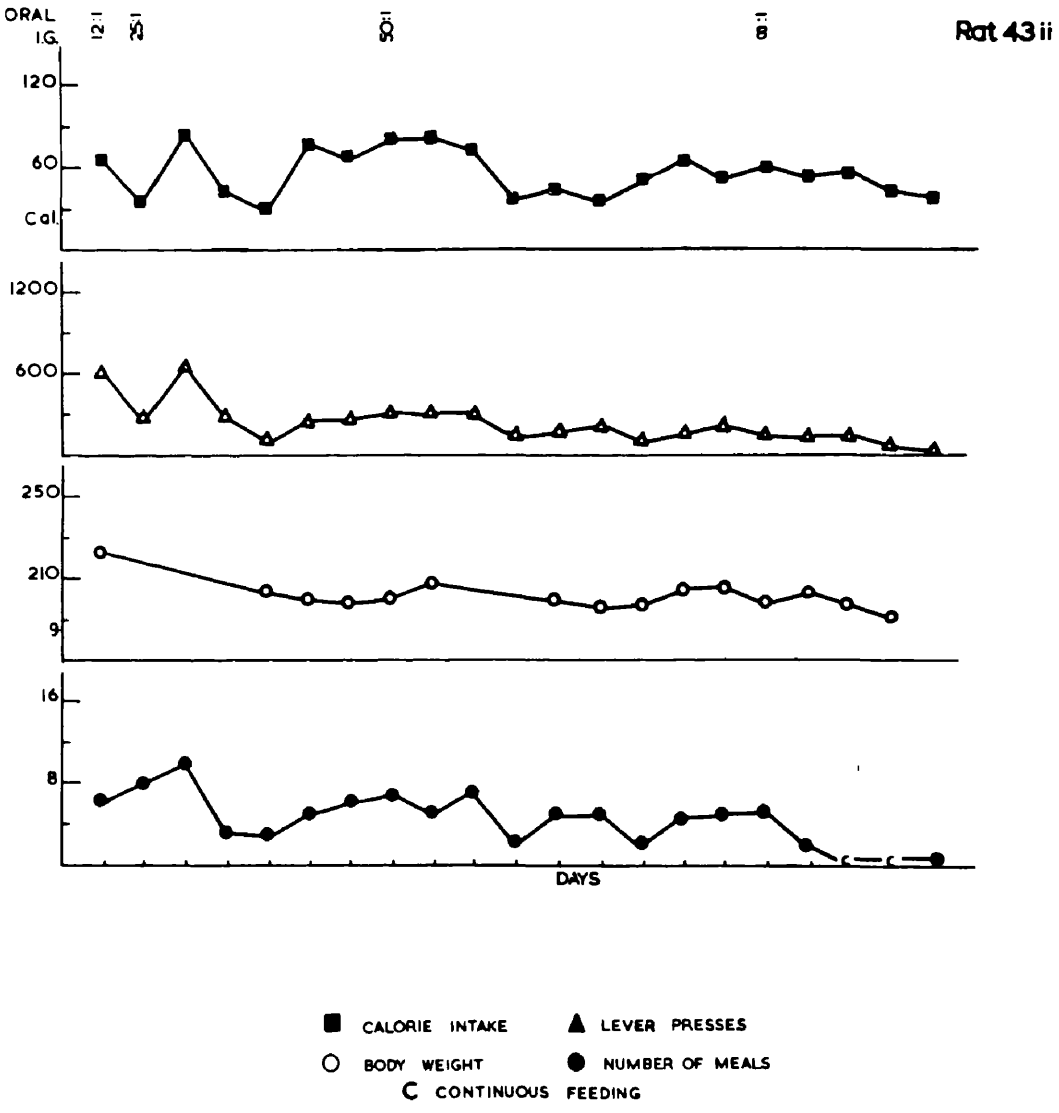
Experimental Period of Rat24 during Changeover from Oral to Purely Intragastric Feeding.

Fig.(28).



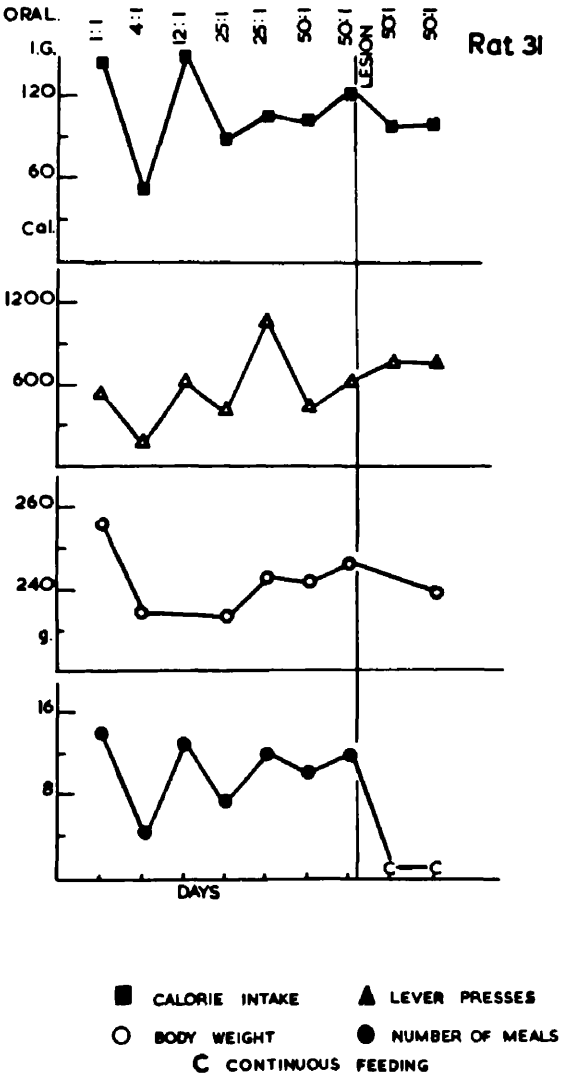
Experimental Period of Rat43 during Changeover from Oral to Purely Intragastric Feeding.

Fig. (29).



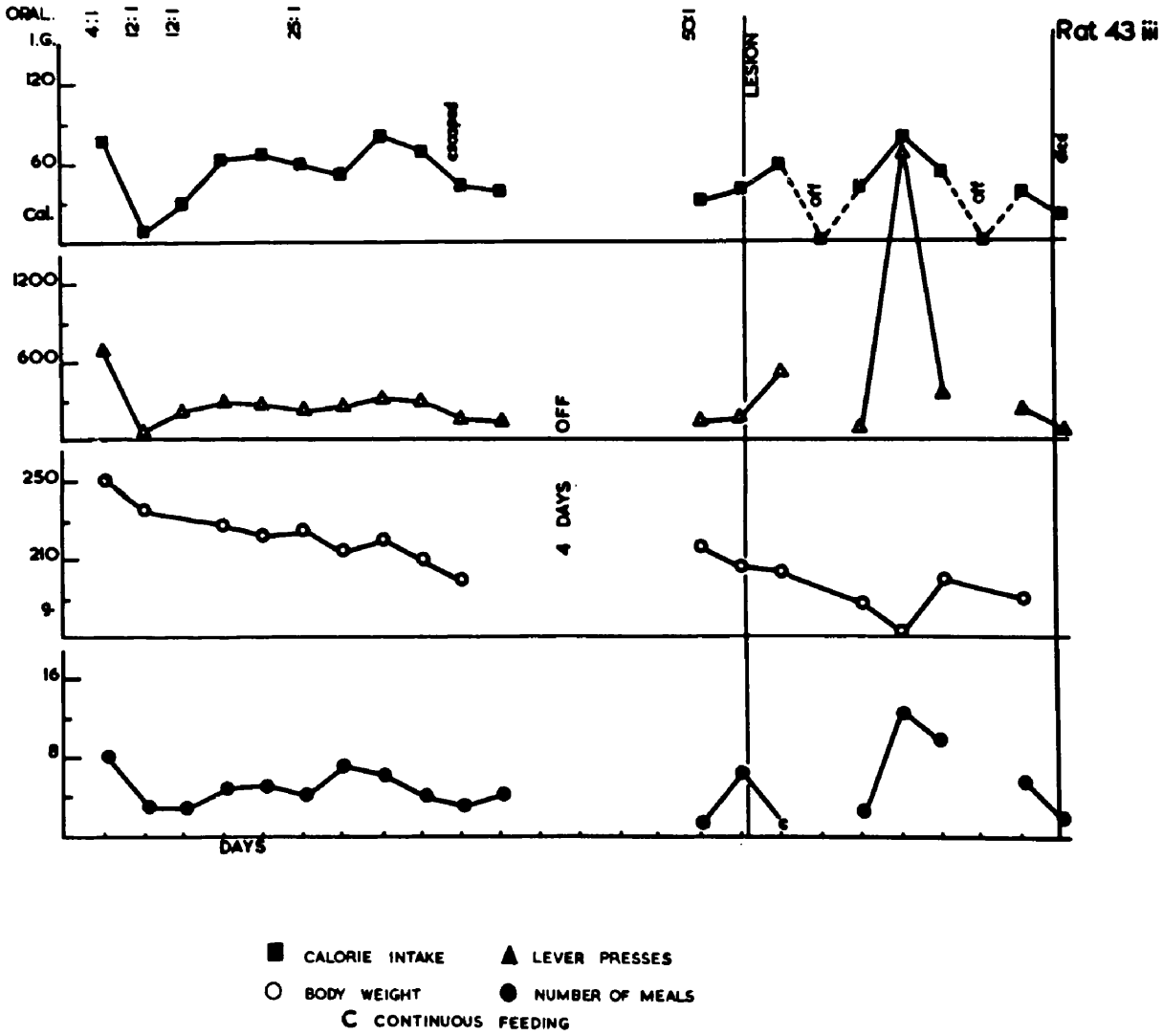
Experimental Period of Rat43 during Changeover from Oral to Purely Intragastric Feeding.

Fig. (30).



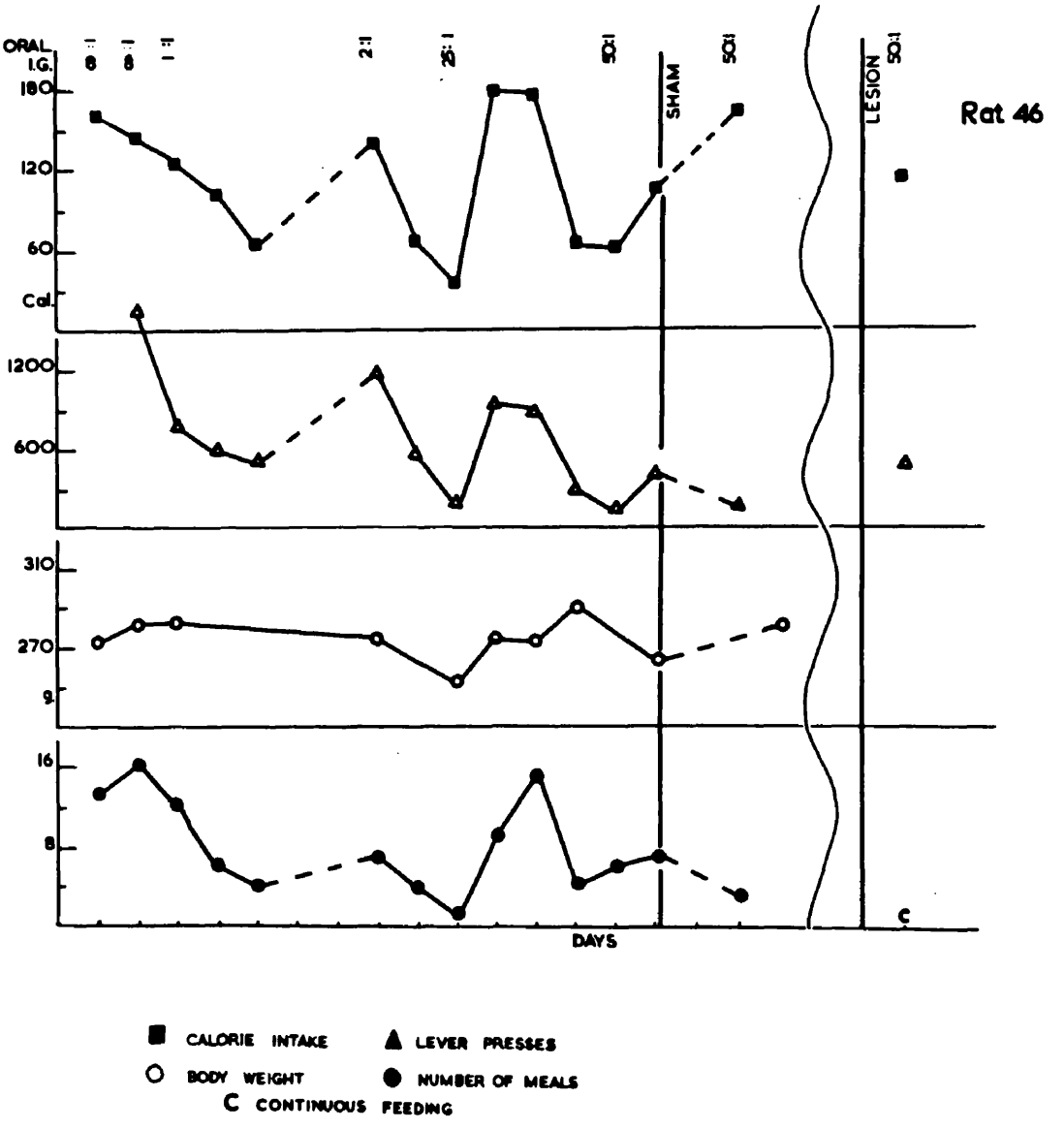
Effect of Lateral Hypothalamic Lesions on Intragastric Feeding of Rat31 in Sleeve Cage Lever System.

Fig. (31).



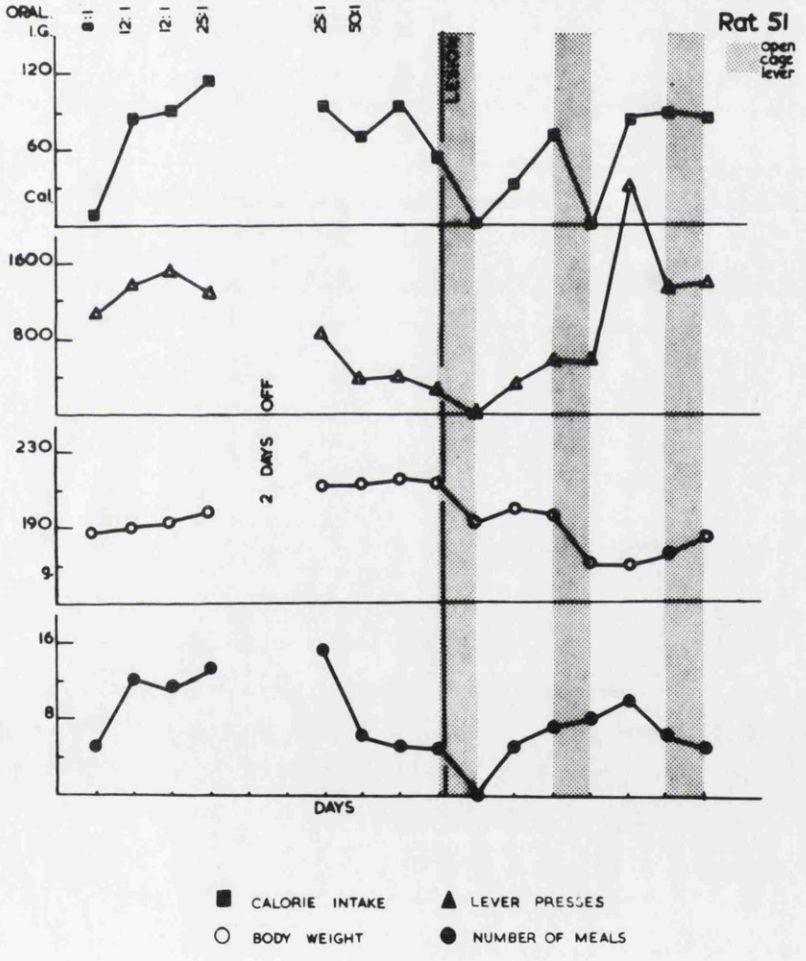
Effect of Lateral Hypothalamic Lesions on Intragastric Feeding of Rat43 in Sleeve Cage Lever System.

Fig. (32).



Effect of Lateral Hypothalamic Lesions on Intragastric Feeding of Rat46 in Sleeve Cage Lever System.

Fig. (33).



Effect of Lateral Hypothalamic Lesions on Intragastric Feeding of Rat51 in Sleeve Cage Lever System.

Fig. (34).

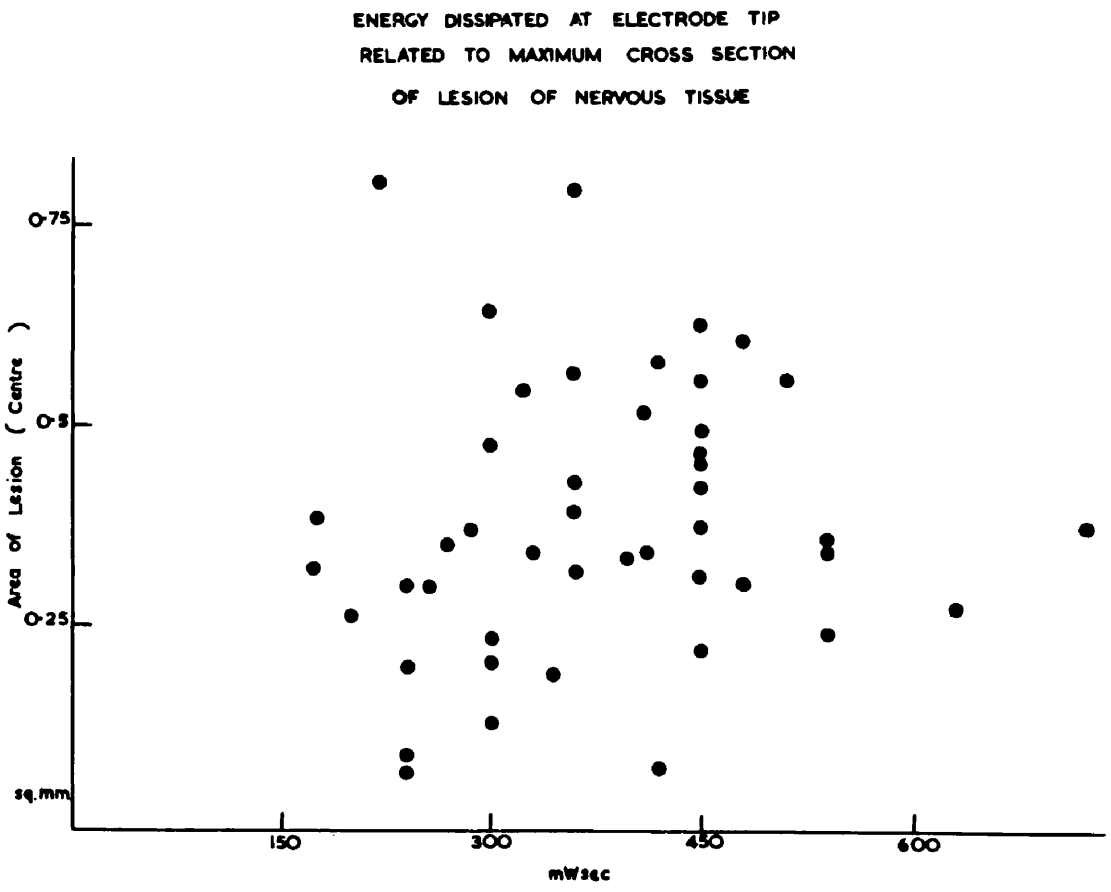
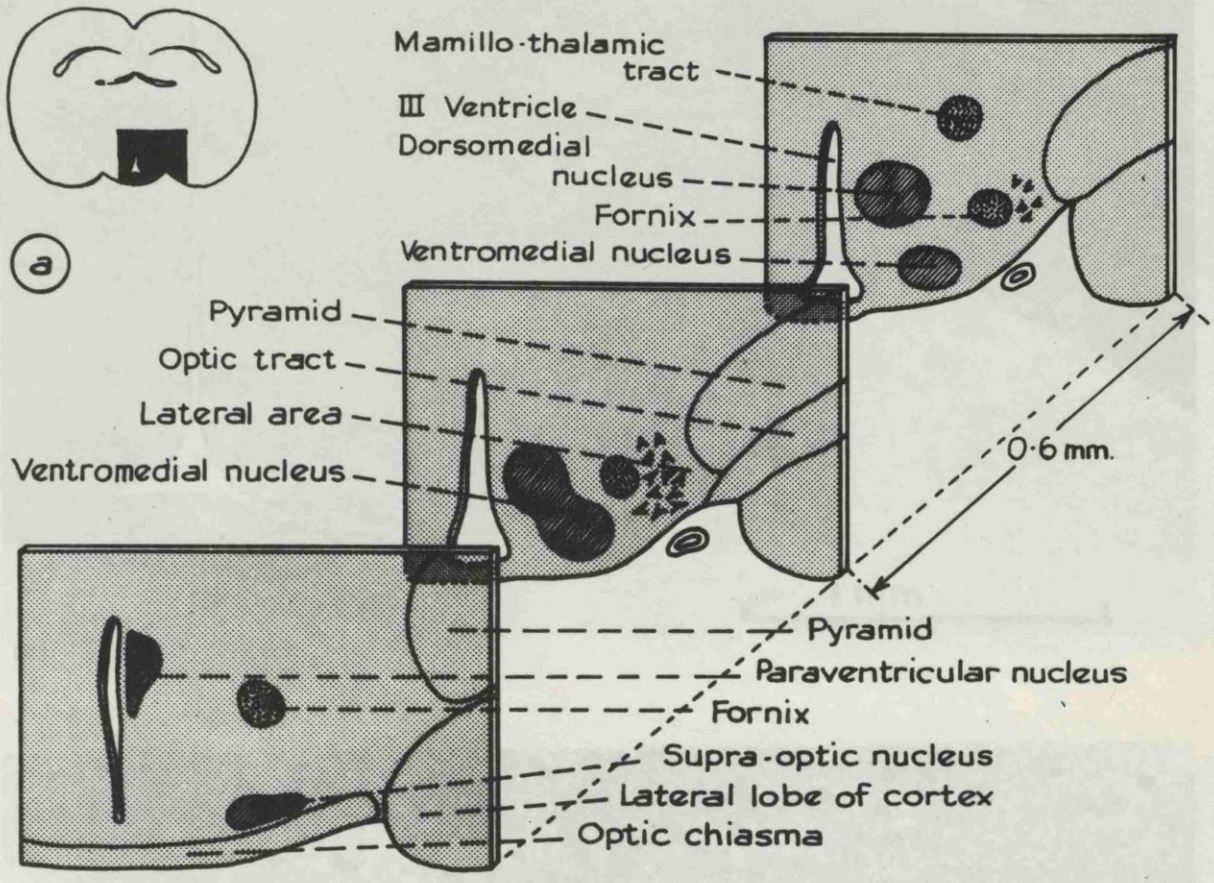
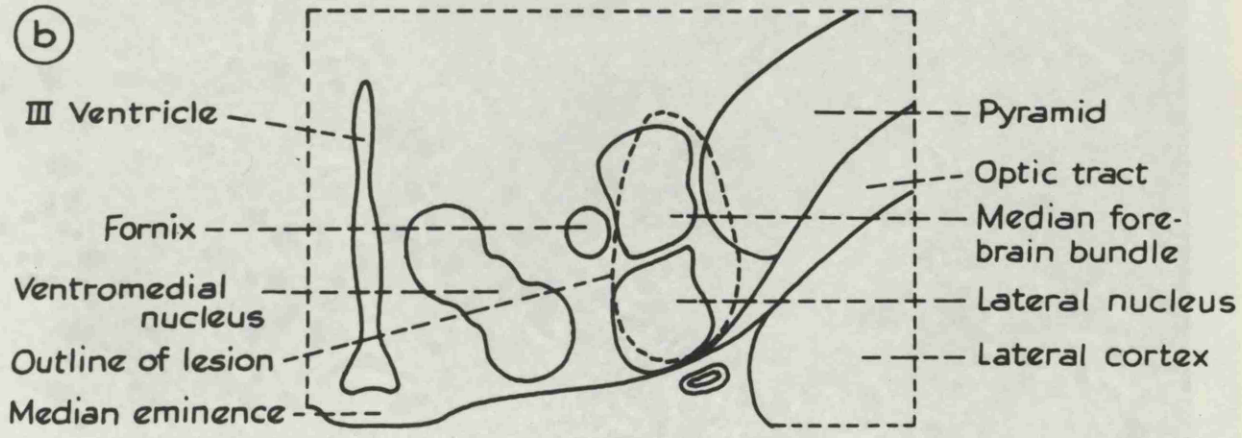

ENERGY DISSIPATED AT ELECTRODE TIP
RELATED TO MAXIMUM CROSS SECTION
OF LESION OF NERVOUS TISSUE
Area of Lesion (Centre)
0·75
0·5
0·25
sq.mm.
150
300
450
600
mWsec

Fig. (35)

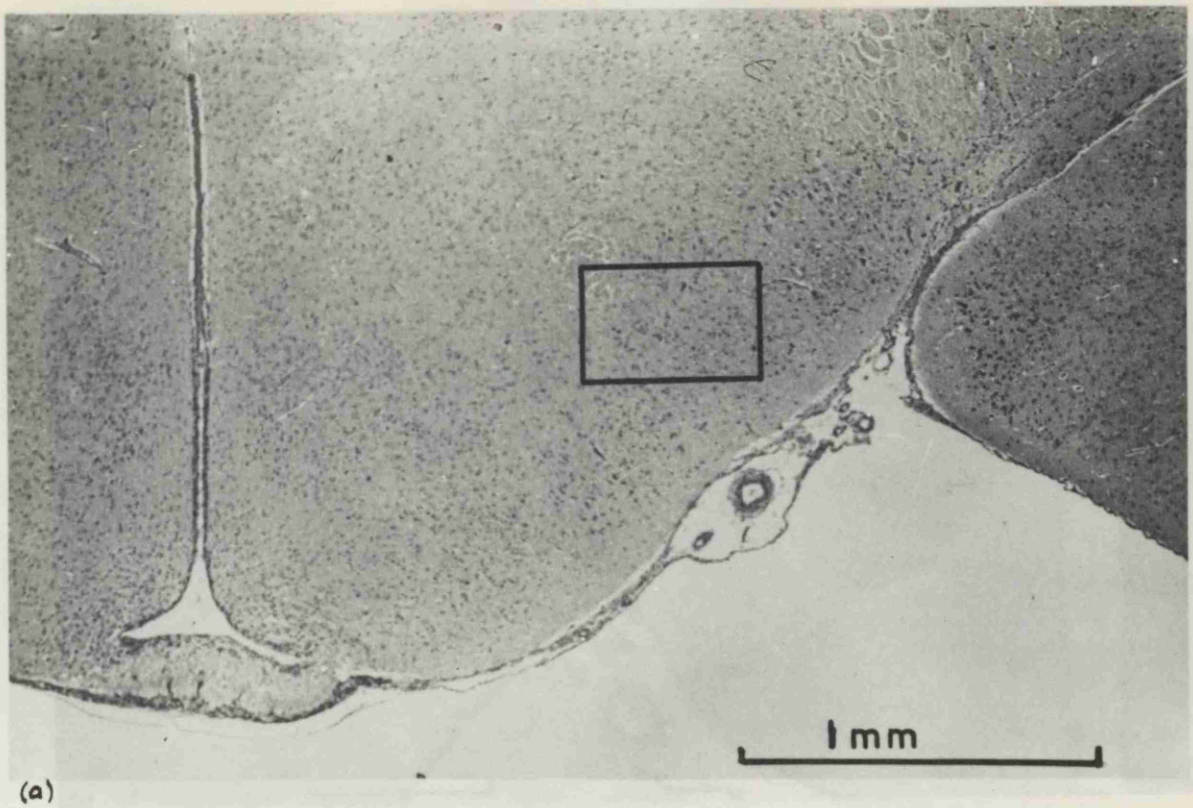


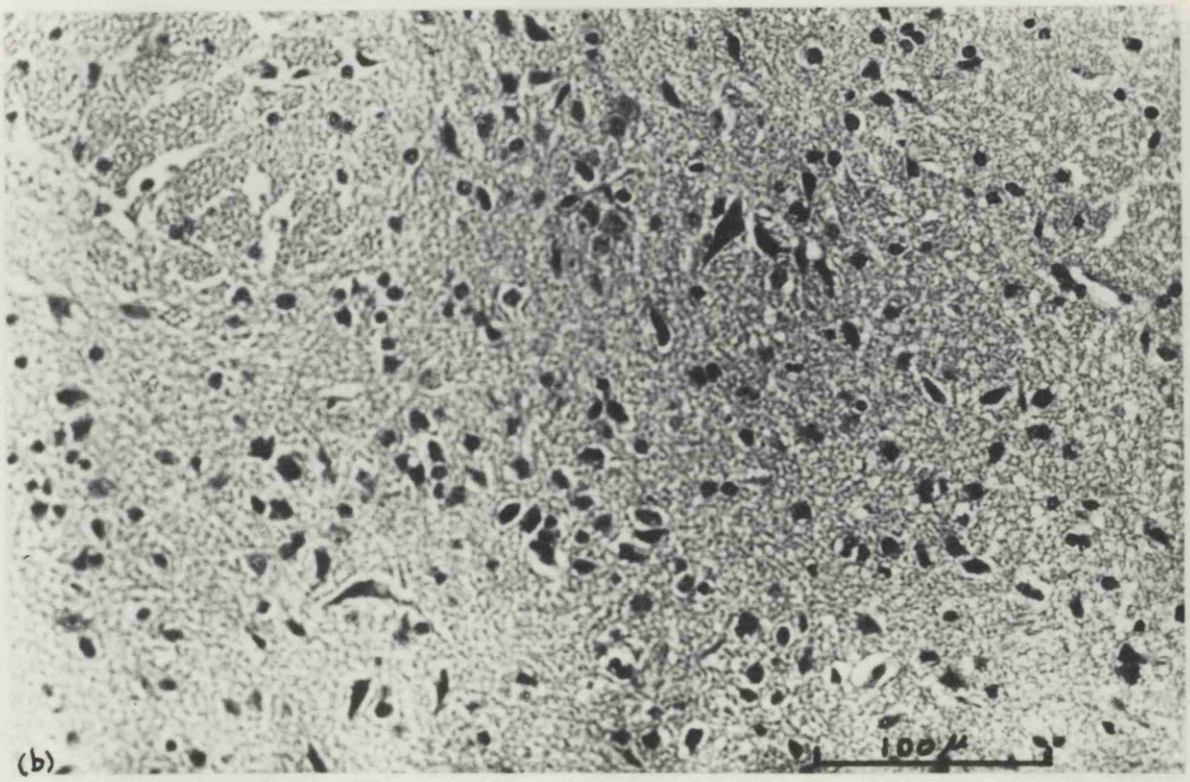
Sections of Rat Hypothalamus — LEFT SIDE



Positions of Lesions relative to Hypothalamic Nuclei.

Fig. (36.)

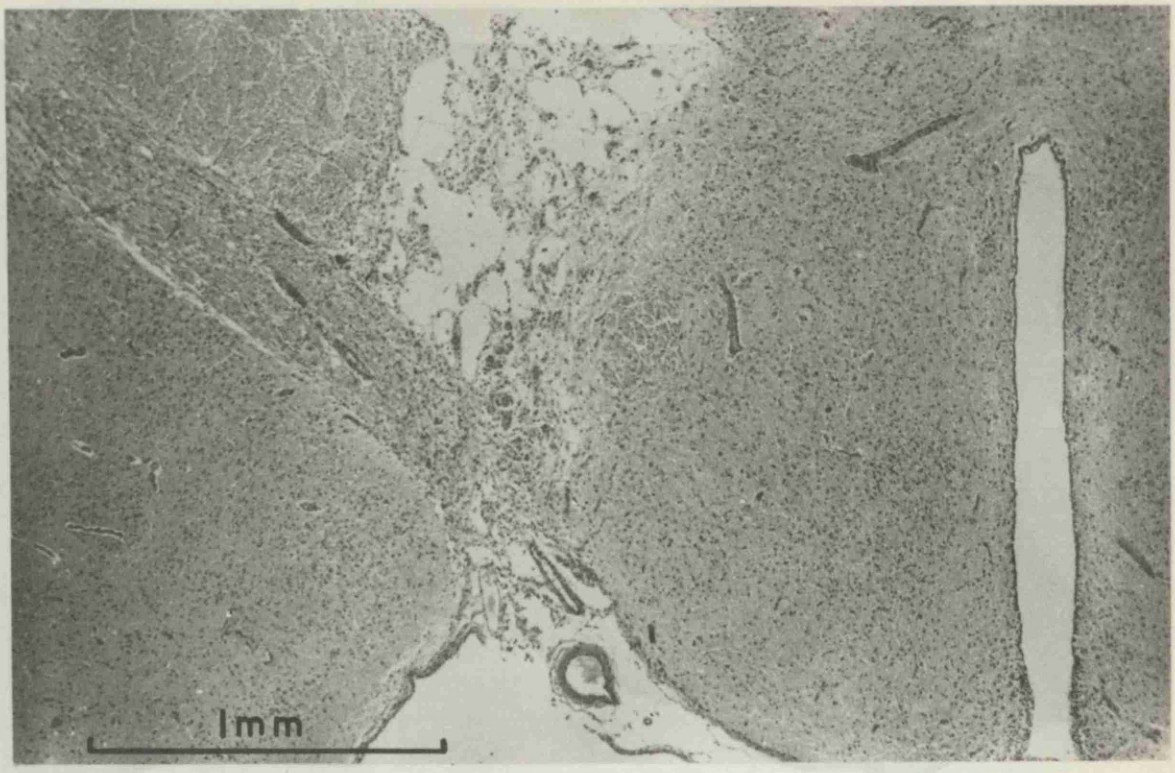




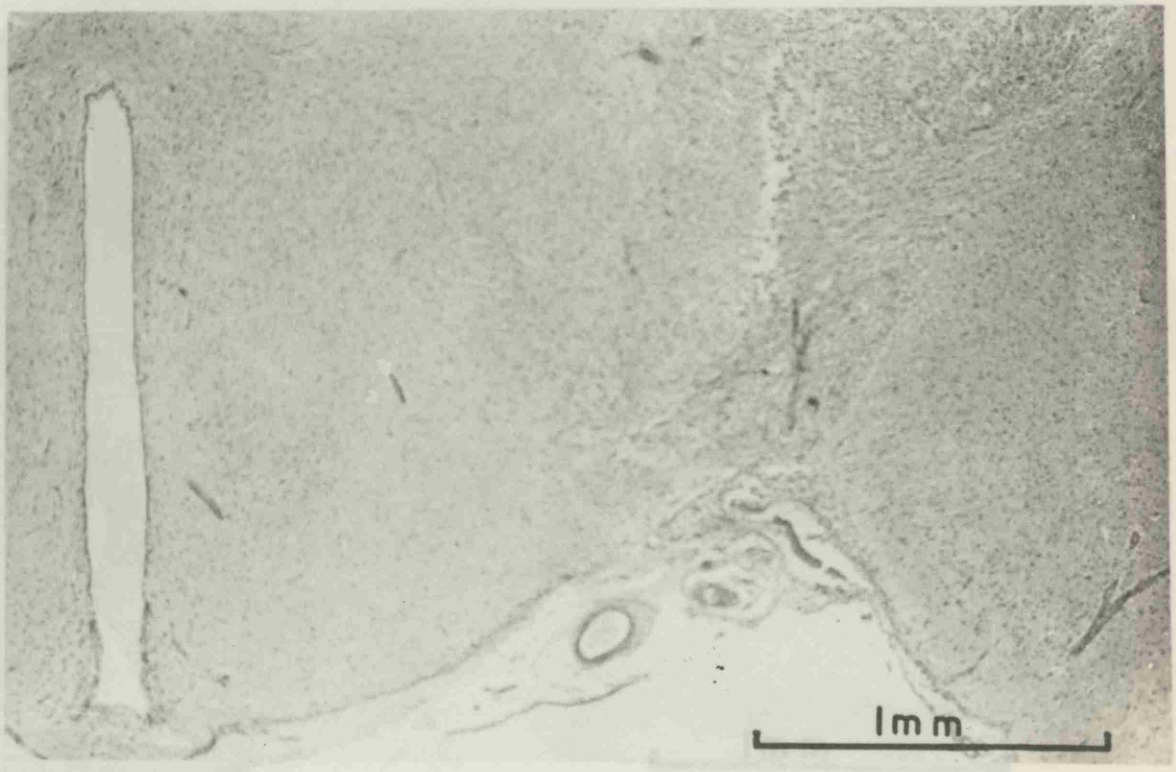
a) Left Side of the Hypothalamus of a Rat at the Level of the Ventro-medial Nucleus.

b) High Power Field to Show Triangular Nuclei of the Lateral Area Cells near the Fornix.

Fig.(37.)



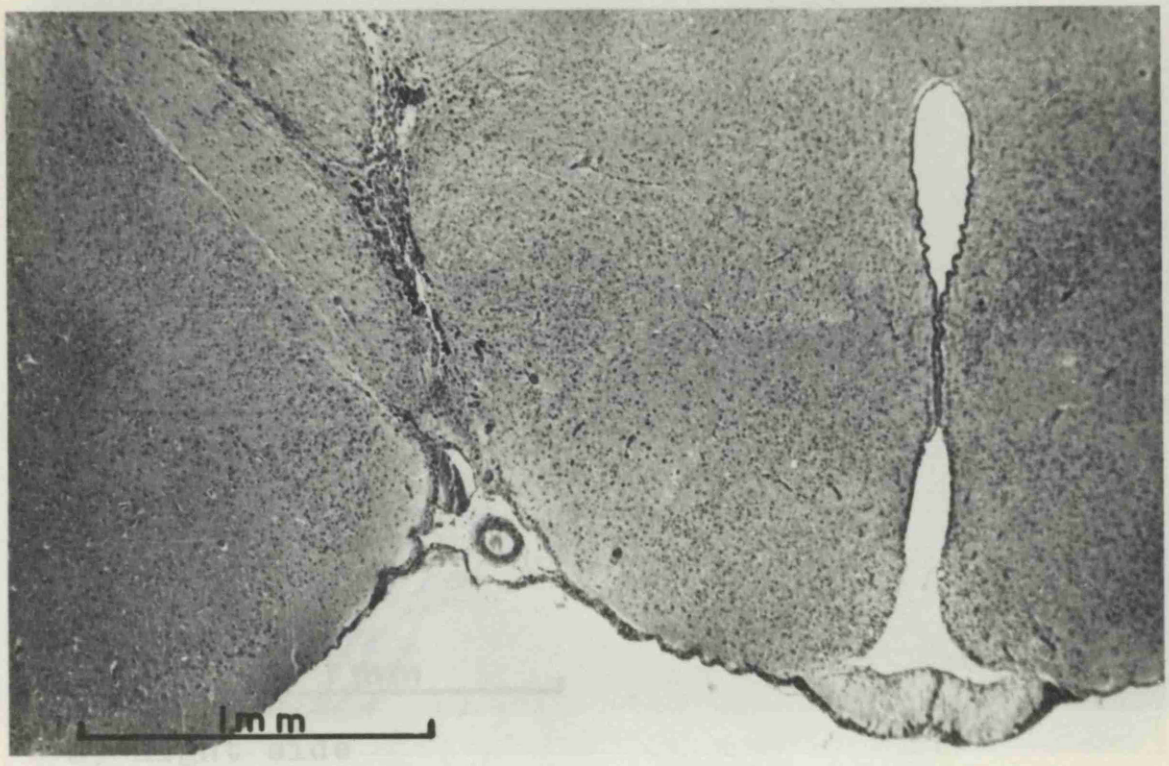
a) Right side



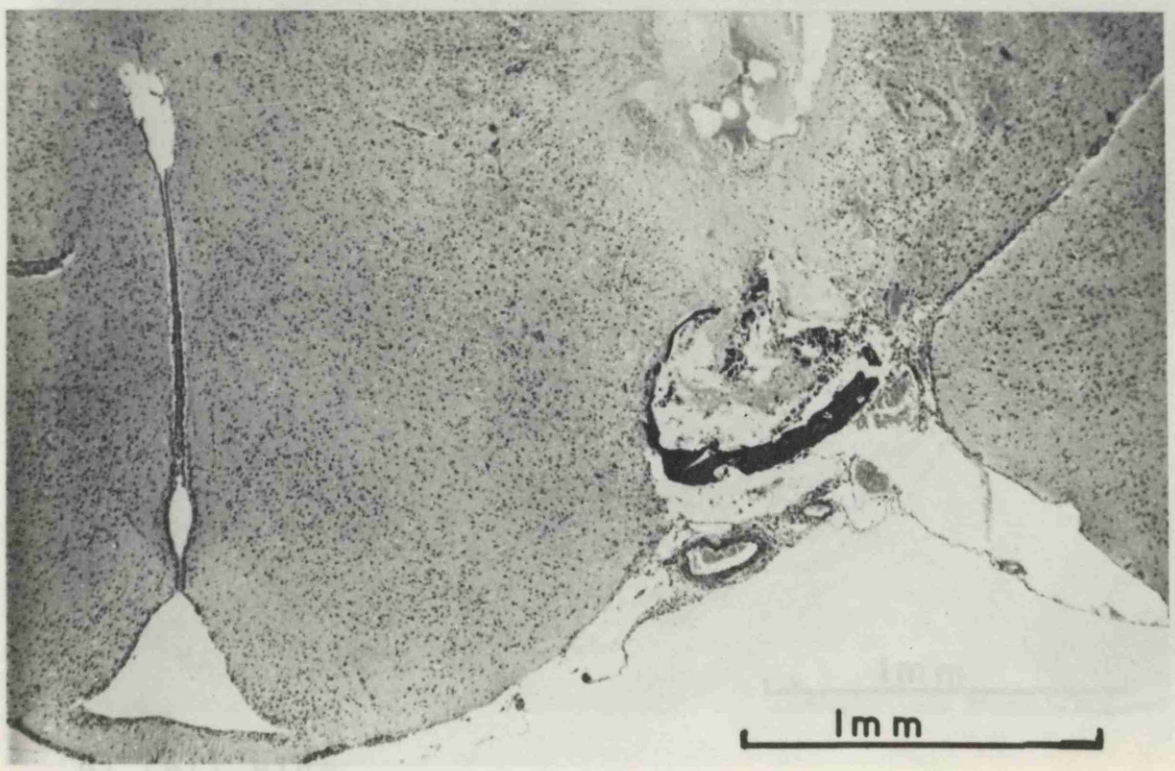
b) Left side

Photomicrograph of the Hypothalamus of Rat 1A at the Mid Level of the Ventromedial Nucleus.

Fig. (38.)



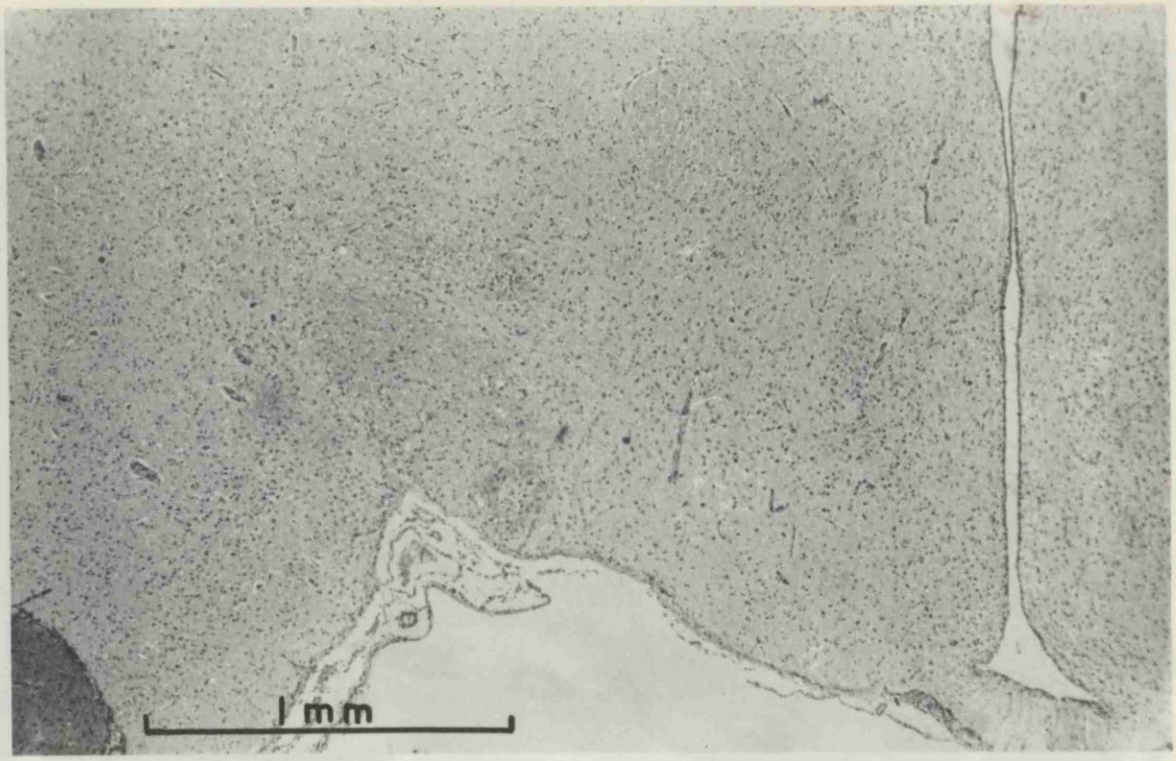
a)Right side



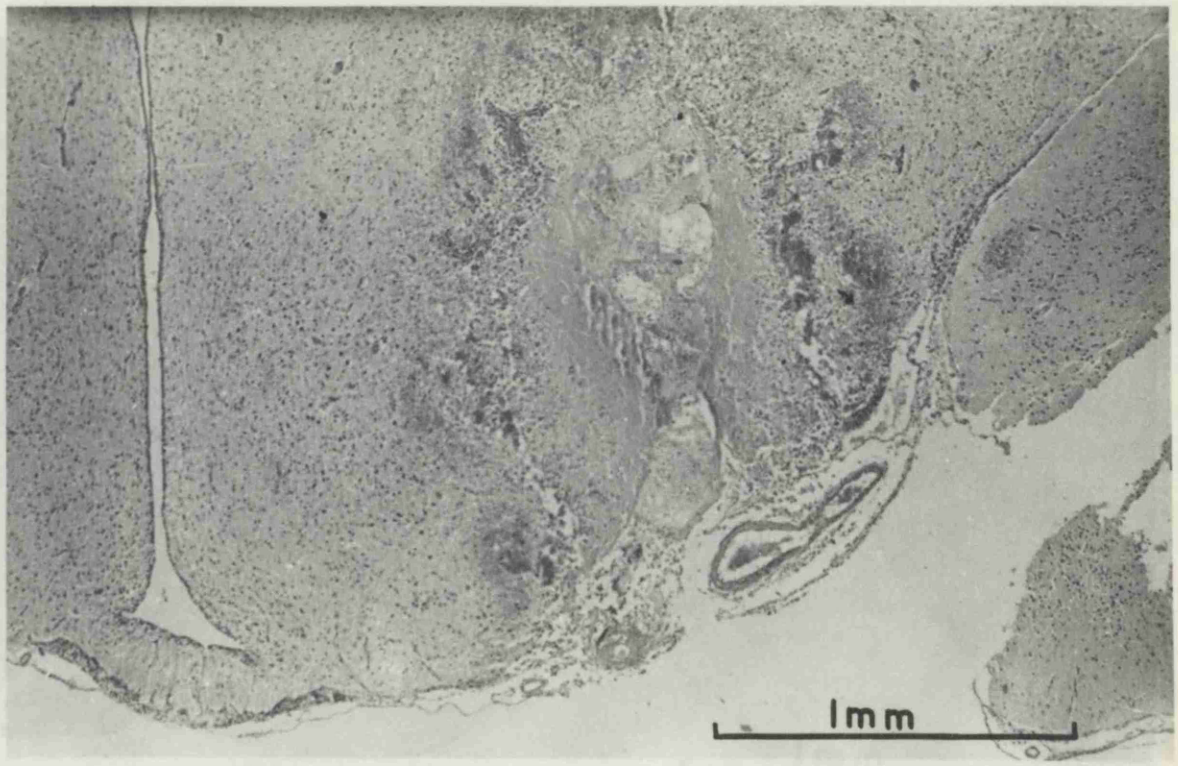
b) Left side

Photomicrograph of the Hypothalamus of Rat 7A at the Mid Level of the Ventromedial Nucleus.

Fig. (39.)



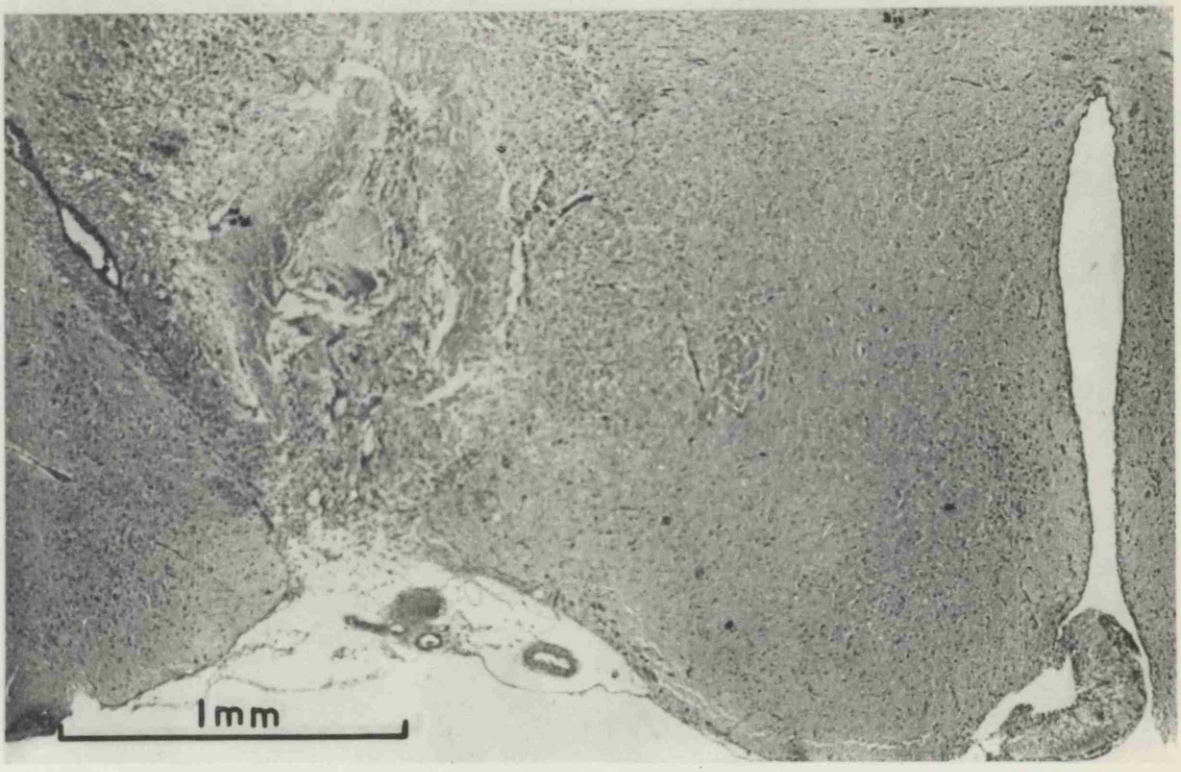
a) Right side



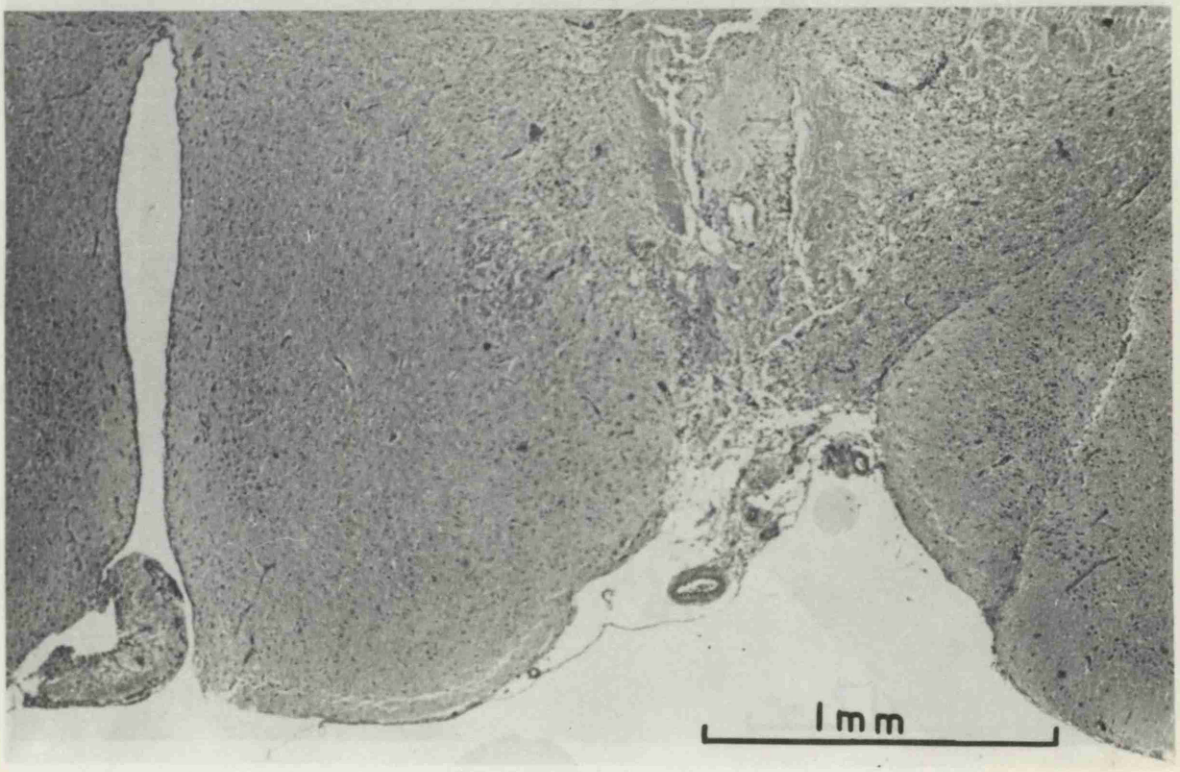
b) Left side

Photomicrograph of the Hypothalamus of Rat 8A at the Mid Level of the Ventromedial Nucleus.

Fig. (40.)



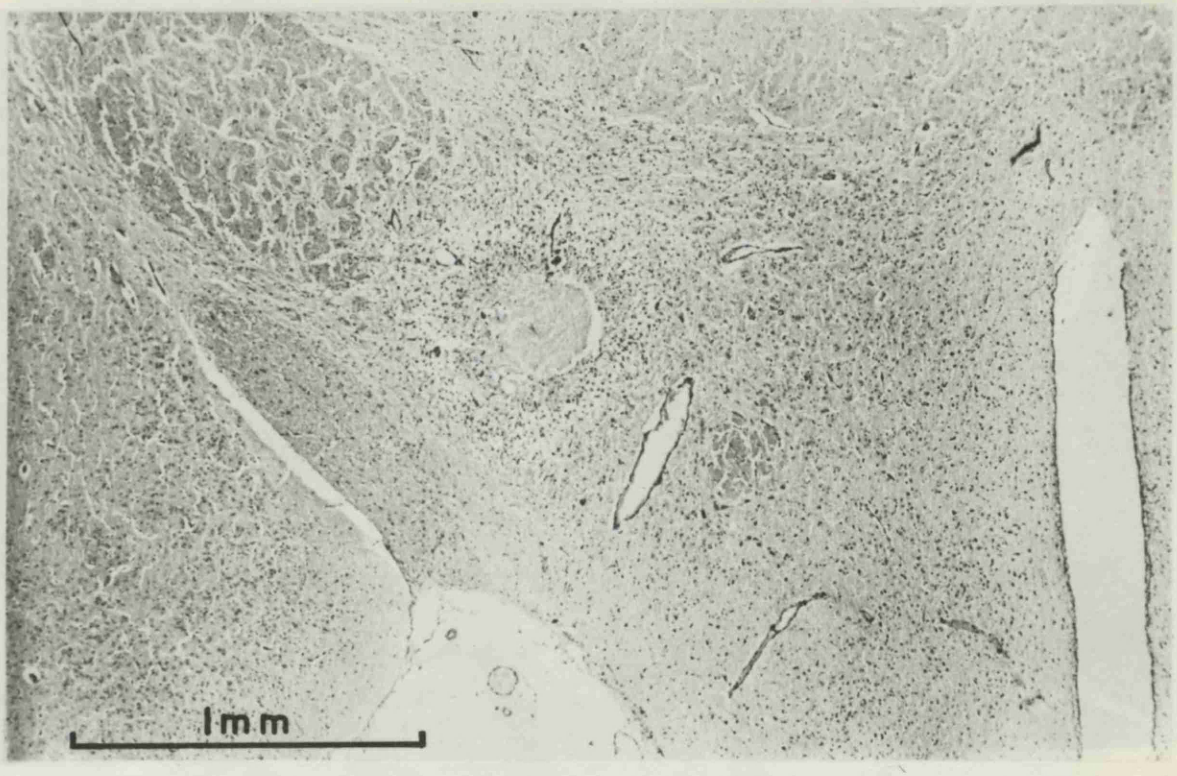
a) Right side



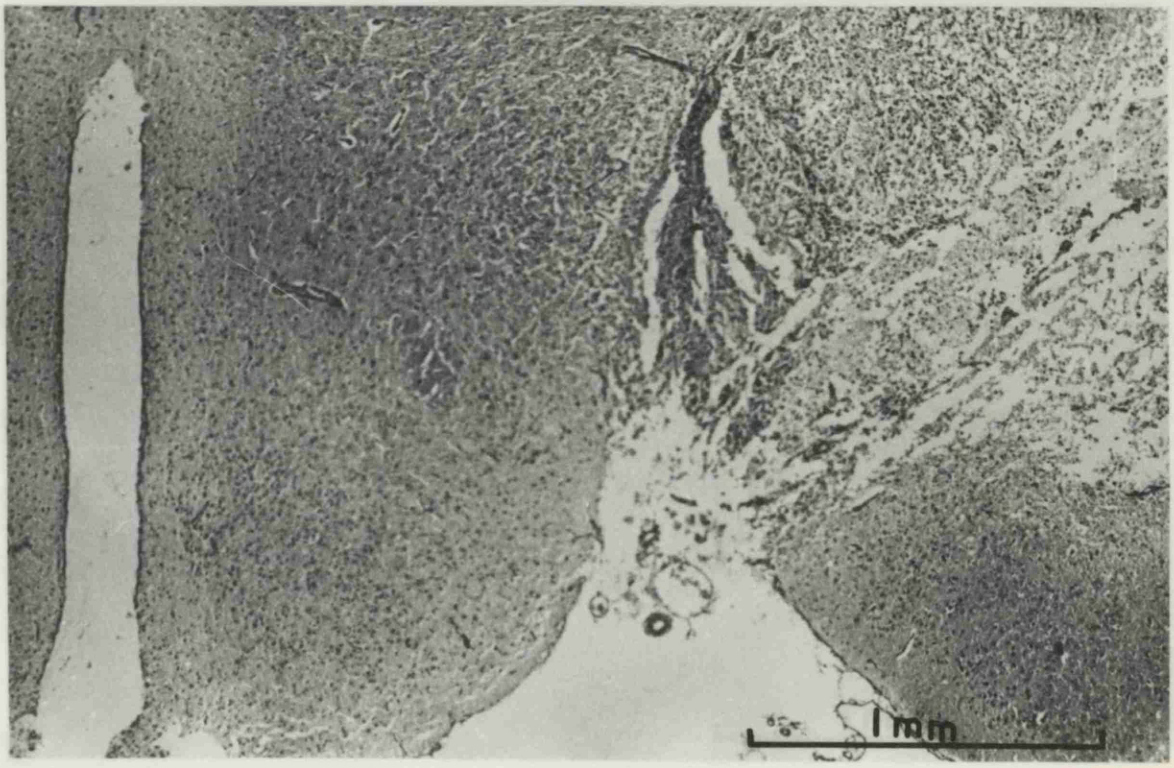
b) Left side

Photomicrograph of the Hypothalamus of Rat 9A at the Mid Level of the Ventromedial Nucleus.

Fig. (41.)



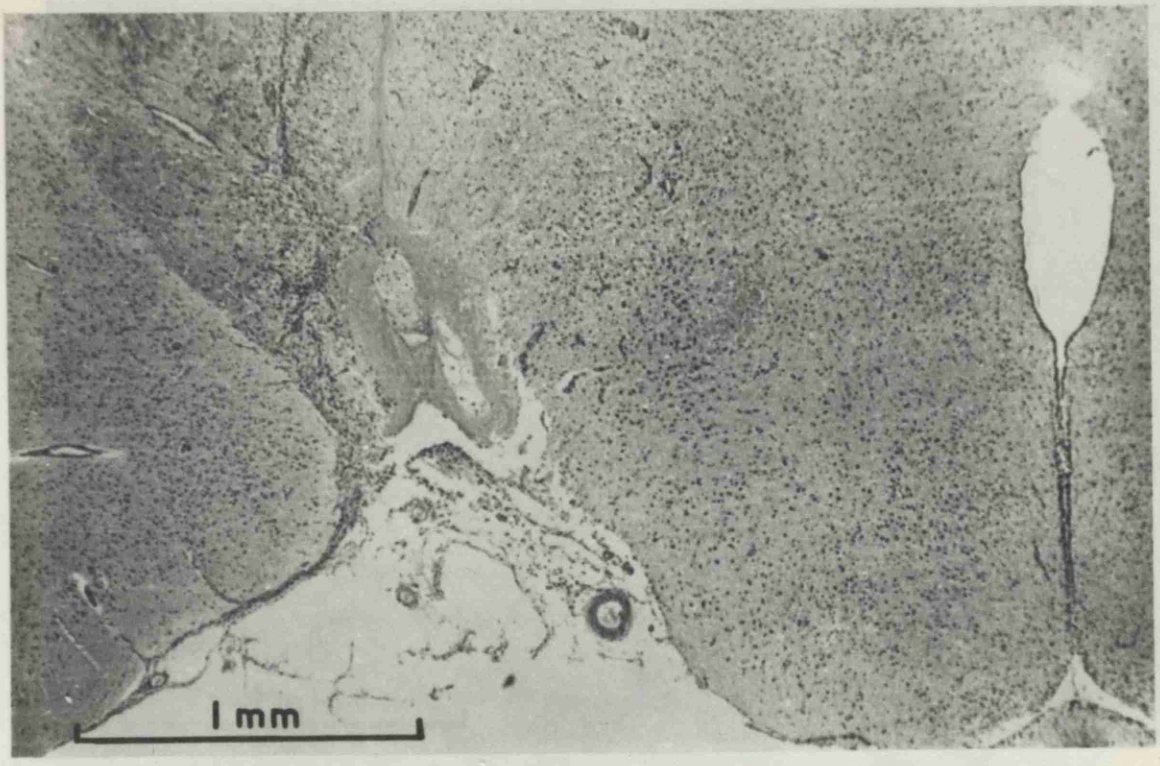
a) Right side



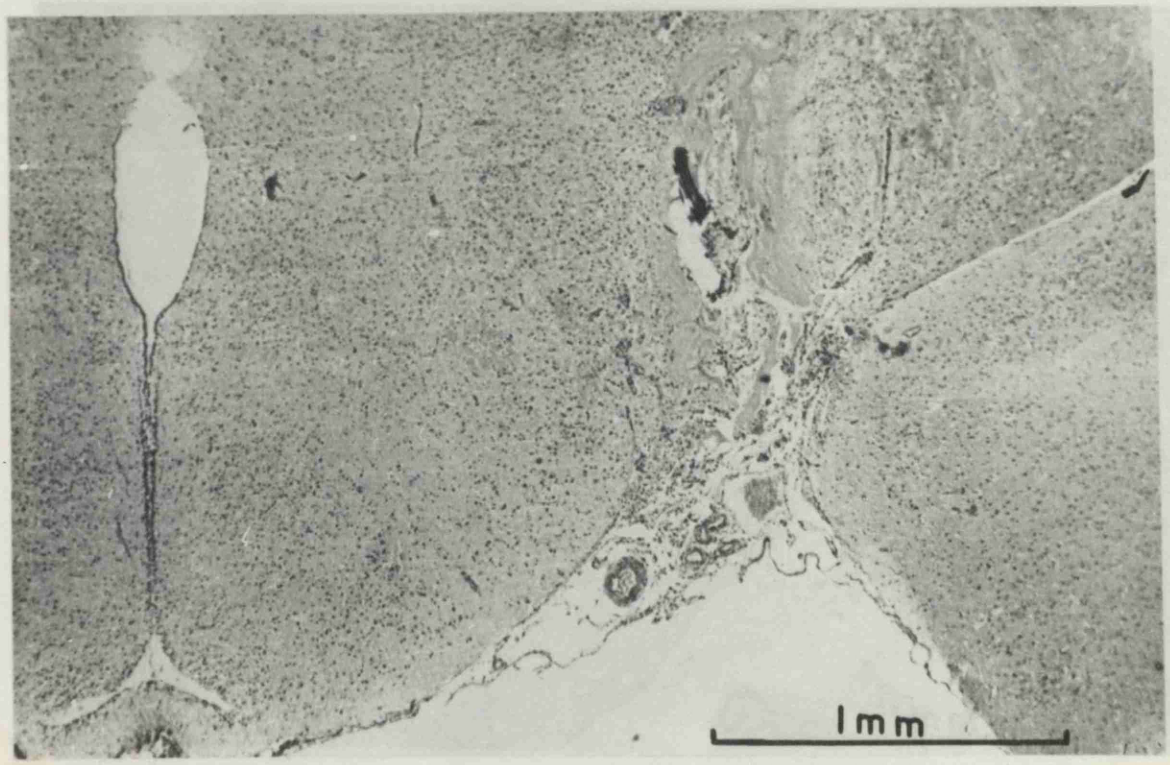
b) Left side

Photomicrograph of the Hypothalamus of Rat 10A at the Mid Level of the Ventromedial Nucleus.

Fig. (42.)



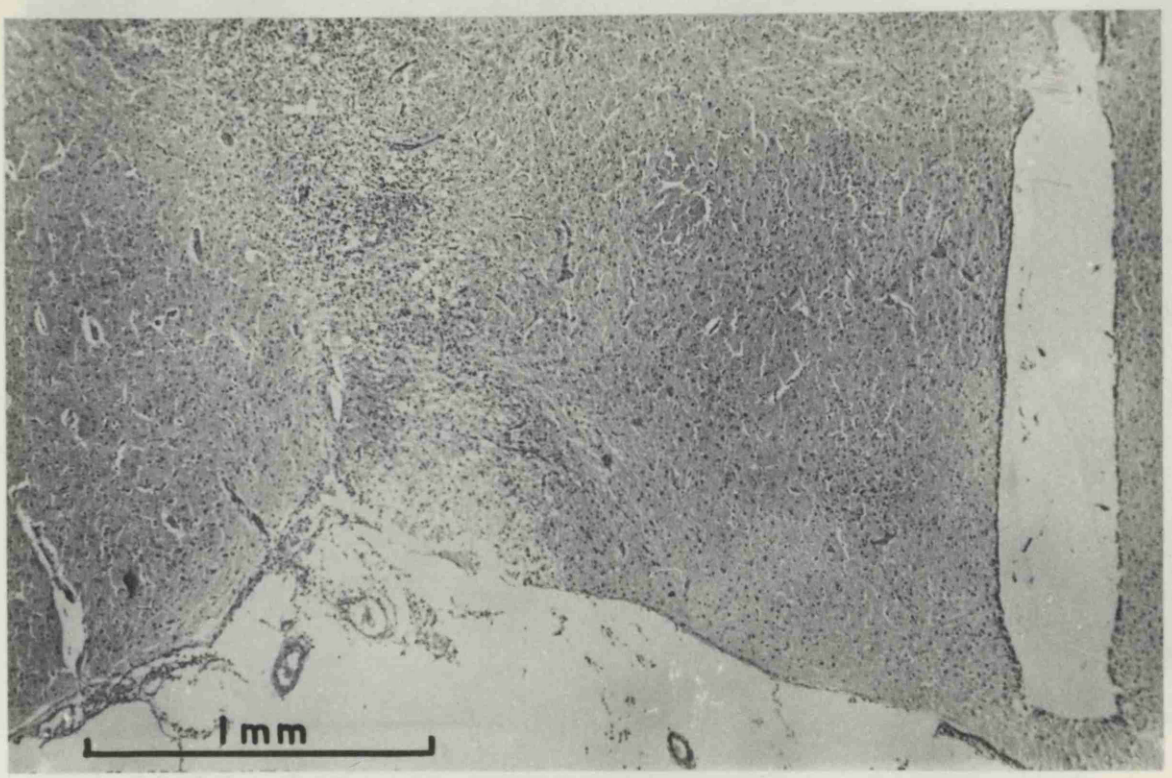
a) Right side



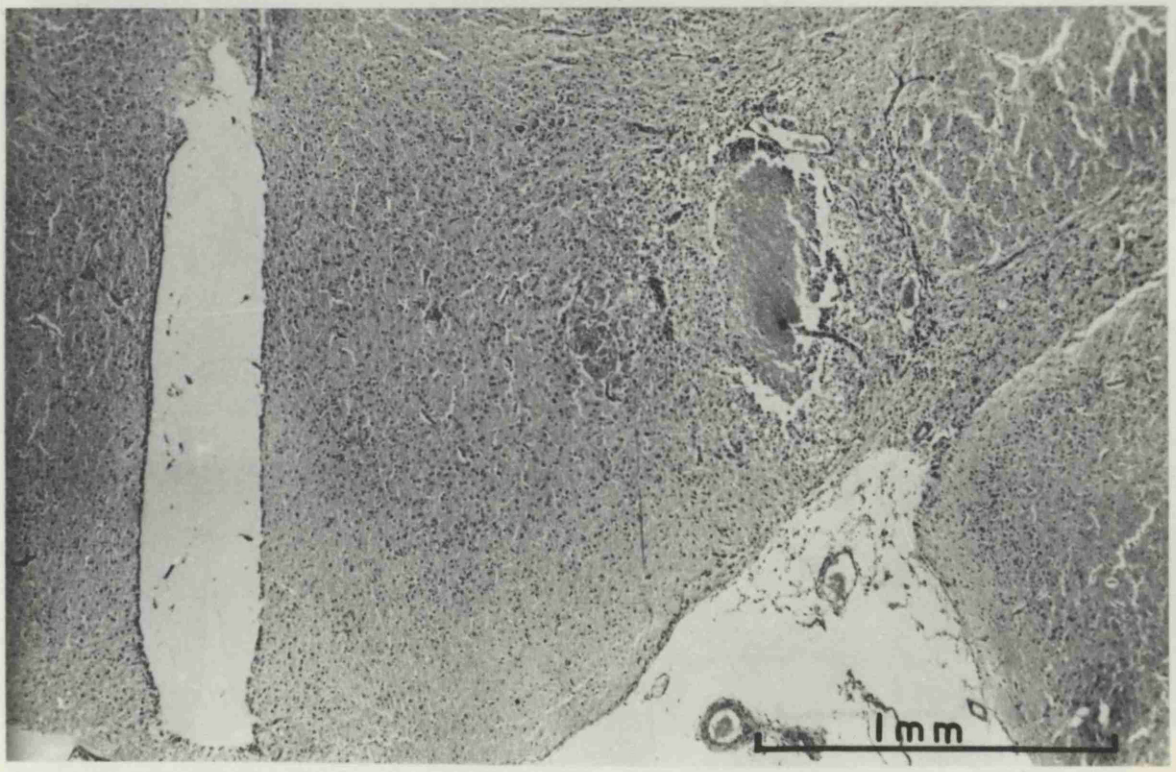
b) Left side

Photomicrograph of the Hypothalamus of Rat 12A at the Mid Level of the Ventromedial Nucleus.

Fig. (43.)



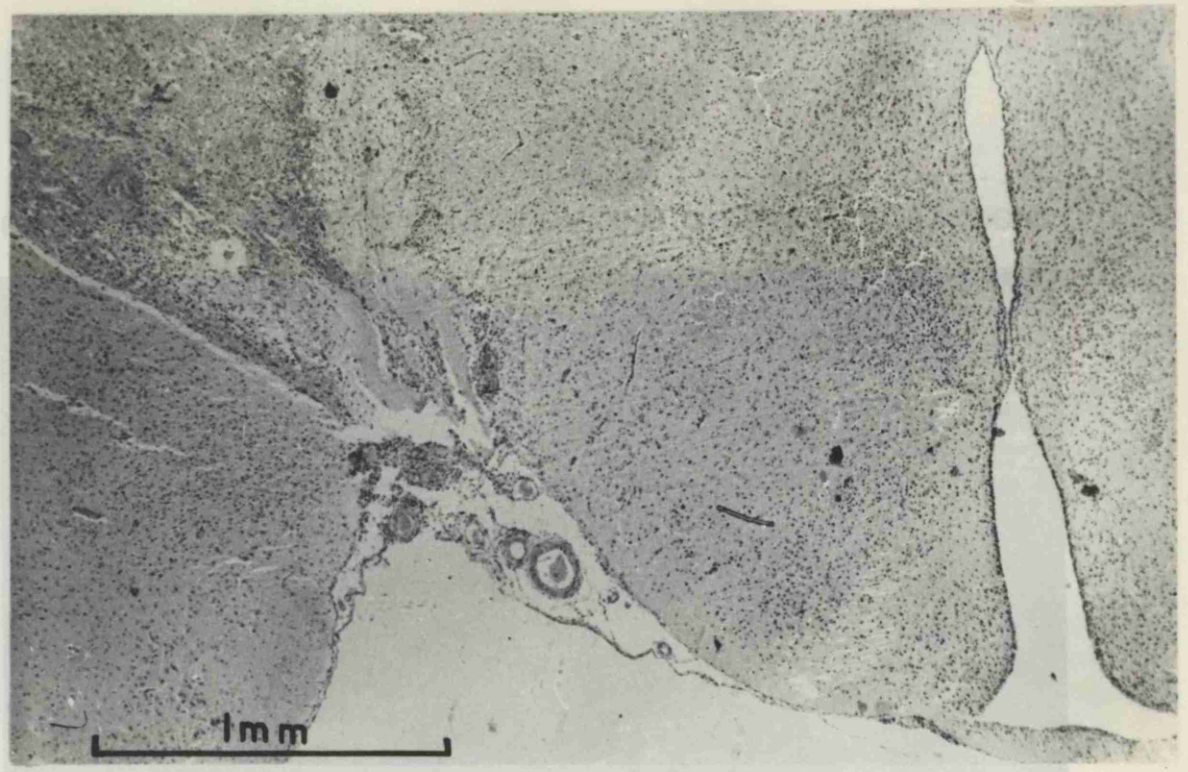
a) Right side



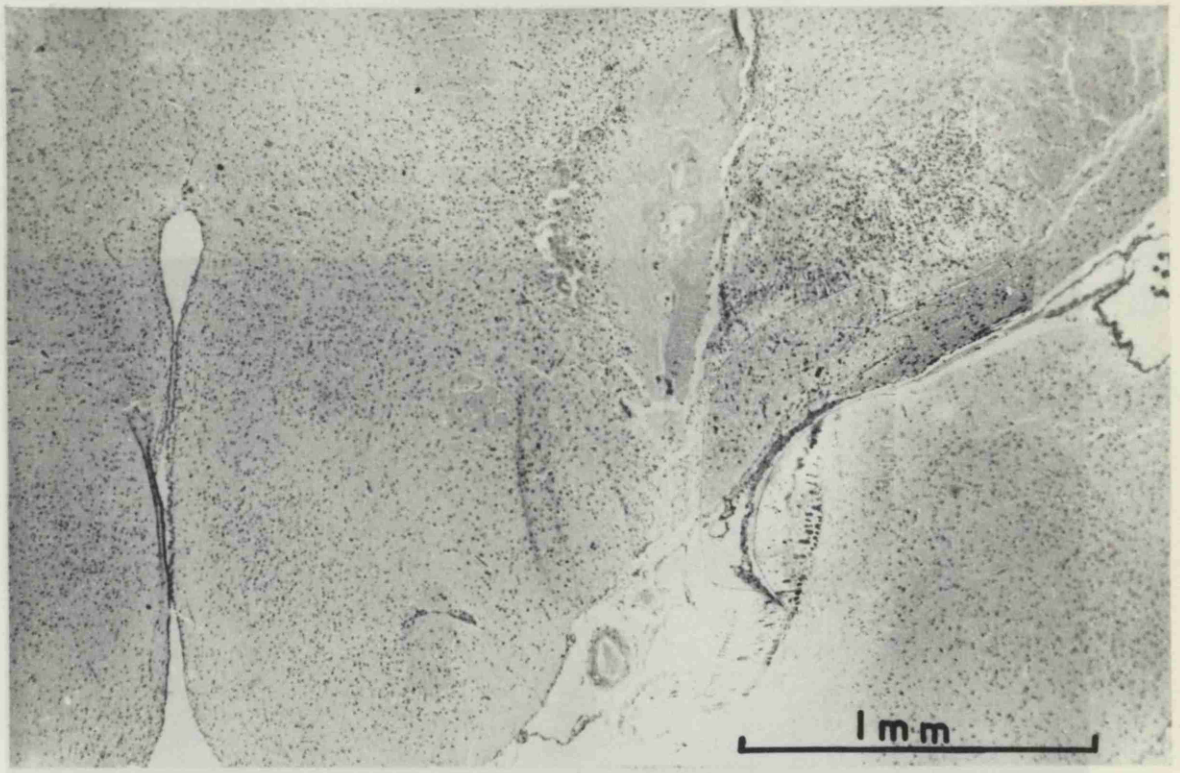
b) Left side

Photomicrograph of the Hypothalamus of Rat 13A at the Mid level of the Ventromedial Nucleus.

Fig. (44.)



a) Right side

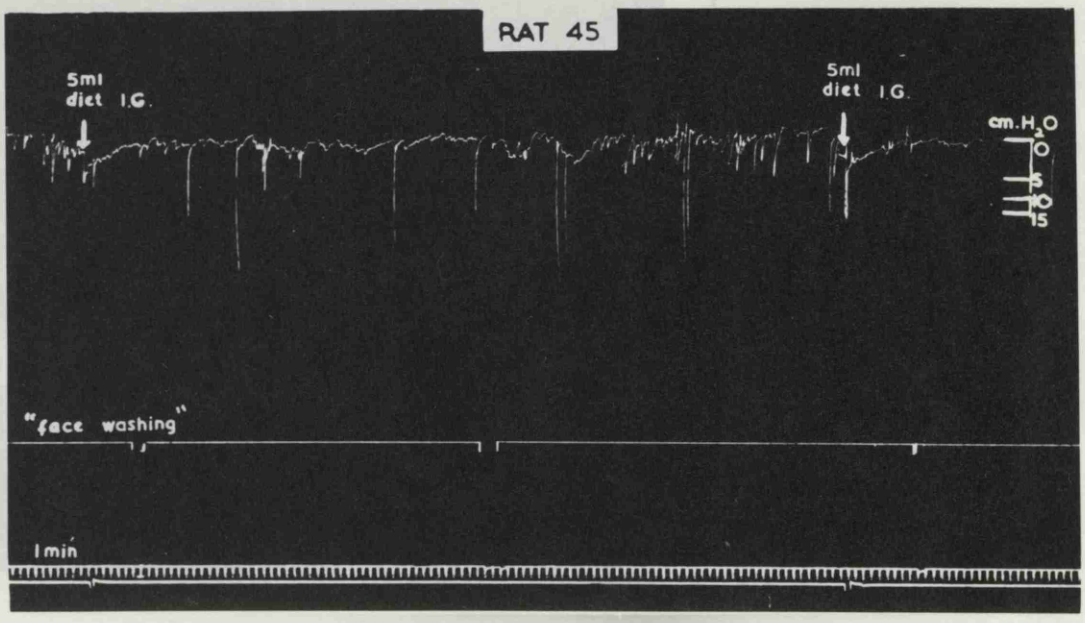


b) Left side

Photomicrograph of the Hypothalamus of Rat 15A at the Mid Level of the Ventromedial Nucleus.

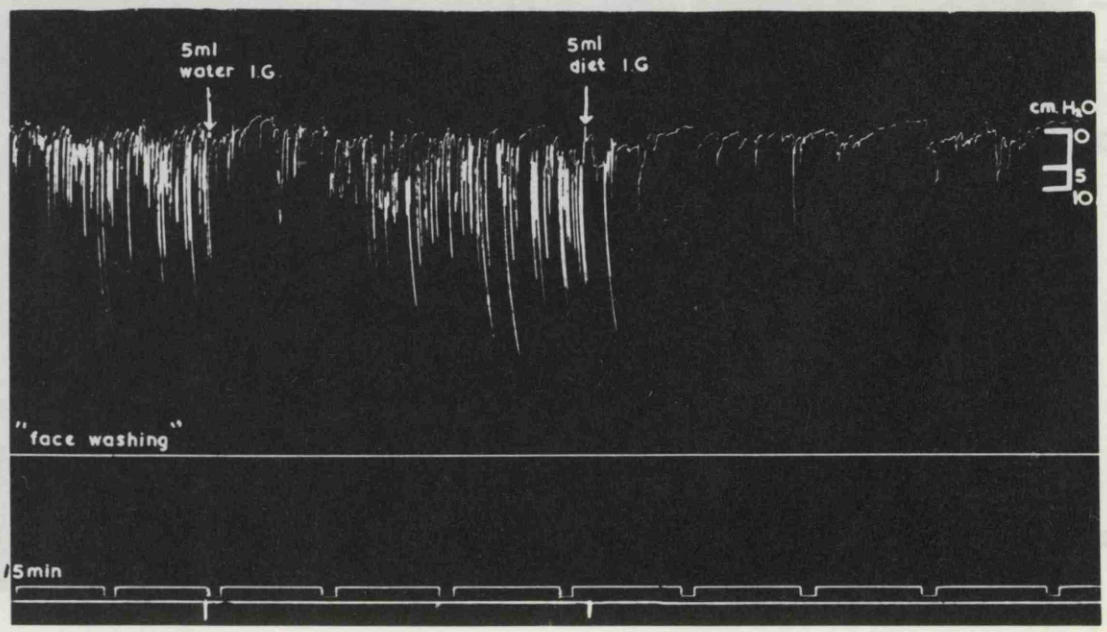
Fig. (45.)

EFFECT OF INTRAGASTRIC INJECTION ON GASTRIC CONTRACTIONS



(a.)

EFFECT OF INTRAGASTRIC INJECTION ON GASTRIC CONTRACTIONS AFTER LATERAL LESIONS RAT 45 FASTED 24hrs.



(b.)

Fig. (46.)

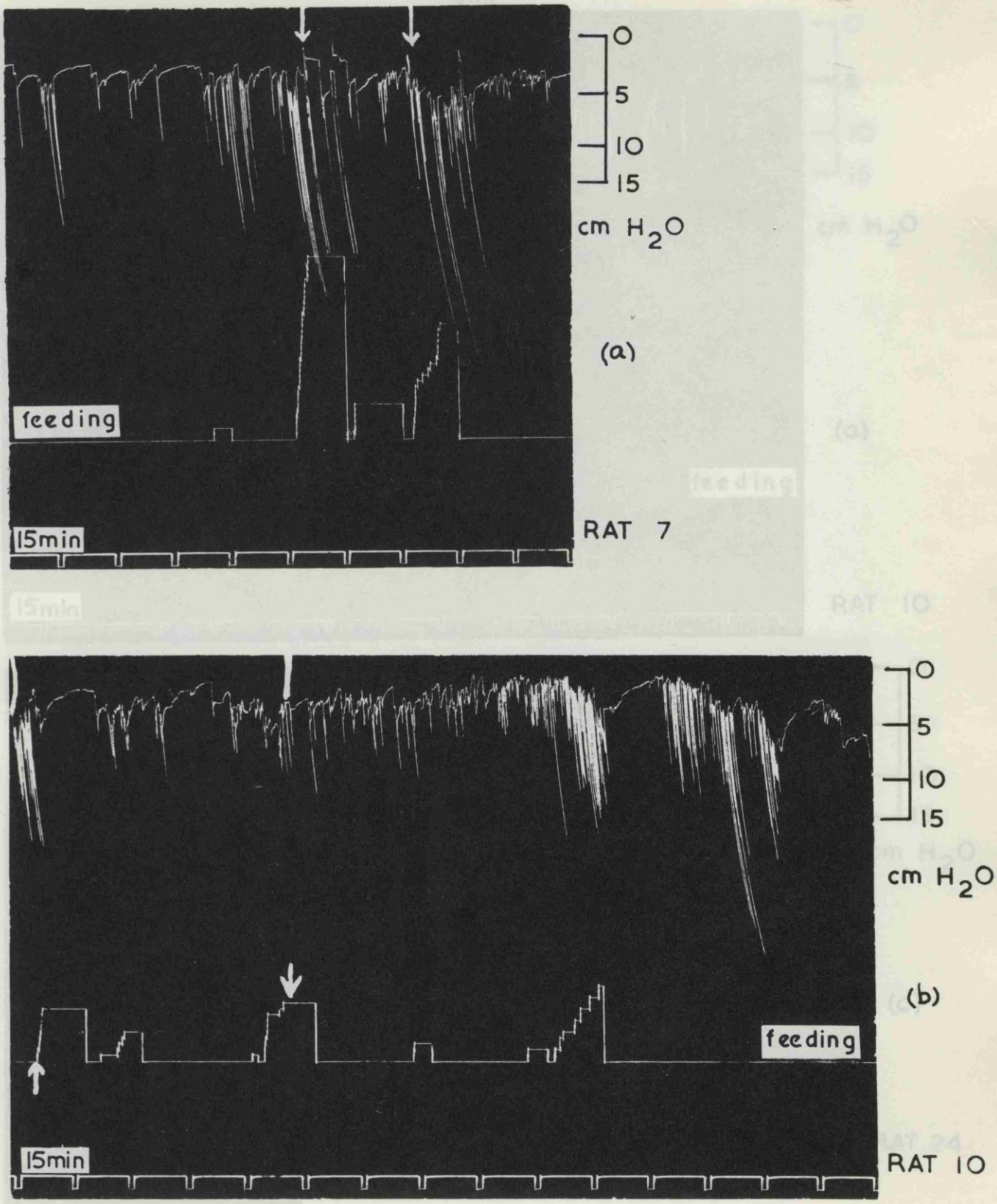


Fig. 47. Normal gastric contractions after intra-gastric feeding.

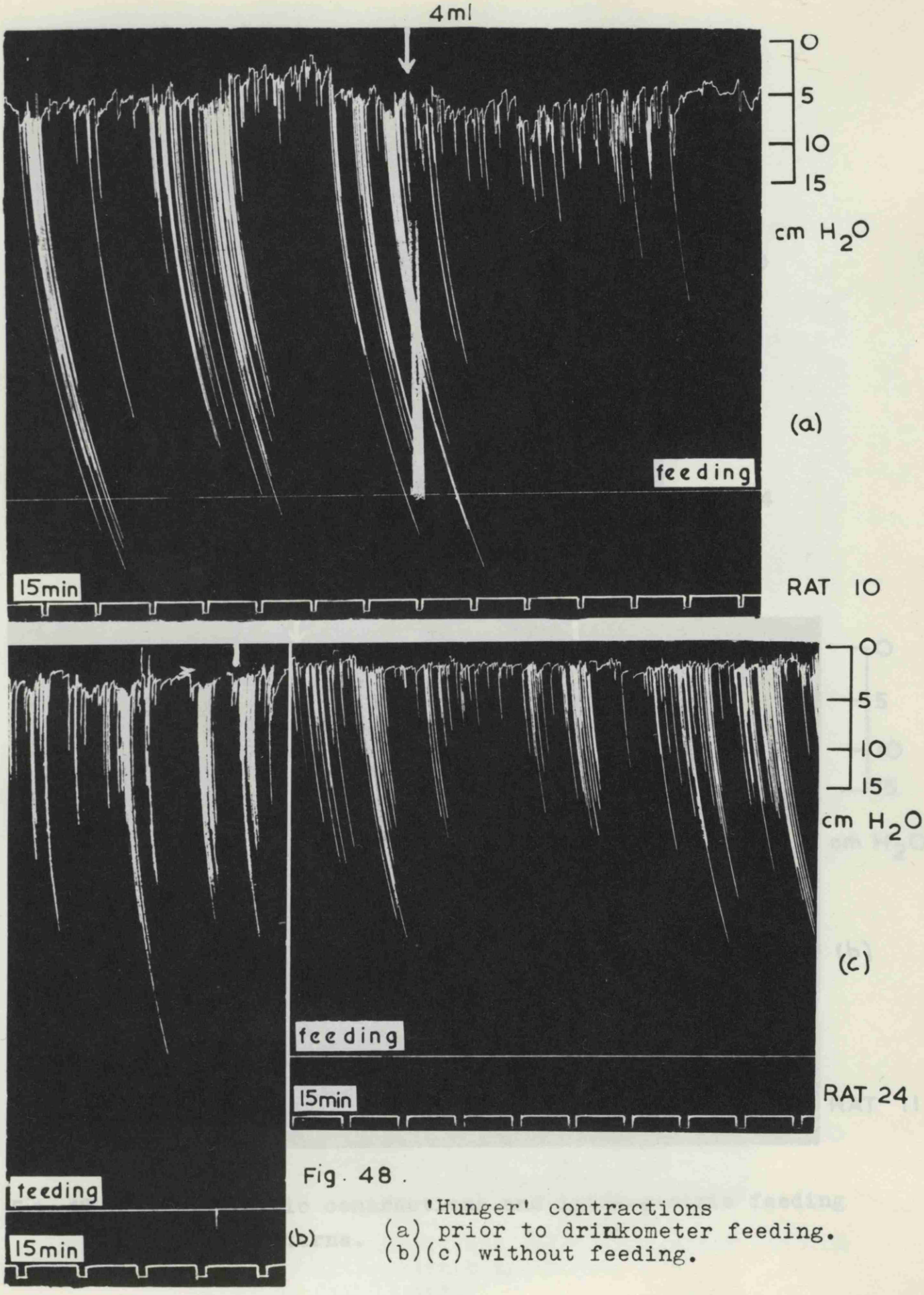


Fig. 48.

'Hunger' contractions
(a) prior to drinkometer feeding.
(b)(c) without feeding.

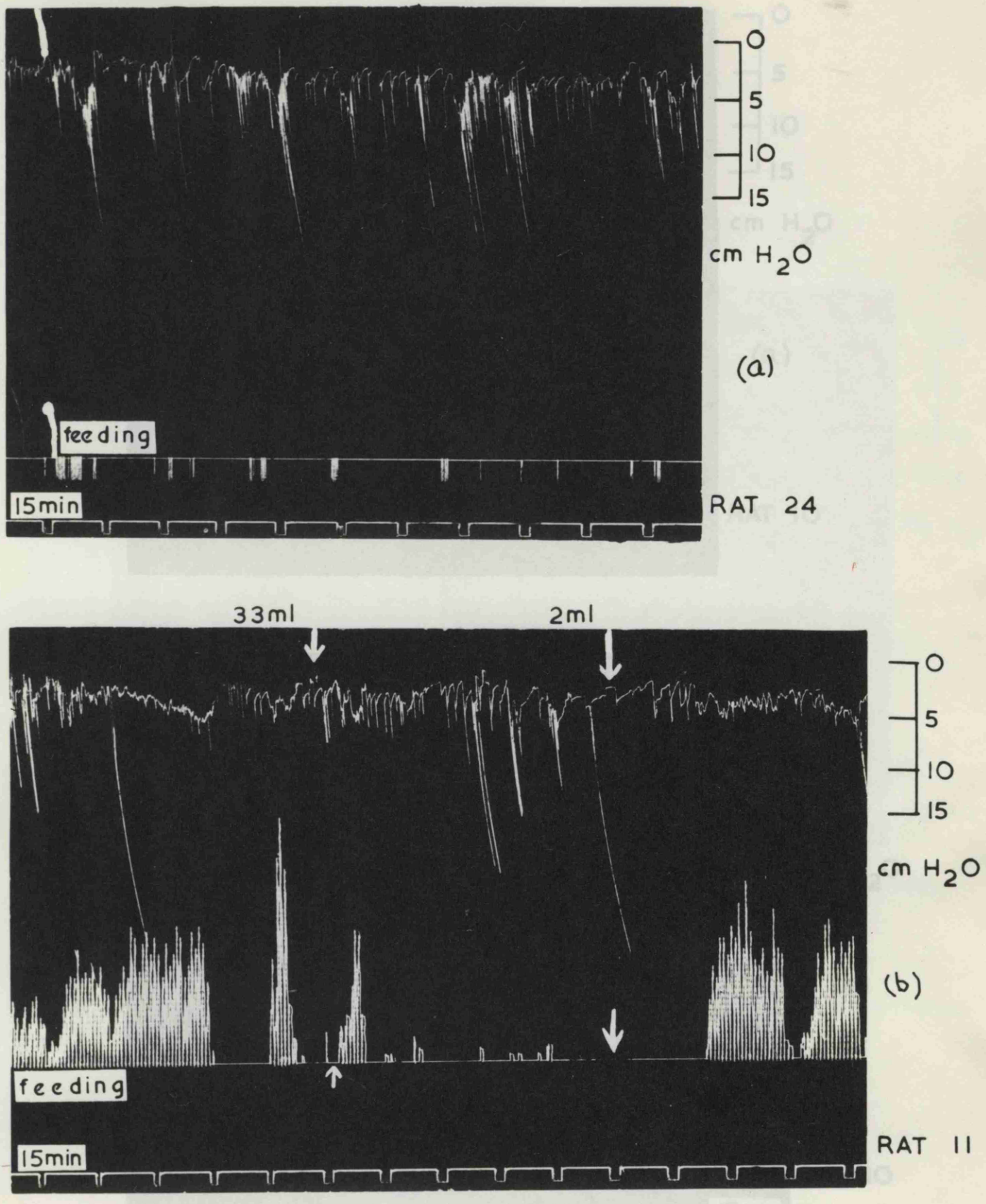


Fig. 49 . (a)(b) Gastric contractions and intra-gastric feeding patterns.

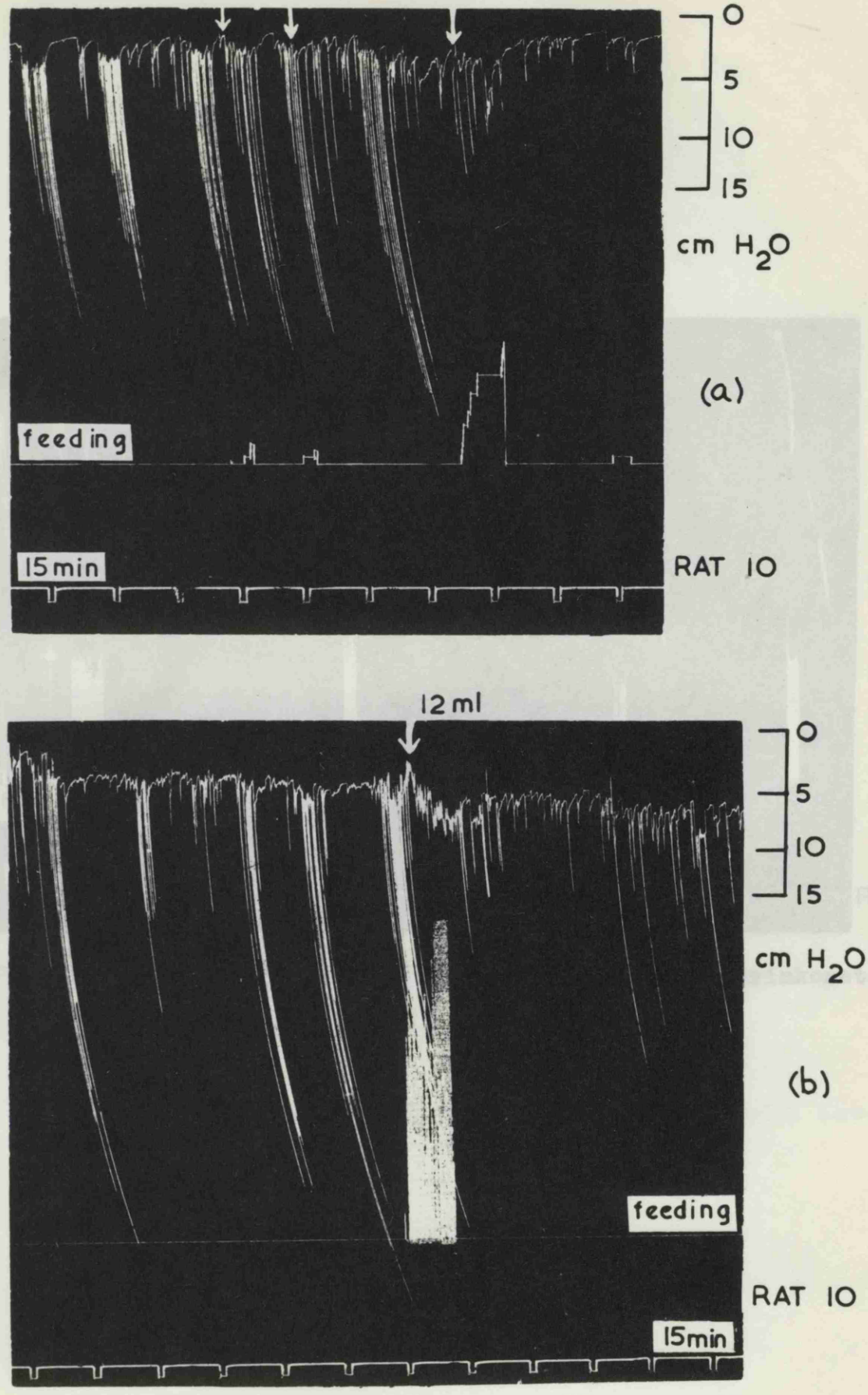


Fig. 50 'Hunger' contractions before and after feeding
(a) by intragastric injection
(b) by drinkometer.

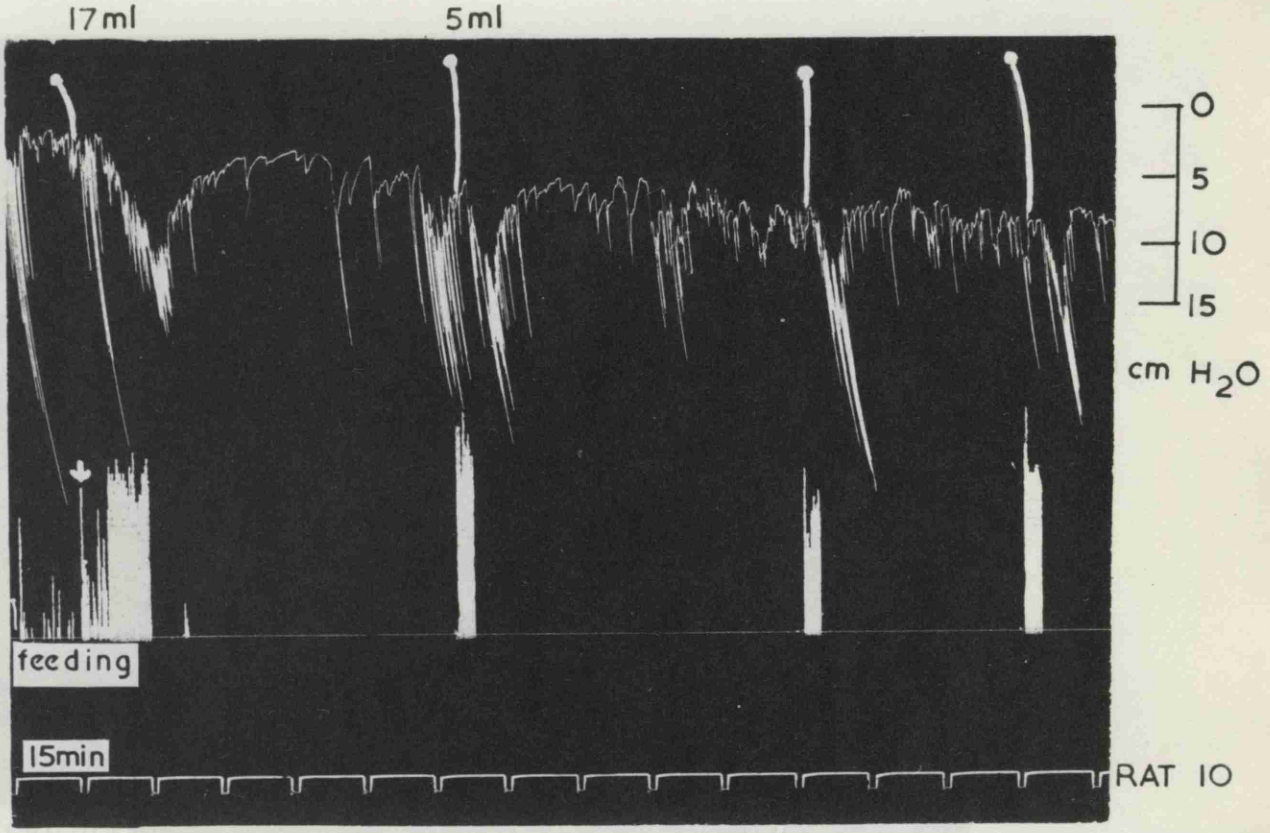


Fig. 51. Increase in gastric tone after feeding by drinkometer.

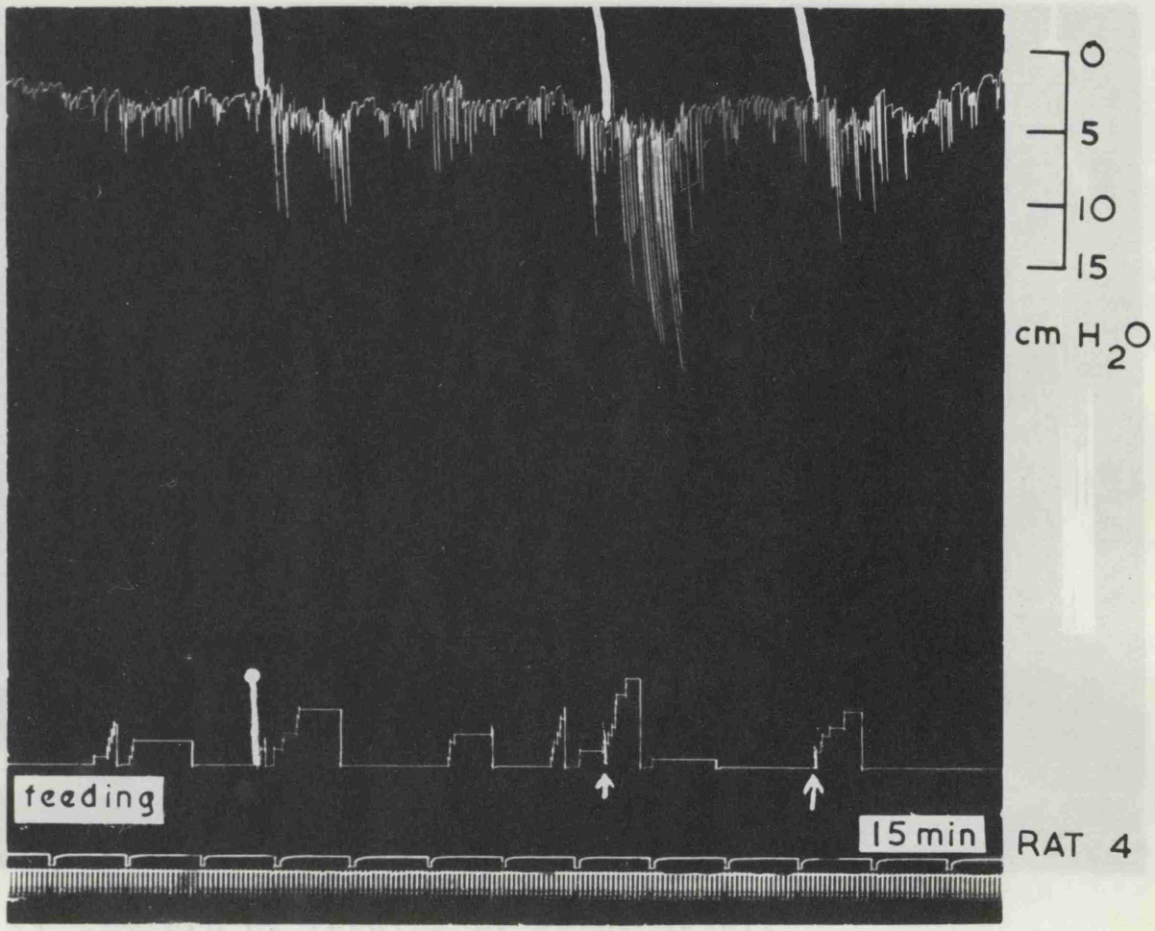


Fig. 52.

Increase in gastric contractions after intra-gastric feeding

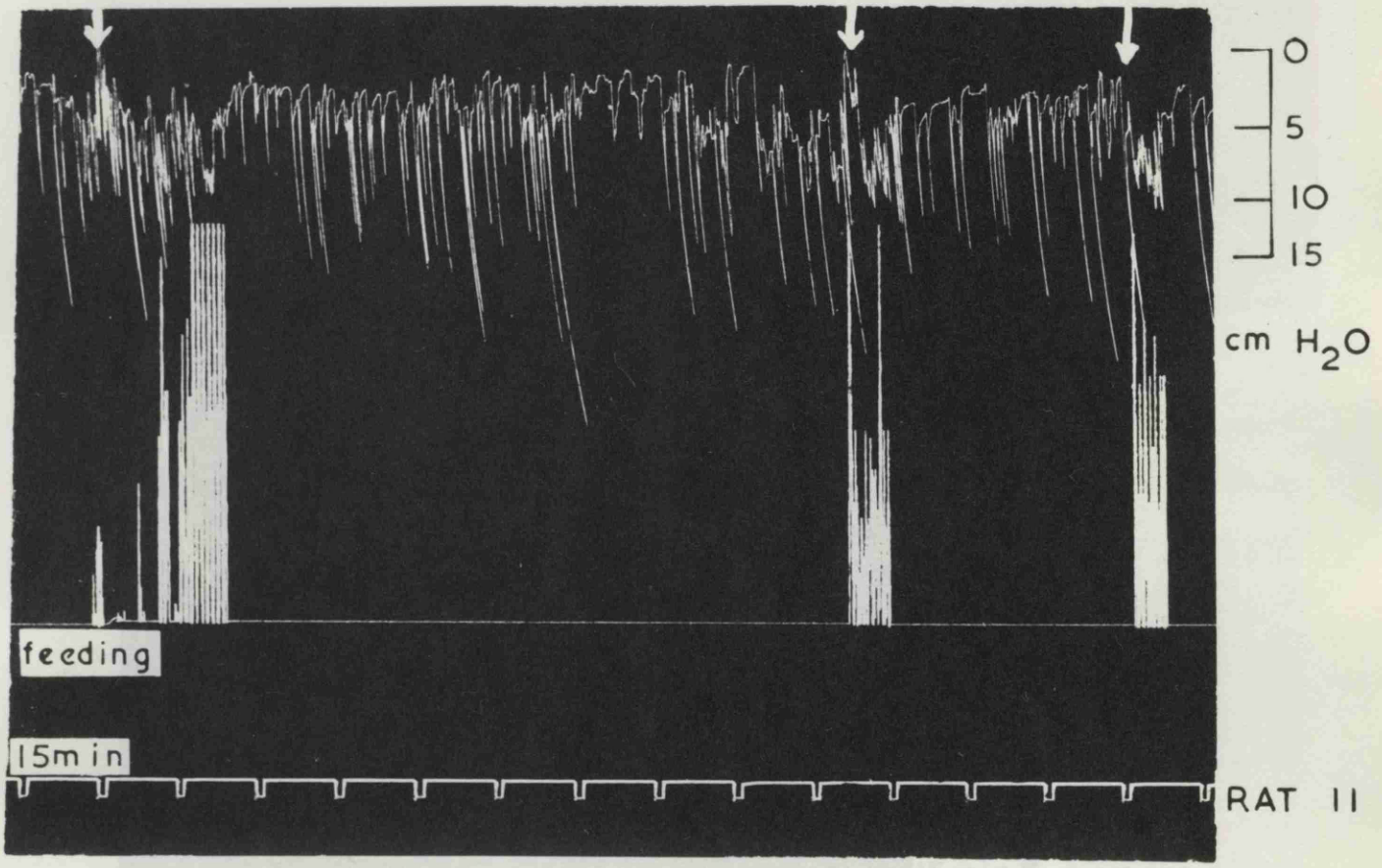


Fig. 53. Pattern of feeding by drinkometer.

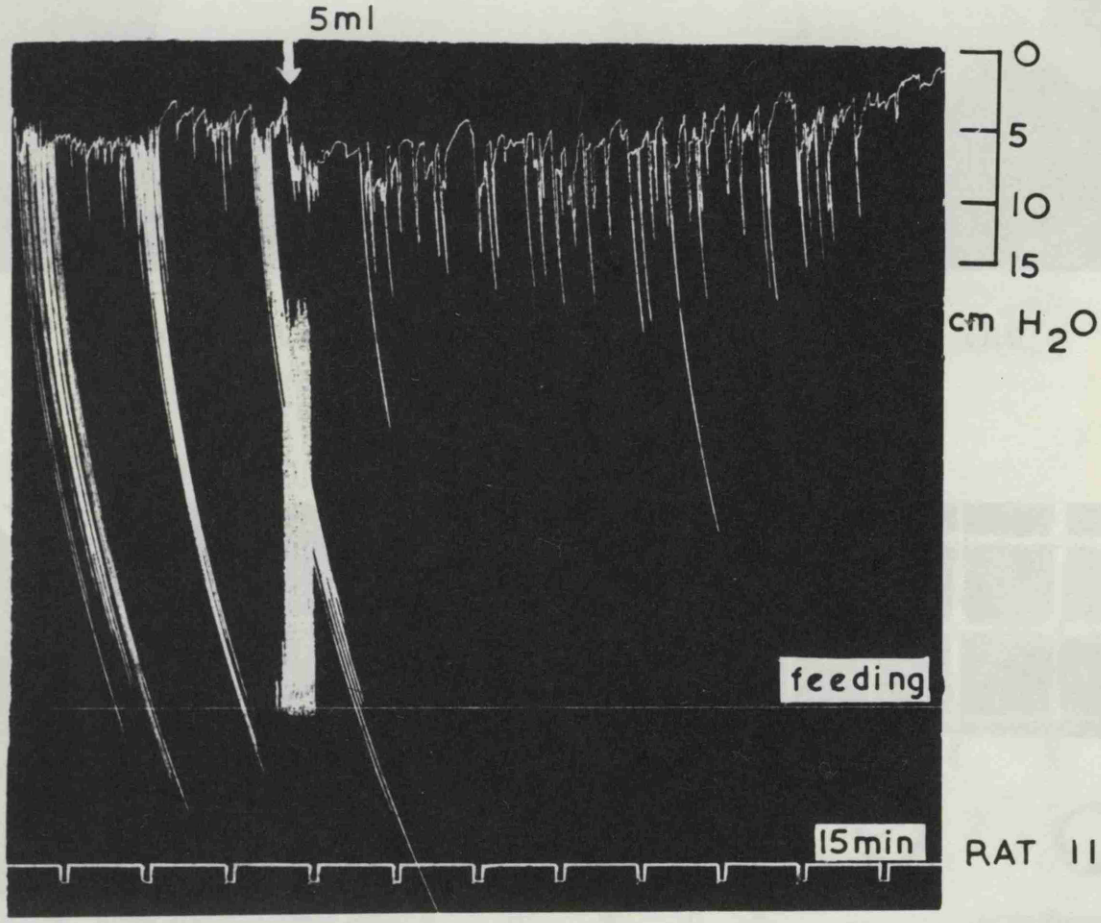
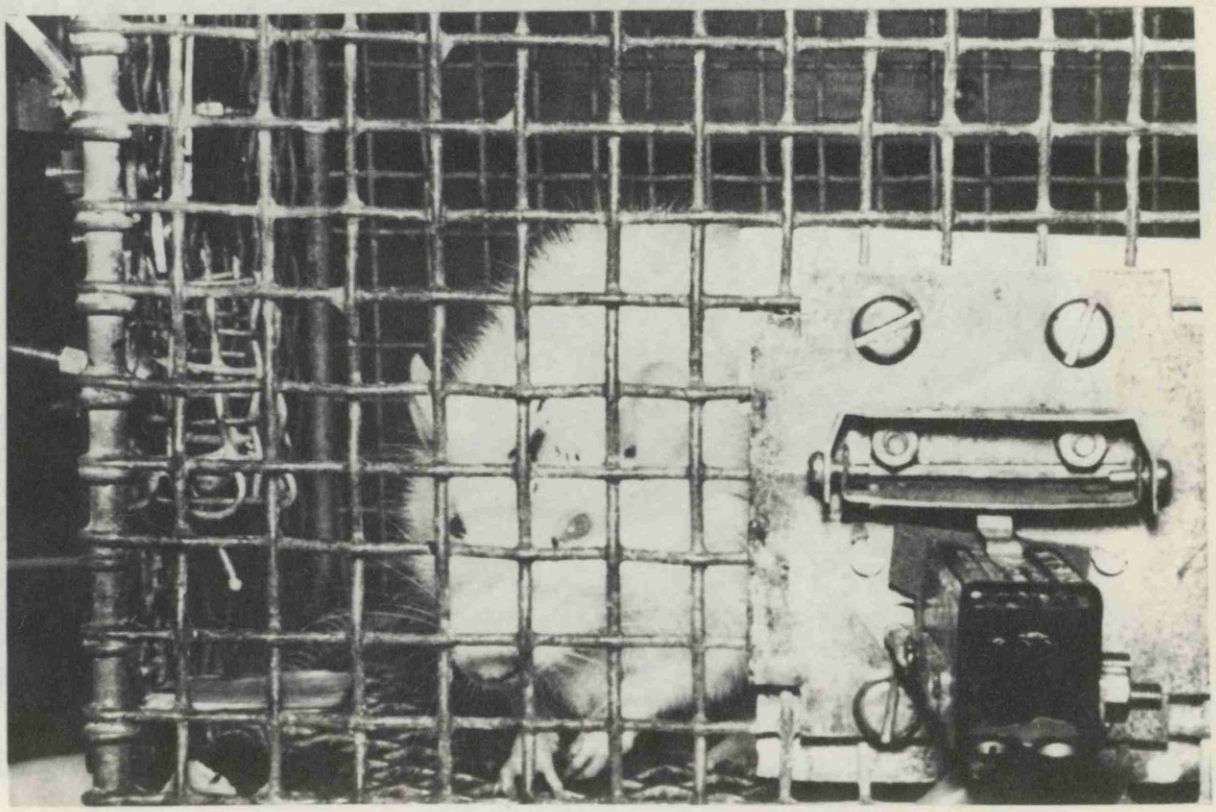


Fig. 54 Feeding by drinkometer after 'hunger' contractions.

Crouch Posture of a Rat with Lesions in the Lateral Hypothalamus.

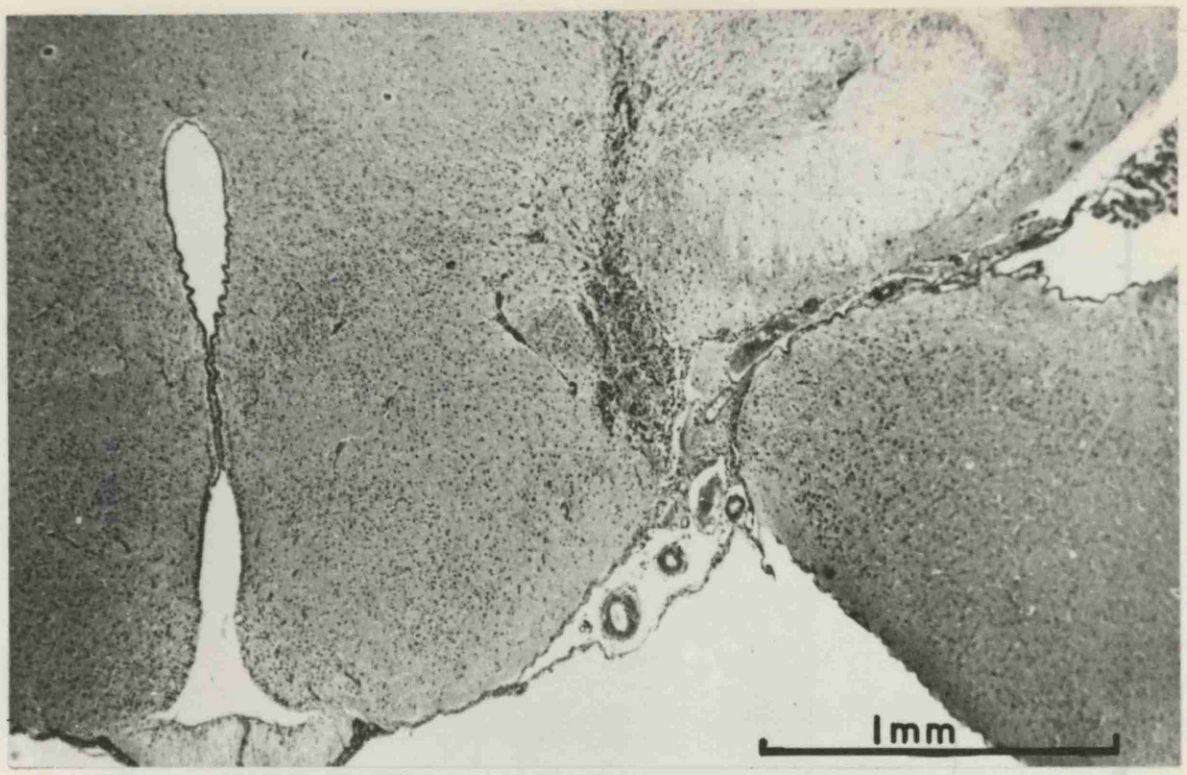
Fig. (55.)



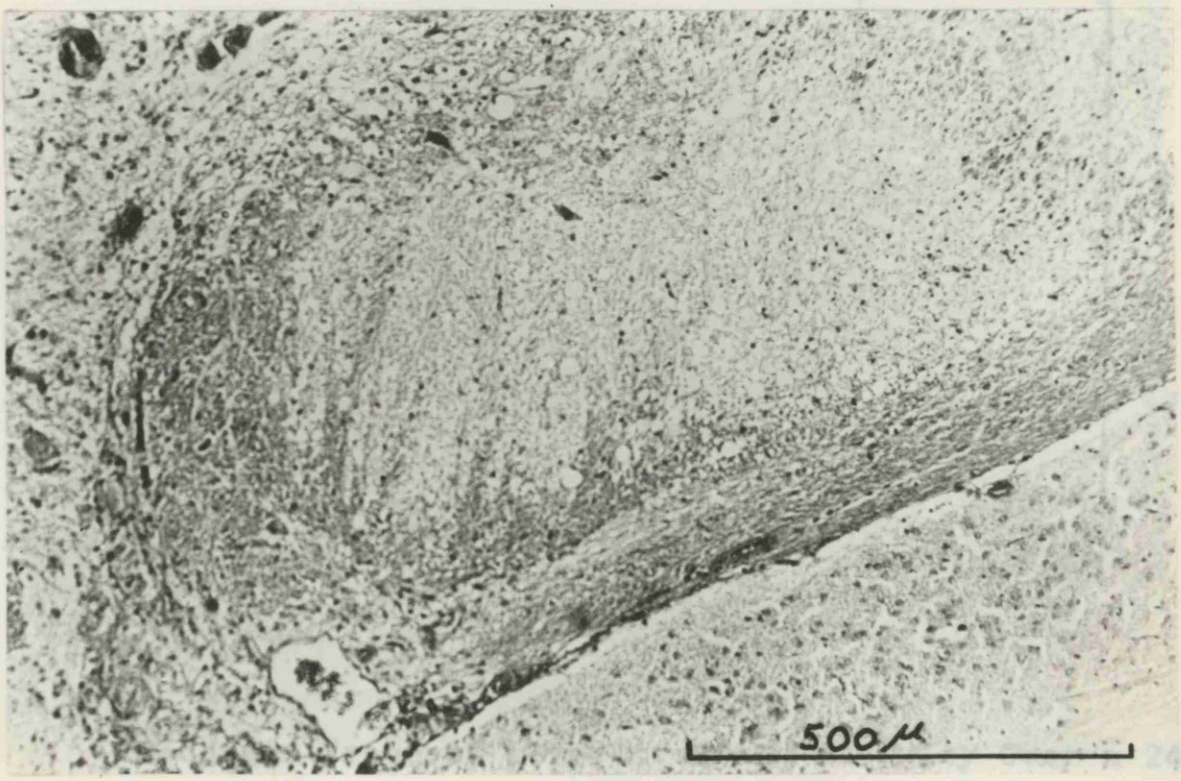
Aphagic Rat During Lever-pressing Activity.

The Diet Was Delivered but <u>not</u> Eaten.

Fig. (56.)

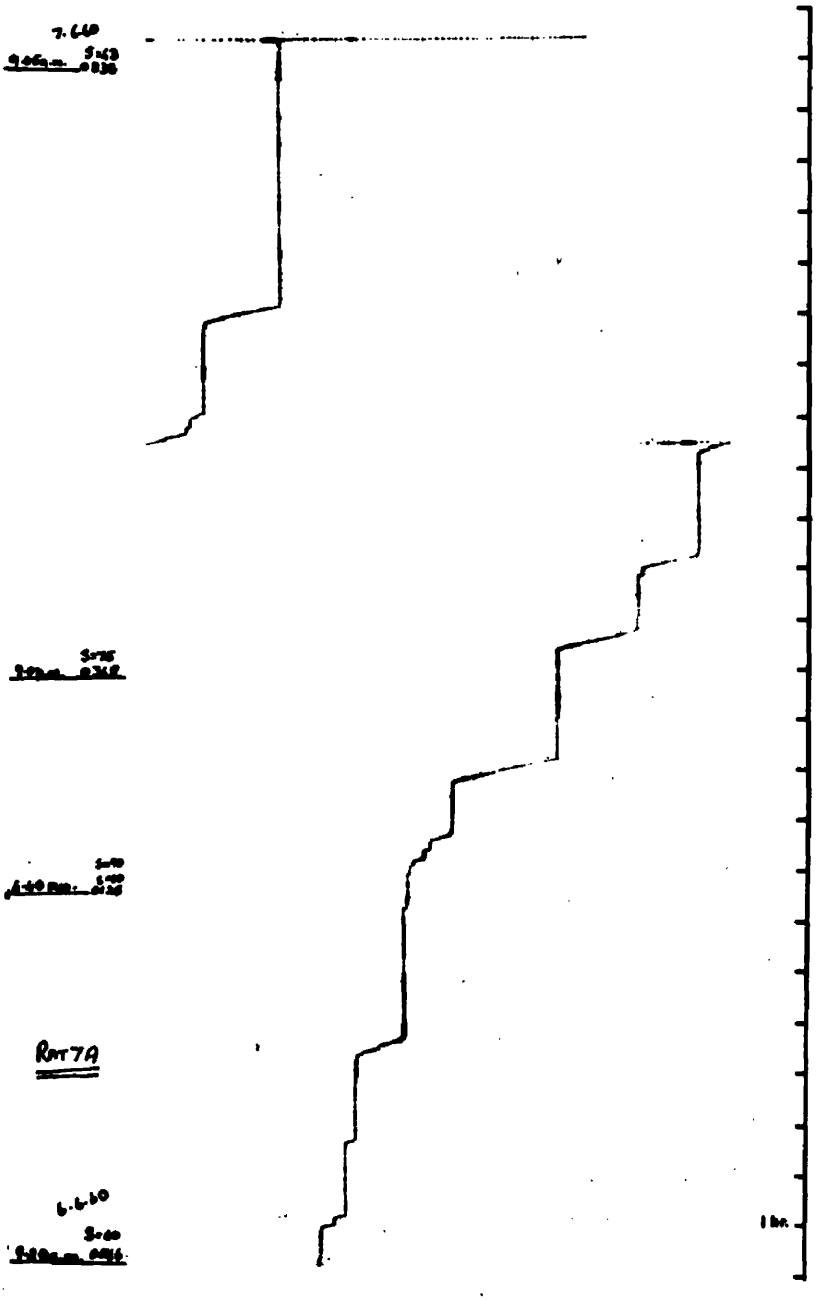


a) Left side of the hypothalamus of Rat 5A.



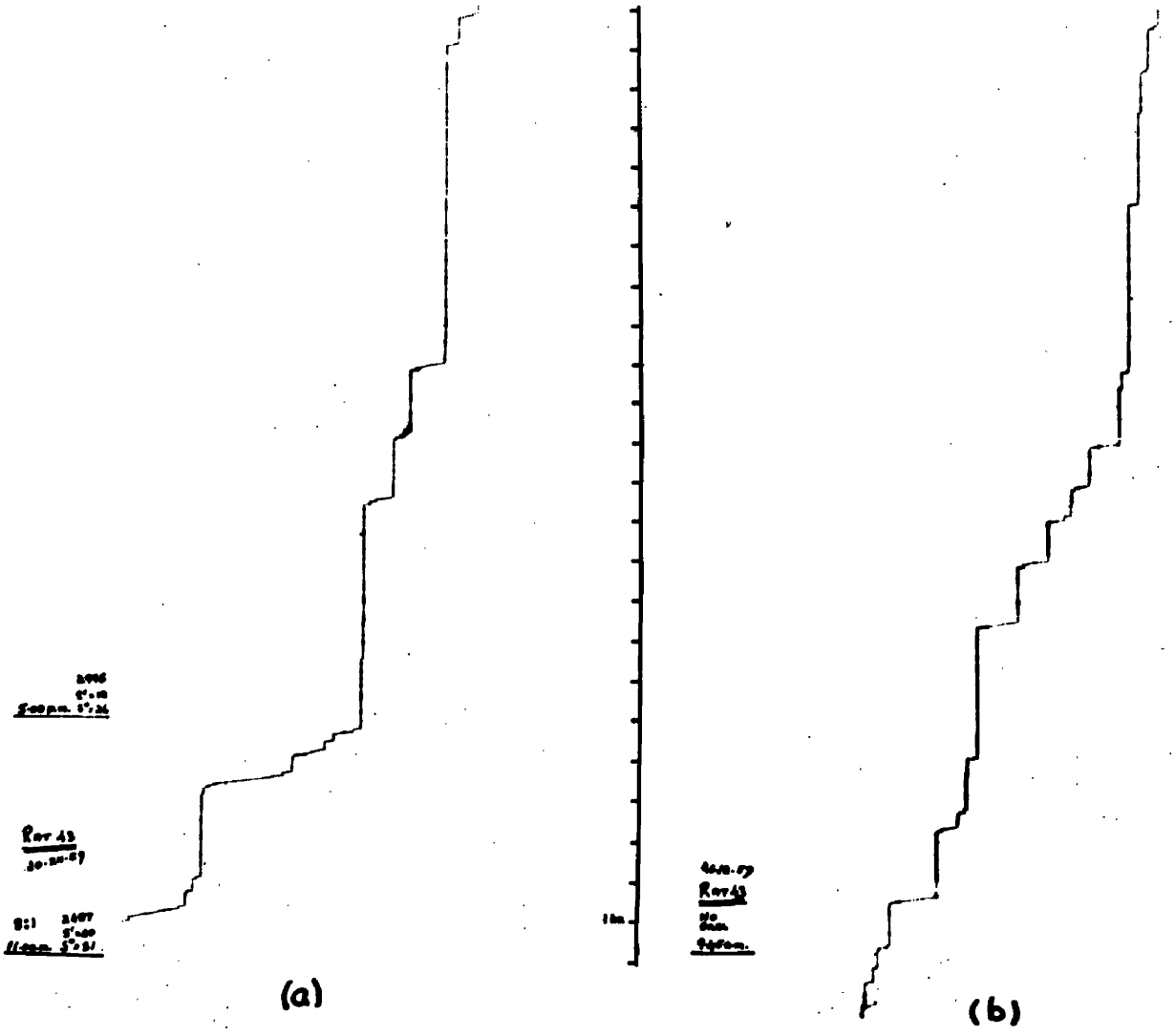
b) High power field to show pyramidal nerve fibre degeneration.

Fig.(57.)



Recorder Trace of Lever-pressing Activity Over a 24hr period.

Fig. (58).

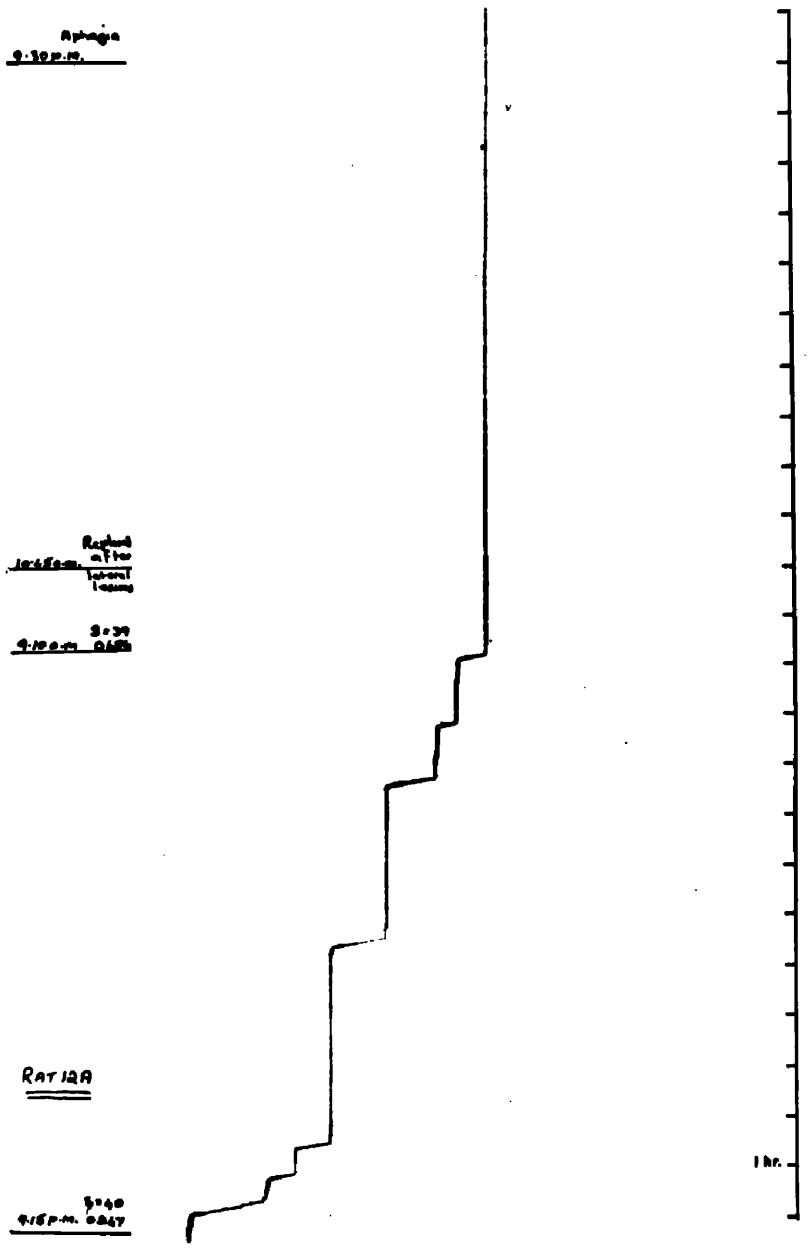


Feeding Patterns of Rat 43

a) feeding orally and intragastrically, and

b) feeding purely intragastrically.

Fig. (59).



Lever-pressing Pattern Before and After Lateral Lesions.

Fig. (60).

Table (6).

CALORIE INTAKE VALUES DURING OPEN CAGE PERIOD.

Selected days for statistical analysis.

RATS

Day	9	10	12	13	15
1	28	73	76	91	69
2	47	76	78	92	79
3	88	70	60	80	78
4	23	34	44	44	63
5	107	86	64	83	80
6	24	0	40	50	22
7	66	82	81	76	12
8	67	64	55	45	70
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	0	43	0	30	0
14	0	76	0	75	42
15	0	62	0	70	33

Table (7).

LEVER-PRESSING VALUES DURING OPEN CAGE PERIOD.

Selected days for statistical analysis.

RATS

Day	9	10	12	13	15
1	406	1031	1143	1354	1077
2	640	1096	1156	1269	1183
3	1058	1022	831	1254	1258
4	224	485	681	808	873
5	1496	1256	991	1189	1221
6	338	30	639	869	316
7	1079	1511	1242	1105	167
8	1030	1101	840	713	1035
9	17	0	4	37	0
10	0	3	20	92	1
11	4	8	17	77	13
12	51	217	28	96	143
13	848	644	633	1241	364
14	1124	1117	2089	1583	621
15	236	1116	445	1184	641

Table (8)

a) Analysis of Variance of Calorie Intake

N = 40 Days 1 - 8

Source of Variance	Sum of Squares	D.F.	Mean Squares	Variance Ratio	P
Between animals	870	4	217.5	< 1	Not significant
Between days	12364	7	1764.8	5.19	$P < 0.001$
Residual	9509	28	339.6		
Total	22743	39			

Standard Error of a single observation = $\sqrt{339.6}$

= 18.42

b) Analysis of Variance of Lever-pressing

N = 40 Days 1 - 8

Source of Variance	Sum of Squares	D.F.	Mean Squares	Variance Ratio	P
Between animals	340995	4	85248.75	< 1	Not significant
Between days	2442038	7	348862.6	3.91	$P < 0.01$
Residual	2496459	28	89159.25		
Total	5279492	39			

Standard Error of a single observation = $\sqrt{89159.25}$

= 298.6

Table (9)

(a) Analysis of Variance of Calorie Intake

N = 15 DAYS 1 - 3

Source of Variance	Sum of Squares	D.F.	Mean Squares	Variance Ratio	P
Between days	185	2	92.5	< 1	Not significant
Between Animals	1719	4	429.75	1.67	P > 0.2
Residual	2058	8	257.25		
Total	3952	14			

Standard error of a single observation = $\sqrt{257.25}$

= 16.04

(b) Analysis of Variance of Lever-pressing

N = 15 DAYS 1 - 3

Source of Variance	Sum of Squares	D.F.	Mean Squares	Variance Ratio	P
Between Days	19125	2	9562.5	< 1	Not significant
Between Animals	586105	4	146526	4.00	P > 0.05
Residual	292400	8	36550		
Total	897630	14			

Standard error of a single observation = $\sqrt{36550}$

= 189.7

Table (10)

(a) Analysis of Variance of Calorie Intake

N = 18 DAYS 1, 2, 3, 13, 14, 15

(3 Rats selected)

Source of Variance	Sum of Squares	D.F.	Mean Squares	Variance Ratio	P
Between Days	6955	5	1391	7.55	$0.001<P<0.01$
Between Animals	1672	2	836.	4.54	$0.01<P<0.05$
Residual	1843	10	184.3		
Total	9470	17			

Standard error of a single observation = $\sqrt{184.3}$

= 13.58

(b) Analysis of Variance of Calorie Intake

N = 30 DAYS 1, 2, 3, 13, 14, 15

Source of Variance	Sum of Squares	D.F.	Mean Squares	Variance Ratio	P
Between Days	1228702	5	245740	2.434	$0.05<P<0.1$
Between Animals	1200269	4	300067	2.973	$0.01<P<0.05$
Residual	2018784	20	100939.2		
Total	447755	29			

Standard error of a single observation = $\sqrt{100939.2}$

= 317.7

Table (11)

Analysis of Variance of Lever-pressing

N = 20 DAYS 9, 10, 11, 12

Source of Variance	Sums of Squares	D.F.	Mean Squares	Variance Ratio	P
Between Days	29162	3	9720.6	4.95	$0.01<P<0.05$
Between Animals	10166.3	4	2541.5	1.295	$P>0.2$
Residual	23546.5	12	1962.4		
Total	62874.8	19			

Standard error of a single observation $= \sqrt{1962.4}$

$= 44.295$

Table (12)

Comparison of Means of Daily Calorie Intake Values

	Days	D.F.	t	P
I	1,2,3,v 4	19	3.228	$P < 0.01$
II	1,2,3,v 5	19	1.23	$0.2 < P < 0.3$
III	1,2,3, v 6	19	4.742	$P < 0.001$
IV	1,2,3,v 7,8	24	1.396	$0.1 < P < 0.2$
V	4 v 6	19	0.874	$0.3 < P < 0.4$
VI	9,10,11,v 12			No difference
VII	1,2,3, v 13	11	5.303	$P < 0.001$
VIII	1,2,3, v 14	11	0.883	$0.3 < P < 0.4$
IX	1,2,3 v 15	11	1.911	$0.05 < P < 0.1$
X	14 v 15	5	0.840	$0.4 < P < 0.5$

Table (13)

Comparison of means of Daily Lever-pressing Values

	Days	D.F.	t	P
I	1,2,3, v 4	19	2.84	$0.01 < P < 0.02$
II	1,2,3, v 5	19	1.1	$0.2 < P < 0.3$
III	1,2,3,v 6	19	3.97	$P < 0.01$
IV	1,2,3, v 7,8	24	0.571	$0.5 < P < 0.6$
V	4 v 6	9	0.931	$0.3 < P < 0.4$
VI	9,10,11, v 12	19	3.82	$P < 0.01$
VII	1,2,3, v 13	19	1.86	$0.05 < P < 0.1$
VIII	1,2,3, v 14	19	1.55	$0.1 < P < 0.2$
IX	1,2,3, v 15	19	1.996	$0.05 < P < 0.1$
X	14 v 15	9	2.9	$P < 0.02$

APPENDIX I.

DETAILS OF EXPERIMENTAL PROCEDURES

DETAILS OF THE OPERATION TO PRODUCE LESIONS

1) The rat is first given a hypnotic dose of Nembutal, 0.025 mg/100 g body weight, and then ether to ensure complete anaesthesia. When the animal is fully anaesthetised the hair from the head is plucked by hand and the skin swabbed with alcohol between the eyes and the ears. The stereotaxic instrument has the electrode in position and the arc at right angles to the animal platform: the incisor clamp has been removed from the platform. The electrode tip is cleaned and filed straight across before the operation to clean off any sharp metal edges and to ensure no dried blood film is across the metal at the tip. The electrode tip is checked at the zero position before the rat is placed on the platform. The head is then clamped in the instrument in the following manner.

2) The lower margins of the tragi are cut at an angle, running downwards and forwards. The auditory canals are thus exposed. The Perspex earcones are then inserted into the external auditory canals through the external auditory meati. This procedure is best done while holding the earplugs in place with one hand. With the other hand, the adjustable earbars of the headholder are screwed into the Perspex cones. When the earbars are tightened the head is firmly held with the rostro-caudal axis at right angles to the line between

the earbars. The head is lightly held by screwing in the earbars until the clutches begin to slip. When the clutches are properly adjusted they begin to slip when there is just sufficient pressure on the head to hold it firmly in place without distortion of the skull. If the head is securely in place between the earbars, then with the earbars as fulcrum, the head may be tilted to any angle and maintain the new position.

3) The head is then brought into the centre exactly between the earbars by unscrewing one earbar for a small distance while screwing up the other until the readings on the earbar graduations are equal. With the Perspex earcones in place the readings should be about 8.

4) The incisor clamp is then slid into place between the jaws, which are held open by a large pair of forceps. This ensures that the tongue remains on the floor of the mouth. The upper incisors are hooked over the bit of the clamp which fits into the angle between the incisors and the roof of the mouth. The incisor clamp is pulled as far forward as the teeth will allow while the snout is pressed firmly down on the bit. The clamp may then be locked in position. While the bar across the front of the incisors is being screwed tight, the snout should again be pressed firmly over the bit to counteract the tendency for the snout to rise as the bar tightens.

During this part of the operation it is essential that a beaker of ether-soaked cotton-wool be kept over the snout as much as possible to keep the rat completely anaesthetised.

5) As the skull has been plucked and swabbed previously, a midline incision is then made from between the eyes to between the ears. This skin is then reflected and the skull exposed by blunt dissection clearing away all strands of connective tissue and other soft tissue covering the cranium.

The bregma is the external mark on the skull from which all external distances are calculated. The method of using the bregma as the constant point in positioning lateral lesions has been shown to be efficient by Morrison & Mayer (1957a).

The bregma is the point on the skull where the frontal and parietal bones meet: there is an X-shaped mark. This mark is easily shown in the live animal by rubbing the region vigorously with a saline swab and then quickly drying it off. The white markings momentarily show up very clearly.

6) When the animal is ready and the bregma located then the electrode-holding arc is swung into place and locked. The tip of the electrode should be approximately above the midline of the skull, when the platform is central.(See Fig.18)

7) The position of the animal platform is then adjusted by the horizontal movements until the electrode tip is exactly above the bregma. A check is made that the tip is exactly above this point by lowering the electrode tip until it lies exactly on the cross of the bregma. The machine readings are then noted as in Table (19).

8) From the bregma, the headholder is again moved so that the tip of the electrode is lying the desired distance (1.5 mm) caudad to the bregma. A check is made here to see that the electrode tip is still above the superior sagittal suture on the midline.

9) From this point the headholder is again moved so that the electrode moves lateral to the midline, either to the left or to the right for the desired distance (2 mm). Machine readings are noted. The electrode is then raised clear of the skull.

10) Under the noted point on the skull above which the electrode tip lies, a hole is started with a fine dental burr of 2 mm diameter. After enough of the bone is drilled away to leave a mark, a check is made that the electrode tip is above the centre of the hole. The speed of the drill is so adjusted to prevent burning of the bone and a few drops of saline keep the region moist. The electrode is raised clear of the mark and drilling is again begun. The electrode tip is frequently lowered to the skull to see that the hole

is made in the correct position, and raised again. Any adjustments are made to the hole position as they become necessary. This hole has to be made carefully as it must pass completely through the bone but not pierce the dura mater lying immediately below. The position of the hole must also be accurate enough to allow the electrode in its fixed and recorded position to pass through without fouling the sides of the hole. The bony edge can easily damage the electrode insulation.

11) The current supply is connected so that the positive is to the electrode and the negative to the incisor clamp. This is connected up before inserting t he electrode into the brain to minimise further movements of the electrode.

12) The tip of the electrode is then introduced into the hole until it is obstructed by dura and the separation of the dura from the inside of the cranium shows as a whiter area spreading from the hole. The reading on the vertical scale of the electrode is noted at that point.

13) The dura is then pierced as the electrode is passed gently and carefully through the brain for approximately 8 mm until its tip strikes the cranial floor (sphenoid), but not piercing it. This point is obvious when a slight obstruction is felt in lowering the electrode by fingertip manipulation of the knob. If the electrode should pierce the sphenoid then the co-ordinates of the lesion are

calculated from the readings on the opposite side of the brain. When the cranial floor reading is noted the electrode tip is elevated 2 mm and the electrode holder locked in place.

14) A current of known size and time is passed.

15) The electrode is carefully removed.

16) The procedure is then repeated for the other side, after which the current supply is disconnected and removed.

17) Finally, the skin is closed with three fine silk sutures and the animal removed from the headholder.

FINAL OPERATION FOR MAKING THE GASTRIC FISTULA

All tubing, washers etc. shown in fig. (17) are made ready beforehand.

1) Nembutal (0.025 mg/100g body weight) is given and ether anaesthesia is maintained throughout the operation.

2) The hair is removed from the left flank by plucking and the skin is swabbed with alcohol.

3) An incision is made with a scalpel on the left flank 2 cm lateral to the midline for 4 cm caudad to the rib cage. The wound is opened and the skin separated from the underlying tissue by blunt dissection.

4) The muscle layers are separated crosswise, by blunt pointed scissors, just caudad to the ribs. This incision is stretched as far as possible to allow freedom in withdrawing the stomach.

5) The stomach is then brought through the wound and is supported by wet saline swabs as shown in fig. (18,a). An incision is made 0.5 cm long on the dorsal side of the stomach well clear of the blood vessels.

6) A stab wound is made dorsolateral to the incision by passing a spike through the incision and stabbing from the inside. This stab wound is then stretched and the nylon tube inserted (fig. 18, b). It is pulled through from the inside keeping the stomach wall as tight round it as possible

with the steel tube pointing caudad. The nylon cloth washer is thus on the inside and the second nylon cloth washer with a pursestring suture round it is passed down to lie on the outside of the stomach wall.

7) When this is in position the pursestring is drawn tight and tied. The nylon nut is then screwed down and before fitting into place a little "Nobecutane" is applied to the fistula to seal the threads. The nut can then be screwed tight on the stomach wall (fig. 18,c) but care must be taken that the tissue is not severed. While all this is taking place it is essential to see that the stomach contents do not spill into the abdominal cavity. The incision in the stomach wall is then sutured with a running stitch using 000 silk.

8) A stab wound is then made in the abdominal wall lateral to the incision (fig. 18,d) and the nylon tube is pulled through.

9) The second nylon nut is then fitted over the muscle wall as in fig. (18,e). "Polybactrin" is sprayed into the abdominal cavity.

10) The muscle wall is then closed with interrupted stitches.

11) A stab wound through the skin just above the bend of the fistula is made with a new spike as in fig. (18,f). The fistula is then pulled through the skin which fits

closely round the steel tubing. The skin is then closed with interrupted stitches and the open end of the fistula sealed temporarily with a polythene cap (fig. 18,g). This prevents the gastric juices from dripping on to the skin.

12) Each animal is then kept in a sleeve cage for 4 days. On the first 2 days no food is given and on the last 2 days only a moist cube diet is fed. This allows the wound to heal without interference from the fibrous material in the cube diet.

Antibiotics may be injected intragastrically and a few ml of water are injected daily into the stomach through the fistula to keep it patent.

ORIGINAL OPERATION TO MAKE A GASTRIC FISTULA

Rat anaesthetised with 0.025 mg Nembutal per 100g body weight + ether.

Hair plucked from left flank and at root of tail. Left dorsolateral incision.

Blunt dissection to separate skin from muscle.

Cut through muscle wall and display stomach.

Two large (3 mm) holes for the polythene nipples made in the stomach wall.

Run pursestring sutures with fine silk around the holes and insert tubing and draw sutures tight within the flange.

Dust wound with Penicillin et Sulphathiazol powder.

Spike the muscle wall twice and pull polythene tubes through. Suture muscle wall.

Spikes passed subcutaneously caudad to two holes in the skin above the tail.

Tubes brought through the skin and pursestring sutures drawn tight around tubes.

Flank incision sutured.

2 ml Procaine Penicillin G injected intramuscularly.

MODIFIED OPERATION USING SILVER/SILICONE RUBBER/STEEL SYSTEM

Rat pretreated 24 hrs previously with 1 ml Proc. Pen. G. Nembutal 0.025 mg/100g body weight.

Flank and tail region plucked.

Dorsolateral incision on left flank.

Muscle wall cut and opened and stomach displayed.

Lateral incision and two stab wounds made in stomach wall.

Silver tubes with nylon cloth washers on nipple pulled through and large double holed nylon cloth washers pulled over tubes and down on to stomach wall.

Silicone rubber washers pulled tight over nylon on stomach and lateral incision sutured.

Second spike wounds through muscle wall and silver tubes led through.

Stomach dusted with Pen. et Sulphath. powder and Polybactrin sprayed inside abdominal cavity.

Silicone rubber washers outside muscle wall and third spike from incision back through skin at base of tail by stab wound.

Steel tube with attached silicone tubes led through from inside. Attach silicone rubber to the silver and secure with silk suture.

Silicone rubber washers on steel tubes outside.

Muscle wall sutured.

Skin sutured.

Changeover routine - with sleeve cage and gastric recording apparatus

a.m. 9.30.

1) Check whether kymograph record is correct and that pointers are all recording. Mark the time and date on the graphic and also corresponding points.

2) Switch lower 3-way tap to disconnect bellows from the recording tube and connect the syringe reservoir to the stomach. Clear the recording tube if possible.

3) Turn the tap to disconnect the recording tube from the recording system.

4) Note the syringe (diet) readings and record on the cumulative recorder trace. Also record counter figures, time and date and switch off the input recorder.

5) Check both oral and intragastric tubes for blockage.

6) Disconnect lever switch from the relays.

7) Remove the polythene tubes from syringe adaptors and the recording system.

8) Remove the laboratory clamp holding the oral tube in place.

9) Remove the tray and cage with the animal in it. Open the rear door and entice the animal out backwards. If this fails, glass rods may be pushed through the mesh one by one in front of the animal so forcing it towards the rear of the cage.

10) The animal is checked for infections on the flank etc. and the bent polythene tube is removed by gripping the steel

tube of the fistula with artery forceps and unscrewing the polythene tube. The animal is then wiped with "Dettol" round the wounds.

11) The rat is weighed and its body weight recorded. Weighing is done in a card box of known weight on a laboratory balance. The animal is then allowed freedom to run about on the bench top.

12) Lever and switch are then unscrewed from the cage and the cage washed in hot water. A new switch and lever is attached to a clean cage.

13) Soiled paper is replaced by fresh paper on the tray which has been washed and dried.

14) Syringes are removed and adaptors and tubes are cleared and washed out. New syringes are refilled if required from the beaker of diet. The diet is only removed from the refrigerator long enough for the refilling of syringes. The cleaned adaptors and syringes are then replaced.

15) The cage is replaced on the tray and the oral tube connected to the syringe.

16) The tubes to the fistula are fitted to the animal and cleared through. The animal is then replaced in the cage.

17) The tubes are connected to the syringes and recording system. All tubes are primed, from either the syringes, or in the case of the gastric recording tube, from the reservoir syringe.

18) The lever switch is then reconnected to the relays and tested to see that the injectors are working. The syringe and counter readings are noted and the recorder input is switched on. The tap on the gastric recording system is turned to allow pressure changes in the stomach to be recorded. Corresponding points are marked on the graphic.

APPENDIX II.

EXPERIMENTAL DATA

Table (14).

CALIBRATION OF SLOW INJECTION APPARATUS

100 ml metal piston syringe - Injector 1

ml/lever-press (from 20 presses)

	Speed		
sec	8	16	32
1	0.08	0.05	0.025
2	0.15	0.075	0.040
3	0.22	0.11	0.055
4	0.25	0.14	0.065
5	0.31	0.15	0.09

100 ml metal piston syringe - Injector 2

ml/lever-press (from 20 presses)

	Speed		
sec	8	16	32
1	0.07	0.035	0.02
2	0.13	0.055	0.035
3	0.19	0.01	0.05
4	0.23	0.12	0.06
5	0.28	0.14	0.08

CALIBRATION OF SLOW INJECTION APPARATUS

50 ml glass barrel and piston syringe - Injector 1

ml/lever-press (from 50 presses)

	Speed		
sec	1	2	4
0.5	0.20	0.11	0.06
1.0	0.35	0.20	0.10
1.5	0.464	0.268	0.132
2.0	0.648	0.324	0.172
2.5	0.736	0.412	0.212
3.0	0.868	0.448	0.240
3.5	1.00	0.512	0.280
4.0	1.14	0.60	0.30
4.5	1.28	0.65	0.30
5.0	1.43	0.72	0.40
10.0	2.60	1.40	0.66
20.0	5.26	2.66	1.40
30.0	7.80	4.00	2.00

Table (15).

FLUID DIETS

First formula : first used in October, 1958

	g
Casein	20.0
Sucrose	53.0
Margarine	19.5
Cod Liver Oil	0.5
Salt Mixture	5.0
Hepamino	2.0
Water to 500 ml	

Second formula : first used 24th February, 1959

	g
Casein	25.0
Glucose	25.0
Margarine	30.0
Cod Liver Oil	0.5
Salt Mixture	5.0
Hepamino	2.0
Water to 500 ml	

Third formula : first used in December, 1959

1 g Penicil. et Sulphathiazol added to each 500 ml

FINAL FLUID DIET USED IN RAT FEEDING EXPERIMENTS

Based on Diet "M.S.1." (Cumming, 1958).

Commencing October 1959, constituents:-

	g
Casein	25.0
Glucose	25.0
Margarine	30.0
Cod Liver Oil	0.5
Salt Mixture	5.0
Hepamino	2.0
Na benzoate	0.5
Na tauroglycocholate	0.5
Cholesterol	0.25

Penicillin et Sulphathiazol added, approx. 1 g to each 500 ml diet

Distilled water to 500 ml.

SALT MIXTURE	g
Na Cl	168.6
Basic calcium phosphate	167.3
Potassium citrate	111.5
Calcium carbonate	77.0
Dipotassium phosphate	36.0
Magnesium carbonate	19.2
Ferric citrate	7.5
Manganous sulphate	0.59
Copper sulphate (Anh.)	0.051
Potash alum	0.04
Cobalt chloride (Anh.)	0.025
Potassium iodide	0.02
Zinc carbonate	0.02
Sodium fluoride	0.0004

Fluid diet (contd.)

HEPAMINO (per 100g)

	mg
Thiamin	1.0
Riboflavin	13.0
Piridoxin	2.0
Pantothenic acid	100.0
Folic acid	3.0
Biotin	0.4
Inositol	250.0
Nicotinic acid	60.0
Hydrolysed protein	80.00
Iron	230 parts/Million
Copper	40 parts/Million

Table (16).

ELECTRODES TESTED

Metal	mm Uncoated	mm Coated	sec * Polarisation
Piano wire	0.46	0.54	48 cleaned 21 uncleaned
	(Heat-stretched - coated with polyurethane)		
Piano wire	0.32	0.44	8 cleaned 7 uncleaned
	(Heat-stretched - coated with polyurethane)		
Minalfa	0.58	0.66	
	(Nylon lacquer coated - cold drawn)		
Minalfa	0.38	0.42	
	(Nylon lacquer coated - cold drawn)		
Stainless Steel	0.26	0.32	
	(Cold drawn - coated with polyurethane)		
Nichrome	0.46	0.56	
	(Cold drawn - coated with polyurethane)		

* time to cause increase in resistance when immersed in solution of casein in saline while constant current passed.

APPENDIX III.

PRESENTATION OF RESULTS in extenso

Table (17).

OPEN CAGE RESULTS

RAT 1A Open Cage 3 sec speed 32

After previous lateral lesions

Lever presses corrected for 4 sec time

(a)	(b)	(c)	(d)	(e)	(f)
2.10.59		46	1119	2	
3.10.59	202	98	1298	7	
4.10.59	205	97	1285	2	
5.10.59	205	80	1023	5	
6.10.59	207	79	1004	3	
7.10.59	206	72	923	6	
8.10.59	204	52	532	5	
9.10.59	203	75			Recorder not switched on
10.10.59	201	59	559	6	
13.10.59	206	67	870	9	Control
14.10.59	207	66	870	9	Control
15.10.59	185	0	0	0	14.10.69 Lateral lesions
16.10.59	174	0	4	0	Still Adipsic and Aphagic
17.10.59	167	0	21	0	Adipsic and Aphagic
20.10.59	147	0	2710	7	Not feeding
21.10.59	149	0	3940	4	Still unable to feed
22.10.59	156	0	740	2	Drinking water Some food eaten
23.10.59	168	49	825	5	Recovery almost complete
24.10.59	162	48	820	4	Recovered

RAT 4A Open Cage 3 sec speed 32

Second lever installed

(a)	(b)	(c)	(d)	(e)	(f)
3.11.59	216	34	664	3	
4.11.59	216	44	891	7	
5.11.59	212	52	1020	3	
6.11.59		50	928	4	$L^2 = 9$ (Second Lever)
7.11.59		81	1390	7	$L^2 = 11$
10.11.59	228	60	856	8	$L^2 = 12$
11.11.59	217	48	886	8	$L^2 = 18$
12.11.59	214	74	1225	7	$L^2 = 15$
13.11.59		54	987	5	$L^2 = 14$
14.11.59		54	998	7	13.11.59 10.55 a.m. Ether and Nembutal $L^2 = 8$
20.11.59		62	1169	7	$L^2 = 1$
21.11.59	241	71	1322	10	$L^2 = 14$
24.11.59	245	57	1063	7	$L^2 = 2$
25.11.59	235	71	831	6	$L^2 = 8$
26.11.59	226	15	204	2	$L^2 = 2$ 25.11.59 Sham Lateral lesion
27.11.59		26	365	5	$L^2 = 4$ Recovery
28.11.59	240	51	994	10	$L^2 = 9$ Recovery
1.12.59	230	45	840	5	$L^2 = 47$
2.12.59	222	62	1142	9	$L^2 = 31$
3.12.59	207	4	2	0	2.12.59 10.30 a.m. $L^2 = 2$ Lateral lesions Aphagia

RAT 4A

(a)	(b)	(c)	(d)	(e)	(f)
4.12.59	195	2	16	0	L^2 = 67
5.12.59	189	7	53	1	L^2 = 132
8.12.59	170	18	79	1	L^2 = 242
9.12.59	189	28	443	3	L^2 = 124 Able to lick Diet
10.12.59	190	63	1120	4	L^2 = 180
11.12.59	188	55	1023	5	L^2 = 96
12.12.59	193	69	1365	4	L^2 = 60
28.1.60	268	28	329	5	
29.1.60	261	60	946	6	L^2 = 18
30.1.60	259	47	819	5	L^2 = 11
1.2.60	263				
2.2.60	267	43	724	4	L^2 = 239
3.2.60	264	47	897	6	L^2 = 18
4.2.60	241	4	7	0	L^2 = 2 Sham Lateral lesions
5.2.60	225	0	0	0	L^2 = 7 Adipsia and Aphagia
6.2.60	212	0	8	0	L^2 = 2

RAT 5A Open cage 4 sec speed 32

(a)	(b)	(c)	(d)	(e)	(f)
20.4.60	219	69	870	5	
21.4.60	218	89	1155	10	
22.4.60	218	73	1080	8	
23.4.60	220	80	1135	10	
24.4.60	225	81	1040	11	
25.4.60	221	66	1160	10	Control period
26.4.60	221	77	1140	10	Control
27.4.60	222	83	1080	10	Control
28.4.60	223	81	1105	10	Spout 8" from Mid Lever
29.4.60	227	88	1260	8	
30.4.60	228	77	1155	10	
1.5.60	227	67	870	8	30.4.60. 11.30 a.m. 0.07 ml Nembutal and Ether
2.5.60	224	65	990	11	
3.5.60	223	25	390	4	2.5.60, 2.30p.m. Sham Lateral lesions - Recovery
4.5.60	229	74	1080	10	
5.5.60	207	10	180	5	4.5.60, Sham Lateral lesions
6.5.60	223	82	1044	10	
7.5.60	219	70	995	8	
8.5.60		50	854	9	
9.5.60	215	64	1173	10	
10.5.60	213	64	873	8	
11.5.60	213	50	799	11	
12.5.60	200	0	339	0	11.5.60, 1.30 p.m. Lateral lesions
13.5.60	192	0	23	0	Able to eat and drink

RAT 5A

(a)	(b)	(c)	(d)	(e)	(f)
14.5.60	196	0	19	0	Oral food from graduated tube
15.5.60	203	76	1260	6	Recovered
26.5.60	231	80	1328	4	
27.5.60	226	51	975	4	Tendency to roll about in cage
28.5.60	210	16	85	0	Ability to take diet
19.7.60			739		Training system
20.7.60	236	57	739		
21.7.60	239	63	827		
22.7.60	243	100	1287	5	
23.7.60	226	0	3	0	22.7.60 Lateral lesions
24.7.60	209	0	18	0	
25.7.60	199	0	180	Continuous	No eating
26.7.60	182	0	38	0	Moribund and cold

RAT 6A Open cage 4 sec Speed 32

(a)	(b)	(c)	(d)	(e)	(f)
16.5.60	194	101	1035	8	Equilibration period
17.5.60	199	74	1165	11	Control Spout 8" from lever
18.5.60	203	76	1109	9	
19.5.60	199	75	1100	10	
20.5.60	197	63	933	9	
21.5.60	209	80	1218	13	Spout 4" from lever
22.5.60	209	53	764	10	
23.5.60	214	70	987	16	
24.5.60	210	46	641	7	23.5.60. 0.05 ml Nembutal and ether
25.5.60	212	71	907	13	25.5.60. Sham lesions. Died before recovery

RAT 7A Open cage 4 sec Speed 32

Pretrained

(a)	(b)	(c)	(d)	(e)	(f)
31.5.60	233	90	1224	7	Equilibration
1.6.60	231	69	1076	8	Control
2.6.60	233	79	1150	8	
3.6.60	234	60	851	8	2.6.60 10.30a.m 0.05ml Nembutal + Ether
4.6.60	234	66	980	6	
5.6.60	233	39	628	3	4.6.60 Sham Lateral lesions
6.6.60	232	44	546	9	
7.6.60	234	61	833	9	
21.6.60	263	90	1237	7	Equilibration
22.6.60	265	86	1335	7	Control
23.6.60	241	0	2	0	22.6.60 Lateral lesions
24.6.60	226	0	16	0	Adipsic and Aphagic
25.6.60	216	5	33	0	Recovered from Aphagia
26.6.60	207	10	130	3	

RAT 8A Open cage 4 sec Speed 32

Pretrained

(a)	(b)	(c)	(d)	(e)	(f)
7.6.60	239	71	1063		
8.6.60	239	73	1023	10	Equilibration
9.6.60	239	44	628	7	Control
10.6.60	240	63	871	10	
11.6.60	240	53	739	10	
12.6.60	241	45	626	7	11.6.60. 0.5ml Nembutal and Ether
13.6.60	240	42	545	9	
14.6.60	224	0	1	0	13.6.60 Sham lesions.
15.6.60	229	52	732	6	
16.6.60	230	56	950	7	
27.6.60	257	58	974	10	Equilibration
28.6.60	260	47	658	9	
29.6.60	260	68	1080	9	28.6.60 Lateral lesions
30.6.60	260	54	796	11	
1.7.60	259	35	457	8	30.6.60 Lateral lesions. Eating immediately afterwards
2.7.60	257	53	795	12	
3.7.60	257	60	980	11	

RAT 9A Open cage 4 sec Speed 32

Pretrained

(a)	(b)	(c)	(d)	(e)	(f)
4.7.60	261	28	406	2	Equilibration
5.7.60	262	47	640	6	Control
6.7.60	264	88	1058	11	
7.7.60	255	23	224	4	6.7.60 Ether and 0.05 ml Nembutal
8.7.60	261	107	1496	12	
9.7.60	259	24	338	4	8.7.60 Sham lesions
10.7.60		66	1079	9	
11.7.60	263	67	1030	10	
12.7.60	269	78	1108	13	
13.7.60	262	76	1052	16	
14.7.60	242	0	17	0	13.7.60 Lateral lesions
15.7.60	222	0	0	0	Aphagia and Adipsia
16.7.60	210	0	4	0	Aphagia and Adipsia
17.7.60	197	0	16	0	Aphagia and Adipsia
18.7.60	187	0	51	0	Aphagia
19.7.60	177	0	848	7	Aphagia
20.7.60	166	0	1124	6	Aphagia
21.7.60	160	0	263	1	Moribund and Aphagia

RAT 10A Open cage 4 sec Speed 32

Pretrained

(a)	(b)	(c)	(d)	(e)	(f)
27.7.60	293	76	1096	7	
28.7.60	289	70	1022	6	
29.7.60	282	34	485	5	28.7.60. 0.06ml Nembutal and Ether
30.7.60	286	86	1256	11	
31.7.60	265	0	30	0	30.7.60 Sham operation
1.8.60	283	82	1511	7	
2.8.60	279	64	1101	10	
10.8.60	296	57	841	8	
11.8.60	297	51	710	5	
12.8.60	295	72	941	6	
13.8.60	295	75	992	9	
14.8.60	272	0	0	0	13.8.60 Lateral lesions
15.8.60	254	0	3	0	Aphagic
16.8.60	248	0	8	0	Aphagic
17.8.60	233	0	1	0	Aphagic
18.8.60	224	0	20	0	Aphagic
19.8.60	215	0	50	0	Aphagic
20.8.60	194	0	319	Continuous	Aphagic
21.8.60	192	0	283	Continuous	Aphagic
22.8.60	185	0	217	Continuous	Aphagic
23.8.60	192	43	644	4	22.8.60 Recovered
24.8.60	185	76	1117	7	
25.8.60	184	62	1116	4	

RAT 11A Open cage 4 sec Speed 32

Pretrained

(a)	(b)	(c)	(d)	(e)	(f)
21.10.60	247	45	652	9	Control
22.10.60	248	76	1087	7	
23.10.60	232	61	922	8	
24.10.60	244	88	1283	7	
25.10.60	240	61	889	9	24.10.60 Nembutal and Ether
26.10.60	247	74	1189	10	
27.10.60	236	41	587	5	26.10.60 Sham operation
28.10.60	239	64	869	9	
29.10.60	239	64	946	10	
30.10.60	236	86	1207	15	
31.10.60	232	80	1148	10	
1.12.60	232	64	965	10	
2.12.60	240	58	1262	10	Died under anaesthetic

RAT 12A Open cage 4 sec Speed 32

Pretrained

(a)	(b)	(c)	(d)	(e)	(f)
30.9.60	240	58	813	12	Control
1.10.60.	240	76	1143	12	
2.10.60	240	78	1156	11	
3.10.60	238	60	831	10	
4.10.60	232	44	681	11	3.10.60. 0.05ml Nembutal and Ether
5.10.60	231	64	991	12	
6.10.60	227	40	639	7	5.10.60 Sham operation
7.10.60	233	81	1242	10	
8.10.60	230	55	840	10	
11.10.50	239	59	831	13	
12.10.60	239	60	834	13	
13.10.60	238	47	686	13	
14.10.60	219	0	4	0	13.10.60 Lateral lesions
15.10.60	207	0	20	0	Aphagic
16.10.60	195	0	17	0	Aphagic
17.10.60	186	0	28	0	Aphagic
18.10.60	180	0	633	8	Aphagic - but Lever pressing
19.10.60	168	0	2089	8	Aphagic - but Lever pressing
20.10.60	159	0	445	5	Aphagic - but Lever pressing

RAT 13A Open cage 4 sec Speed 32

(a)	(b)	(c)	(d)	(e)	(f)
30.8.60	226	72	1018	6	
31.8.60	223	80	1268	8	Control
1.9.60	224	91	1354	8	
2.9.60	229	92	1269	5	
3.9.60	233	80	1254	7	
4.9.60	228	44	808	6	3.9.60 Nembutal & Ether
5.9.60	234	83	1189	5	
6.9.60	228	50	869	6	5.9.60 Sham operation
7.9.60	234	76	1105	7	
8.9.60	239	45	713	5	
9.9.60	235	67	1061	8	
17.9.60	257	76	1132	9	
18.9.60	250	70	1089	5	
19.9.60	255	81	1134	10	
20.9.60	254	67	1011	9	
21.9.60	231	0	37	0	20.9.60 Lateral lesions
22.9.60	216	0	92	0	
23.9.60	202	0	77	0	
24.9.60	189	0	48	0	
25.9.60	179	0	47	0	
26.9.60	169	0	96	0	
27.9.60	198	30	1241	7	26.9.60 Pressing lever but initially no eating
28.9.60	195	75	1583	9	Ate all diet
29.9.60	196	70	1184	5	Ate all diet

RAT 14A Open cage 4 sec Speed 32

Pretrained

(a)	(b)	(c)	(d)	(e)	(f)
10.9.60	233	4	95	0	Equilibration
11.9.60	234	114	1575	8	Control
12.9.60	245	86	1354	9	
13.9.60	243	73	1067	9	
14.9.60	236	52	842	7	
15.9.60	236	54	834	6	14.9.60 Ether, and 0.05 ml Nembutal
16.9.60	246	90	1387	8	16.9.60 Sham operation Animal died after operation

RAT 15A Open cage 4 sec Speed 32

(Recorder initially defective)

(a)	(b)	(c)	(d)	(e)	(f)
3.11.60	223	40	526	2	
4.11.60	225	97	1554	4	
5.11.60	231	101	1509	7	
6.11.60	231	90	1948	11	Counter reading incorrect
7.11.60	233	66	2526	8	Counter reading incorrect
8.11.60	229	72	1164	7	7.11.60, 0.05ml Nembutal and Ether. Recorder still incorrect
9.11.60	239	88	1350	9	
10.11.60	220	20	347	2	9.11.60. Sham operation
11.11.60	233	73	1141	4	Paper Drive slipping
12.11.60	233	69	1087	0	
13.11.60	230	80	1157	17	Control
14.11.60	235	69	1077	13	
15.11.60	234	79	1183	12	
16.11.60	239	78	1258	9	
17.11.60	235	63	873	10	16.11.60. 0.06ml Nembutal and Ether
18.11.60	239	80	1221	11	
19.11.60	228	22	316	3	18.11.60. Sham operation
20.11.60	215	12	167	2	
21.11.60	224	70	1035	6	
22.11.60	222	56	769	6	
23.11.60	222	51	680	7	
24.11.60	222	35	560	8	
25.11.60	202	0	0	0	24.11.60 Lateral lesions
26.11.60	190	0	1	0	Aphagic

RAT 15A

(a)	(b)	(c)	(d)	(e)	(f)
27.11.60	181	0	13	0	Aphagic
28.11.60	173	0	143	3	Aphagic
29.11.60	171	0	364	3	Aphagic
30.11.60	180	42	621	6	Recovering
1.12.60	176	33	641	5	

Table (18).

SLEEVE CAGE RESULTS

RAT 18 Sleeve Cage Oral + Intragastric Feeding

4 sec times

(a) Date	(b) Body Wt. g.	(c) Calorie Intake Cal.	(d) Lever Presses	(e) No. of Meals	(f) Remarks
12.5.59		94	1852	16	Oral only sp 8 - 4 sec
13.5.59	245	34	714	7	Oral = sp 32 4 sec I.G. = sp 8 4 sec
14.5.59.		20	921	7	Oral = sp 32
15.5.59.		13	1114	14	
16.5.59.		35	2053	7	
17.5.59.		30	1774	7	
18.5.59.		41	2132	6	
19.5.59.		60	3097	12	
20.5.59.	228	83	1118	13	I.G. = sp 32 Oral = sp 32
21.5.59.	227	86	1027	8	Oral = sp 32 I.G. = sp 16
22.5.59.	235	101	732	15	I.G. = sp 16 Oral = sp 32
23.5.59.	230	59	403	10	I.G. alone
24.5.59.		22	148	5	
25.5.59.	211	20	134	2	Ether & Nembutal Upper Fistula Tube replaced
26.5.59.		28	132	1	I.G. alone
27.5.59	211	34	200	5	
28.5.59	200	49	225	3	
29.5.59		24	204	4	Complan Diet
30.5.59	191	36	251	5	Complan Diet
31.5.59		39	332	3	Complan Diet
1.6.59	192	56	279	3	Undigested Diet in Faeces
2.6.59	191	6	46	0	Animal not able to assimilate Complan

RAT 23 Sleeve Cage Oral Feeding

Complan Diet

(a)	(b)	(c)	(d)	(e)	(f)
5.6.59.	187	38	2272	9	50% Dilute Oral Complan
6.6.59.	182	47	3044	12	
7.6.59.		102	2821	13	100% Diet
8.6.59.	198	97	2768	15	

RAT 24 Sleeve Cage Oral + Intragastric Feeding

Complan & Own Synthetic Diet : Uniselector

(a)	(b)	(c)	(d)	(e)	(f)
9.6.59	207	50	1576	11	Oral only 'Complan
10.6.59	205	59	1683	12	Complan
11.6.59	199	66	1907	16	Diet passing out in faeces
12.6.59	207	96	2609	14	Original Diet
13.6.59	211	92	2561	19	
16.6.59	215	84	1072	13	I.G. = sp 32 Oral = sp 32
17.6.59	211	43	556	9	
18.6.59	215	60	702	11	
19.6.59		25	376	4	
20.6.59		0	245	4	I.G. alone sp 16
21.6.59	214	56	697	15	
22.6.59	211	71	850	15	
25.6.59	221	0	467	8	2 : 1 Uniselector system used I.G. = sp 16 Oral = sp 32
26.6.59		54	380	5	I.G. = 16 Oral = sp 16 2 : 1
27.6.59	219	72	832	11	Silicone-Steel system replaced in upper tube
28.6.59					Animal off
29.6.59					Animal off
30.6.59		72	539	11	4 : 1
1.7.59		72	931	15	8 : 1 sp 8

RAT 24

(a)	(b)	(c)	(d)	(e)	(f)
2.7.59	214	95	857	13	12:1
3.7.59	214	72	504	11	25:1
4.7.59	214	62	298	5	25:1
5.7.59	209	38	385	5	No oral
6.7.59	219	92	431	13	
7.7.59	210	71	296	6	
8.7.59	204	29	130	2	
9.7.59	201	25	98	1	
10.7.59	207	86	528	8	
11.7.59	211	106	445	8	
12.7.59	204	36	249	5	
13.7.59	207	23	91	1	
14.7.59	197	78	350	5	
15.7.59	195	40	170	2	Lever pressing calculated
16.7.59	197	86	293	3	
17.7.59	199	51	272	3	

RAT 31 Sleeve Cage Oral + Intragastric Feeding

Uniselector Lateral Lesions

(a)	(b)	(c)	(d)	(e)	(f)
3.9.59	256	143	532	14	I.G. = sp 8 Oral = sp 32 1:1
4.9.59	235	51	185	4	4:1
5.9.59	233	147	620	13	12:1
6.9.59	233	87	409	7	25:1
7.9.59	243	105	1068	12	25:1
8.9.59	242	100	442	10	50:1
9.9.59	247	124	622	12	50:1
10.9.59		97	770	Constant	9.9.59 Lateral lesions 50:1
11.9.59	240	99	765	Constant	50:1

RAT 36 Sleeve Cage Oral + Intragastric Feeding

Uniselector

(a)	(b)	(c)	(d)	(e)	(f)
16.9.59		67	602	Continuous	I.G. = 1 sec sp 8 Oral = 1 sec sp 8
17.9.59		69	903	Continuous	4:1
18.9.59		66	699	13	4:1
19.9.59		45	387	8	4:1

RAT 43 Sleeve Cage Oral + Intragastric Feeding

Uniselector Lateral Lesions

(a)	(b)	(c)	(d)	(e)	(f)
15.12.59	249	82	1162	10	I.G. = sp 32 Oral = sp 32 1:1
16.12.59	245	93	1242	13	
17.12.59	242	65	1210	11	2:1
18.12.59	230	21	184	4	I.G. = sp 32 Oral = sp 16 12:1
19.12.59	222	45	368	5	
20.12.59		71	1078	12	8:1
21.12.59	213	42	665	7	12:1
22.12.59	214	60	1004	17	
23.12.59	211	51	848	9	
24.12.59		55	854	12	25:1 Power cut
25.12.59		63	516	9	
26.12.69	209	72	771	12	
27.12.59	205	64	611	7	50:1
28.12.59	202	50	518	14	
29.12.59	196	41	252	5	No oral
30.12.59	195	59	444	4	
31.12.59	190	70	673	10	
7.1.60	223	65	610	6	12:1
8.1.60		37	253	8	25:1
9.1.60		84	654	10	25:1
10.1.60		42	292	3	
11.1.60	203	29	127	3	
12.1.60	200	78	273	5	
13.1.60	199	67	295	6	
14.1.60	200	81	332	7	50:1
15.1.60	209	79	322	5	

RAT 43

(a)	(b)	(c)	(d)	(e)	(f)
16.1.60		73	313	7	
17.1.60		38	148	2	
18.1.60	200	44	181	5	
19.1.60	197	37	221	5	
20.1.60	198	50	126	2	
21.1.60	206	66	175	5	
22.1.60	206	50	242	5	
23.1.60	198	58	172	5	No. oral
24.1.60	204	53	158	2	
25.1.60	198	56	162	Continuous	
26.1.60	192	41	102	Continuous	
27.1.60		35	86	1	Tubes bitten
9.2.60	250	75	687	8	4:1
10.2.60	235	10	73	3	12:1
11.2.60		31	218	3	
12.2.60	228	63	298	5	
13.2.60	222	67	286	5	
14.2.60	225	59	243	4	25:1
15.2.60	214	52	276	7	
16.2.60	220	82	325	6	
17.2.60	210	69	294	4	
18.2.60	199	42	166	3	Escaped
19.2.60		38	149	4	
23.2.60	215	31	127	1	
24.2.60	205	40	156	6	
25.2.60	202	58	509	Continuous	24.2.60 Lateral Lesions
26.2.60					Open cage Adipsia - Aphagia

RAT 43

(a)	(b)	(c)	(d)	(e)	(f)
27.2.60	185	40	94	2	
28.2.60	171	75	2180	12	
29.2.60	198	47	325	9	Off
1.3.60					Open cage - Adipsia Aphagia
2.3.60	188	34	206	5	
3.3.60		16	52	1	Animal died overnight

RAT 46 Sleeve Cage Oral + Intragastric Feeding

Uniselector Lateral Lesions

(a)	(b)	(c)	(d)	(e)	(f)
15.3.60	272	165	2355	13	Oral = sp 16
16.3.60	282	145	1663	16	
17.3.60	283	125	783	12	
18.3.60		101	595	6	
19.3.60		63	497	4	
22.3.60	275	142	1168	7	2:1
23.3.60		65	576	4	
24.3.60	252	32	161	1	25:1
25.3.60	275	180	958	9	
26.3.60	273	174	915	15	Figures for Pressing calculated
27.3.60	291	63	315	4	
28.3.60		60	135	6	50:1
29.3.60	263	105	425	7	50:1
29.3.60					10.15 a.m. Sham Lateral Lesion Open cage - Eating and drinking
31.3.60		48	165	3	50:1
6.4.60					Lateral Lesions unsuccessful
8.4.60		112	488	Continuous	50:1

RAT 51 Sleeve Cage Oral + Intragastric Feeding

Uniselector Lateral Lesions

(a)	(b)	(c)	(d)	(e)	(f)
7.12.60	188	9	1051	5	8:1
8.12.60	191	83	1380	12	12:1
9.12.60	193	89	1520	11	
10.12.60	199	113	1274	13	25:1
13.12.60	213	94	854	15	25:1
14.12.60	213	70	356	6	50:1
15.12.60	216	92	397	5	
16.12.60	214	54	255	5	
17.12.60	193	0	17	0	Lateral Lesions Open Cage - Aphagia
18.12.60	201	34	310	5	Sleeve cage
19.12.60	197	72	576	7	Sleeve cage
20.12.60	172	0	580	8	Open cage - Aphagia
21.12.60	171	83	2216	10	Sleeve cage
22.12.60	177	88	1325	6	Sleeve cage
23.12.60	186	86	1391	5	Open cage eating

Table(19).

STEREOTAXIC COORDINATES

STEREOTAXIC COORDINATES OF HYPOTHALAMIC LESIONS
(mm. from machine zero).

(a) RAT	(b) BREGMA Rostrocaudal Lateral	(c) ELECTRODE Rostrocaudal Lat. Rt. Left	(d) DURA Rt. Left	(e) CRANIAL FLOOR Rt. Left	(f) LESION Rt. Left	(g) CURRENT Rt. Left ↙ sec ↓ amp volt	(h) BODY WT. (g)	(i) DAYS OF ADIPSIA & APHAGIA	(j) REMARKS
1A	7.25 Left 0.5	5.75 2.5 1.5	3.05 3.00	4.47 4.45	4.27 4.25	15 15 2 2	185	2	Piano wire electrode
1A	8.00 Rt. 0.65	6.5 2.35 1.65	2.37 2.35	3.40 3.38	3.20 3.18	15 15 2 2	207	8	"
2A	6.75 R 0.25	5.25 2.25 1.75	3.07 3.10	4.05 4.03	3.85 3.83	15 15 2 2	194	0	"
4A	7.50 0.00	6.00 2.00 2.00	0.98 0.98	1.90 1.91		Sham	235	1	"
4A	7.50 L 0.25	6.00 1.90 1.90	1.55 1.60	2.55 2.55	2.35 2.35	15 15 2 2	222	2	"
4A	8.75 R 0.25	7.00 2.25 1.75	1.55 1.58	2.55 2.54	2.35 2.34	15 15 2 2	264	3	"
5A	6.75 L 0.75	5.25 1.50 1.50	3.0 3.02		3.80 3.82	Sham	224	0	Current supply failed

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
5A	6.75 0.00	5.25 1.80 1.80	2.95 2.95		3.85 3.85	Sham	227	0	Current supply failed
5A	6.75 2.01	5,25 2.0 2.0	2.58 2.55	3.43 3.44	3.23 3.24	8 8 1.5 1.5 11 11	213	0	
5A	6.6	5.1 1.8 1.8	Skull 2.78	3.70 3.71	3.50 3.51	15 15 2 2 10 15	243	4	
6A	6.75 L 0.25	5.25 2.00 2.00	2.35 2.41	3.30 2.30		Sham	212	Died	Nichrome electrod
7A	7.5 R 0.5	6.0 2.0 2.0	Skull 3.00	3.7 3.7		Sham	234	0	
7A	6.5 L 0.5	5.0 2.0 2.0	Skull 2.80	3.8 3.76	3.6 3.56	15 15 2 2 10 8	265	3	
8A	6.25 L 0.5	4.75 2.00 2.00	Skull 2.9	3.90 3.90		Sham	240	2	
8A	7.0 R 0.5	5.5 2.0 2.0	Skull 2.98	4.0 4.0	3.8 3.8	15 15 2 2 15 12	260	0	

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
8A	6.75 R 0.5	5.25 2.0 2.0	Skull 2.95	4.0 4.0	3.8 3.8	15 15 2 2 7.5 6	260	0	
9A	8.0 R 0.25	6.5 2.0 2.0	Skull 2.78	3.75 3.75		Sham	261	0	
9A	8.0 L 0.25	6.5 2.0 2.0	Skull 2.78	3.75 3.75	3.55 3.55	15 15 2 2 12 16	262	8	
10A	7.75 R 0.25	6.5 2.0 2.0	Skull 2.8	3.8 3.78		Sham	286	1	
10A	8.25 0.00	6.75 2.0 2.0	Skull 2.8	3.76 3.76	3.56 3.56	15 15 2 2 21 15	295	9	
11A	6.0 R 0.5	4.5 2.0 2.0	Skull 2.55	3.55 3.65		Sham	247	0	
11A	6.0 0.00						240	Died	
12A	8.0 L 0.25	6.5 2.0 2.0	Skull 2.65	3.65 3.61		Sham	231	0	

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
12A	7.75 0.00	6.25 2.0 2.0	Skull 2.60	3.6 3.6	3.4 3.4	15 15 2 2 14 15	238	7	
13A	8.0 R 0.2	6.25 2.0 2.0	Skull 2.78	3.73 3.75		Sham	234	0	
13A	7.75 R 0.5	6.25 2.0 2.0	Skull 2.62	3.6 3.58	3.4 3.38	15 15 2 2 14 14	254	6	
14A	8.25 L 0.25	6.75 2.0 2.0	Skull 2.78	3.8 3.8		Sham	246	Died	
15A	6.75 L 0.25	5.25 2.0 2.0	Skull 2.69	3.67 3.65		Sham	239	0	Not recorded from
15A	6.5 L 0.25	5.0 2.0 2.0	Skull 2.69	3.6 3.6		Sham	239	0	
15A	7.5 L 0.25	6.0 2.0 2.0	Skull 2.69	3.69 3.69	3.49 3.49	15 15 2 2 12 18	222	5	
31	8.0 R 0.5	6.5 2.5 1.5	3.08 3.12	4.13 4.12	3.93 3.92	15 15 2 2	247	3	Piano wire electrod

(a)	(b)	(c)	(d)	(e)	(f)	(g)		(h)	(i)	(j)
43	7.5 0.0	6.0 2.0 2.0	1.67 1.70	2.62 2.60	2.42 2.40	15 2	15 2	205	5	Piano wire electrod
45	8.75 R 0.25	7.25 2.05 1.55	2.98 2.98		3.78 3.78	8 2	8 2	319	1	"
46	9.0 L.0.2	7.00 1.80 2.20	2.9 2.9		3.7 3.7	Sham		263	0	"
46	8.8 L 0.4	6.30 1.40 2.20	2.95 2.93		3.75 3.73	Sham		282	0	"
46	9.00 L 0.25	7.5 1.55 2.05	2.9 2.9		3.7 3.7	Sham			0	"
46	9.0 L 0.5	7.5 1.3 2.3	2.9 2.9		3.7 3.7	8 2	8 2	307	0	"
51	8.25 R 0.25	6.75 2.0 2.0	Skull 2.69	3.7 3.7	3.5 3.5	15 2 17	15 1.5 18		4	Nichrome electrod
B11	6.5 R 0.25	5.0 2.0 2.0	2.45 2.46	3.30 3.47	3.10 3.27	15 1.5 19	15 1.5 18	240	2	

(a)	(b)	(c)	(d)	(e)	(f)	(g)		(h)	(i)	(j)
B12	7.0 0.0	5.5 2.0 2.0	Skull 3.7	3.30 3.30	3.1 3.1	15 1.5 20	15 1.5 20	232	4	
B13	6.0 R 0.25	4.5 2.0 2.0	Skull 2.32	3.30 3.30	3.1 3.1	15 2 18	Avo Meter	226	2	Unilatera lesion
B14	6.5 R 0.1	5.0 2.0 2.0	Skull 2.29	3.26 3.31	3.06 3.11	8 2 19	8 2 17	227	3	
B15	7.5 L 0.1	6.0 2.0 2.0	Skull 2.3	3.25 3.29	3.05 3.09	8 2 21	8 1.5 18	219	0	
B16	5.75 0.0	4.25 2.0 2.0	Skull 2.35	3.32 3.23	3.12 3.03	8 2 16	8 1 22	208	Died	
B17	6.25 R 0.75	4.75 2.0 2.0	Skull 3.0	4.1 4.1	3.8 3.8	10 1 20	15 2 8	215	Died	Female
B18	8.5 0.0	7.0 2.0 2.0	Skull 3.95	4.0 4.05	3.8 3.85	15 2 18	15 2 8	280	1	Female
B20	7.0 0.0	5.5 2.0 2.0	Skull 3.1	4.05 4.05	3.85 3.85	10 2 15	10 2 18	289	3	

(a)	(b)	(c)	(d)	(e)	(f)	(g)		(h)	(i)	(j)
B21	7.5 R 0.1	6.0 2.0 2.0	Skull 3.1	4.1 4.1	3.9 3.9	10 2 16	10 1.5 21	244	Died	
B22	6.75 R 0.5	5.25 2.0 2.0	Skull 3.2	4.15 4.15	3.95 3.95	10 1.5 20	10 2 15	230	3	
D01	6.2 L 0.1	4.75 2.00 2.00	2.65 2.67	3.47 3.45	3.27 3.25	8 1 22	15 1.5 16	190	2	Minalfa Electrode
D02	6.75 0.0	5.25 2.0 2.0	2.5 2.45	3.3 3.29	3.1 3.09	15 2 18	8 1.5 20	224	2	Nichrome Electrode
D03	6.0 0.0	4.5 2.0 2.0	Skull 2.44	3.35 3.39	3.15 3.19	15 1.5 21	15 2 8	200	6	
D04	6.5 L 0.25	5.0 2.0 2.0	Skull 2.59	3.45 3.45		Sham		254	0	Large Minalfa
D04	8.25 0.0	6.75 2.0 2.0	Skull 2.59	3.60 2.59	3.40 3.39	15 2 14	15 2 19	305	Died	Nichrome
D05	6.75 R 0.25	5.25 2.0 2.0	Skull 2.62	3.45 3.45		Sham		254	0	Large Minalfa

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
D05	8.5 0.0	7.0 2.0 2.0	Skull 2.6	3.6 3.6	3.4 3.4	15 15 1.5 1.5 20 20	265	0	Nichrome
D06	6.25 R 0.25	4.75 2.0 2.0	Skull 2.85	3.9 3.9		Sham	238	1	Nichrome
D06	7.75 0.0	6.25 2.0 2.0	Skull 2.72	2.75 2.75	- 3.55	- 15 - 2 - 14	270	Died	
D07	7.50 0.0	6.0 2.0 2.0	Skull 2.85	2.90 3.85		Sham	245	2	
D07	7.5 0.0	6.0 2.0 2.0	Skull 2.73	3.75 3.7	3.55 3.5	15 15 2 2 11 12	272	3	

Appendix IV

PROTOCOL RECORD OF SERIAL SECTIONS FROM THE HYPOTHALAMUS OF A RAT AFTER PLACEMENT OF LATERAL LESIONS

The sections are from the tissue between the outer levels shown in fig. (36.a). 8μ serial sections were mounted 10 per slide i.e. representing 1.2 mm of hypothalamus in this case of Rat 9A.

Abbreviations used:-

P.V.N.	-	paraventricular nucleus
O.T.	-	optic tract
V.M.	-	ventromedial nucleus
P.M.V.	-	ventral pre-mamillary nucleus
D.M.	-	dorsomedial nucleus.

Rat 9A

Slide No.	Right Side	Left Side
42) 43)	P.V.N. present. O.T. separating laterally. Small lesion in lateral area. Correct plane	P.V.N. present. No lesion.
44) 45)	P.V.N. present. O.T. lateral. Medium lesion centred too far laterally.	P.V.N. present. Medium lesion at level of pyramid.
46) 47)	P.V.N. present. V.M. appeared. Large lesion extending up to height of pyramid.	P.V.N. present. V.M. appeared. Medium large lesion high in lateral area.
48) 49)	P.V.N. and V.M. present. Lesion still large but diminishing lateral.	P.V.N. and V.M. present. Large lesion and electrode track in correct area.
50) 51)	Only V.M. nucleus. Lesion diminishing. O.T. in lateral position.	V.M. present. Large lesion lateral to fornix and in lateral area.
52) 53)	V.M. present. Lesion still large but diminishing. Correctly placed.	V.M. only. Lesion diminishing in size. Still in correct area.
54) 55)	D.M. replaced V.M. Lesion still large. V.M. completely gone.	D.M. present. Lesion diminishing in size.
56) 57)	D.M. and P.M.V. present. Lesion disappearing quickly	D.M. and P.M.V. present. Lesion also beginning to decrease in size.

Appendix V

BEHAVIOUR AFTER INTRAGASTRIC INJECTION OF DIET

18.3.60	Rat 45
Time	
12.55 a.m.	Fasted for 12 hours previously
2.32 p.m.	10 ml diet i.g. Cage chewing and restlessness
2.37	Bar chewing
3.58	5 ml diet i.g.
4.01	Teeth gnashing
4.02	Face washed
5.17	5 ml diet - bar chewing
6.31	5 ml diet - bar chewing
6.34	Teeth gnashing
6.35	Face washed
7.21	5 ml diet - bar chewing
7.26	Face washing
4.4.60	Lateral lesions made
5.4.60	Rat 45
11.15	Recording contractions
12.02	5 ml water i.g. - bar chewing
12.52	5 ml diet i.g. - cage chewing
3.32	5 ml diet i.g. - bar chewing
3.42	5 ml diet i.g. - no result

Diet was injected at the rate of 10 ml/min.

STUDIES ON THE NEURAL CONTROL OF FEEDING IN THE RAT

by Peter Baillie

Summary of the thesis submitted for the degree of Doctor of Philosophy in the Faculty of Science.

Institute of Physiology,
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April, 1961.

Two centres in the hypothalamus are known to exert control over feeding. Bilateral destruction of the ventromedial centres causes an increased intake of food i.e. hyperphagia, and bilateral destruction of the lateral centres results in no food being eaten i.e. aphagia. At present there is only speculation as to the way in which these neural lesions act. An investigation was undertaken to detect the changes in feeding behaviour which result from lateral lesions being made in the hypothalamus of the rat.

Records of feeding behaviour were obtained from a Skinner box system in which rats were motivated by hunger to press a lever. As a result, food was made available to them and the relationship between the total number of lever-presses and the caloric intake in each 24 hour period was derived. From these results it was seen that the mean feeding patterns of rats was constant from day to day.

After anaesthesia, there was a 40% decrease in both calorie intake and lever-pressing activity. There was a 50% decrease in calorie intake and lever-pressing following a sham operation in which no brain lesion was made. Bilateral lesions in the lateral hypothalamus resulted in complete aphagia for at least three days. During the same period, lever-pressing activity was minimal. This result suggests that the urge to eat was non-existent at that period. During recovery from the lesion operation lever-pressing activity increased significantly

in every case, and was presumably due to recovery of the urge to eat. Calorie intake was zero for the first day on which lever-pressing was resumed in each experiment. Thereafter there was a recovery of feeding in some cases while other rats remained aphagic. Lever-pressing was continued throughout that recovery period. Histological examination of the brains of these rats showed that bilateral destruction of a similar area had been achieved in every case.

Rats with gastric fistulae implanted were maintained for long periods and trained to feed by autoinjection of diet through the fistula. Intragastric feeding was as efficient as oral feeding in inhibiting gastric "hunger" contractions in rats with fistulae. Sequences of grooming behaviour also followed intragastric feeding but were not seen after lateral lesions were made in the hypothalamus. Intragastric feeding behaviour did not change after rats with fistulae were made aphagic by lateral hypothalamic lesions. Although unable to ingest food orally those rats maintained their intake of food by the direct intragastric route.

The implications of those results suggest that the aphagia produced by lateral lesions of the hypothalamus is not primarily due to lack of the desire to eat. Recovery of lever-pressing activity during aphagia suggests that the urge to eat is still present although rats cannot ingest food orally. Rats with gastric fistulae are therefore able to

feed by by-passing the oral mechanisms. Further observations suggest that the neural block caused by lateral lesions affects the act of swallowing, rather than the act of mastication. No damage to any discrete nerve pathway could be detected.