AN APPROACH

to the

PHYSIOLOGY OF APPETITE.

by

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D E C L A R A T I O N

The work described herein was performed by the author, except where otherwise stated.

This dissertation has not been previously presented at any University.

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P R E F A C E.

In his study of the literature relevant to the physiology of appetite, the author of this thesis has been impressed with the large amount of work on the control of daily food intake. There are, of course, details to be filled in and final decisions to be reached. Nevertheless, in the hands of experienced workers in this field, knowledge has progressed a long way. The author has been equally impressed with the fact that there is good evidence than an animal can to a large extent balance its diet if given the components thereof separately. Here, it seems to him, the underlying physiology has barely been touched upon. The reasons for this appear to be twofold. In the first place, the study of the control of daily food intake has, from the very outset, been in the hands of physiologists and neurologists, whereas the study of differential, or specific, appetite has been in the hands of experimental psychologists to a large extent. The result of this is that these two facets of the same subject have been approached with two entirely different points of view by people with different scientific backgrounds. In the second place, the measurements used in both lines of study have been limited to the weighing and measuring of food and the weighing of animals. The author's observations of men and animals have led him to the conclusion that there are wide variations in individual patterns of eating. He knows one man, for example, who starts the day at 5 a.m. with rump steak and three fried eggs; this man is not obese. He knows other people who have the greatest difficulty in eating anything before midday and then eat but little; these people

are not emaciated. He has noticed similar though less marked variations among farm animals.

Such thoughts and observations led him to consider a line of approach to appetite in which the weighing and measuring of quantities would be but secondary to the measuring of the temporal distribution of feeding activity, for surely there must be a physiological background to this. Although his main interest is in differential appetite, he feels that this study should not be wholly divorced from the control of daily food intake. With these ideas in mind he initiated the development of the apparatus described herein.

It will be seen, by those with the patience to read this thesis, that the use of this apparatus is beset with difficulties and pitfalls which must be tackled step by step. The author was fully aware of this. He accepted the fact that the project on which he has embarked must extend over a long period of years. It is not possible, therefore, in a single thesis of this sort, to claim to have made large additions to an already large fund of knowledge. Instead, the author has described the thoughts which have led him into the path he intends to follow. He has devoted a large part of this thesis to a description of the development and construction of the apparatus he intends to use in measuring the temporal distribution of feeding activity and has described the type of information which this apparatus yields. Lastly, he has shown what lessons have been learned about the design of future experiments and what further lines of study appear, at this stage, to be profitable.

The author does not, therefore, claim to have solved major problems. He does claim to have initiated an approach which he hopes and believes will ultimately prove fruitful. This is implicit, or even explicit, in the title of this work, An Approach to the Physiology of Appetite.

I N T R O D U C T I O N.

From time to time discussions appear in the scientific press as to whether hunger and appetite are the same thing or whether one can distinguish between the two (Ivy 1945). While the writer inclines to the view of Brobeck (1957) that hunger is an extreme condition which is not normally concerned with regulation, as air hunger is not normally concerned with the regulation of respiration, he feels that the argument is fruitless and therefore has decided to avoid the issue by referring to both as the regulation of food intake. Some breadth of mind is necessary, however, since, especially in the older literature, the terms are frequently used as synonyms, and the writer has decided to take the advice of Mr. Pickwick:-

'"It's best on these occasions to do what the mob do."

"But suppose there are two mobs?" suggested Mr. Snodgrass.

"Shout with the largest," replied Mr. Pickwick.' (Dickens 1836).

While it would be indecorous to describe a body of learned opinion as a "mob", the principle of the thing is the same. Moreover, the subjective components of the states of hunger and appetite are at least as nebulous as were the policies of the "Buffs" and the "Blues" and it were better not to attempt the difficult task of describing either. The two terms will therefore be used somewhat indifferently, governed by the usage of particular authors but the writer of this thesis prefers to use the term "hunger" as representing merely a more intense condition than the term "appetite".

The history of the study of appetite and hunger divides naturally

into three sections. To a certain extent these sections converge and intermingle, but the course of development of knowledge of them followed different paths which are easiest to describe separately. First there was the primitive belief, arising no doubt in the days when learned men felt real hunger, that hunger arose in the region of the stomach. The writer is happy to say that, in spite of spending a war in Europe, he has not experienced this sensation which is described as an unpleasant one in the region of the epigastrium. That the stomach is involved in this state is clear from a considerable body of evidence, but further investigation showed that perhaps the stomach per se was not the important factor, since it might well be controlled from above. This debate led to the peripheral versus central origin of hunger controversy. The second path was the path of obesity. This, after one or two deviations, led to the present concept of the hypothalamic control of food intake and leant such support to the theory of the central control of food intake that the peripheral theory might perhaps have been neglected. Of interest here is the meeting of physiology and psychology in the field of drive and motivation. The third path has hovered on the borders of psychology almost from the start. The facts that animals are alive and that the diet necessary to keep an animal alive is far from simple have led psychologists, nutritionists, and physiologists to the conclusion that animals are able to select their own diets. Experimental evidence supports such a conclusion to a large extent,

and it has become apparent that, in eating, an animal not only answers the question of, "How much?" but the more difficult one of, "How much of what?".

This review of our present knowledge of the regulation of food intake will deal with the subject under these three heads.

THE ROLE OF THE STOMACH

Larsson (1954) undertook an informative review of the early literature. Unfortunately, little of that work is available and first hand comment is therefore impossible. Haller (1776) and Darwin (1801) postulated the peripheral origin of hunger. Although Darwin's view is somewhat at variance with subsequent evidence supporting the role of the stomach in hunger, since he attributed the sensation to quiescence of the empty stomach, these two papers seem to be the natural development of the not unexpected view that since the sensation of hunger is felt in the epigastrium the stomach must be involved. What is more interesting is the large body of opinion that postulated a hunger centre in the brain sensitive to a starvation state of the blood (Magendie 1826, Milne-Edwards 1878, Foster 1891, Roux 1897). Here the absence of the literature is frustrating in that one wonders on what evidence such surprisingly accurate postulations were based. Neither Magendie nor Roux was convinced that there were no peripheral influences at work, but their opinion seems to have been that these influences arose in a somewhat diffuse way and not specifically from the stomach. One would have thought

that the matter was clinched by Sherrington's (1900) demonstration that neither denervation nor removal of the stomach affects the regulation of food intake. The writer has not been able to obtain a copy of Sherrington's paper, the importance of which seems to have been overlooked. It seems, from information gleaned from a variety of reviews, that Sherrington measured food intake over a longish period and found it unchanged. He does not appear to have been interested in the sensation of hunger per se, in contrast to Cannon and Washburne (1912).

Cannon found that in his subject Washburne, hunger pans coincided with contractions of the stomach. His finding was fully confirmed by Carlson (1912). Carlson's subject, a Mr. V., had accidentally swallowed a corrosive liquid in his youth with the result that his oesophagus had closed up completely. He fed himself by chewing his food and then spitting it into a gastric fistula made for that purpose. This singular state of affairs was important in that it was possible for Carlson to insert a ballon into Mr. V's stomach without making him swallow. It could not, therefore, be argued that the gastric contractions were induced by the act of swallowing. The inability of Mr. V. to swallow led Carlson to another important finding. He was able to leave the balloon in situ while Mr. V. was chewing his food, and found that the act of chewing inhibited the gastric contractions. He therefore postulated that gastric motility is to a certain extent controlled reflexly from the mouth.

A significant feature of the work of Cannon and Washburne, and Carlson is that they did not claim to have made a contribution to the understanding of the regulation of food intake. Cannon and Washburne claimed to have made a contribution to the understanding of the sensation of hunger and Carlson to the physiology of the stomach. There is thus no conflict between them and Sherrington who seems to have claimed to have made a contribution to knowledge of the regulation of food intake but to neither the sensation of hunger nor the physiology of the stomach. This induces in one's mind a strong suspicion that the sensation of hunger is not necessarily a factor in the normal, day to day, regulation of food intake.

However that may be, the fact remains that hypermotility of the stomach appears in hunger states and the strong contractions correlate with hunger pangs. It was natural to suspect that the blood glucose level might be the factor determining the intensity of gastric motility. This was investigated by Bulatao and Carlson (1924). They found that intravenous injections of glucose in unfed dogs inhibited hunger contractions, while insulin injections increased gastric motility and tonus, the effects correlating well with the blood glucose level. The effects of the insulin injections could be reversed by glucose.

It is a curious fact that such carefully performed and well controlled experiments should have received only partial support from subsequent workers. Mulinos (1927 and 1933), Quigley (1929),

and Carlson and Quigley (1931) confirmed that insulin injections increase gastric motility, while Short (1929) and McKay et al (1940) found that insulin injections increase food intake. On the other hand Long and Bischoff (1930) and Freyburgh (1935) found that they do not. There seems to be little doubt that insulin does increase gastric motility, but more that it affects food intake. Indeed, Freyburgh reported that in man the suggestion that an injection would increase appetite was as important as the injection itself. However, as McKay et al. pointed out, much depends on the type of insulin used, whether its action is of short duration or long. They found a significant effect on food intake when they used protamine-zinc insulin but not when they used ordinary insulin.

The question of glucose injections and gastric motility is answered as clearly but in the reverse sense. Only Carlson and Quigley (1931) have been able to confirm Eulatao and Carlson's (1924) results. Mulinos (1927) found injections of glucose ineffective in stilling an active stomach, while Mulinos (1933), Quigley and Hallaran (1932) and Scott et al. (1938) could find no correlation between blood sugar levels and the occurrence of hunger contractions. Janowitz and Grossman (1948, 1949 a, b) and Janowitz et al. (1949) showed that parenteral administration of glucose did not depress appetite either at the following meal or over longer periods, and that intragastric administration of glucose was less effective in

reducing food intake than administration by mouth. This latter finding is reminiscent of Carlson's (1912) finding with Mr. V. that chewing inhibited gastric hunger contractions. The sum of the evidence leads to the rather contradictory conclusion that low blood sugar is a stimulant to appetite and hunger contractions, while high blood sugar does not lead to satiety.

Allied to the role of hunger contractions and blood sugar, is the question as to the origin of hunger contractions. Are they a local, autogenic phenomenon, or is there a neural mechanism at work?

Much of the work on gastric motility and its control had a clinical bias, which is not surprising in view of the desire to gain control of ulcerative conditions. Thus in 1920, Ivy reported that in dogs with acute duodenal ulcers, gastric motility, particularly gastric tone, is reduced by extrinsic denervation. Much of the evidence quoted in support of the vagal origin of gastric hunger contractions is in fact not relevant thereto. For example, Robins and Boyd (1923) made only the following reference to the vagi, "In one animal the vagi nerves were sectioned above the diaphragm. This procedure did not affect in any way the rhythmical activity of the pouch and inhibition from the stomach was as easily obtained after the operation as before." The pouch referred to was a Heidenhain pouch. Again, Beroovitz (1925) is quoted in a similar context, but his paper was concerned purely with a denervated Heidenhain pouch which was compared with an innervated pouch only to show that the

effect of ergotoxin was altered by the presence or absence of extrinsic nerves. M'Crea et al. (1927) came to the conclusion that "a decrease in the initial emptying time is the only pronounced and constant result of double vagotomy." Quigley et al. (1934) were more concerned with the humoral inhibition of gastric motility than with vagal control. Finally, Quigley in 1942 made the following statement, "The mechanism controlling the spontaneous initiation and termination of hunger contractions is unknown. Vagotomy tends to depress and splanchnicotomy to augment this motility in a characteristic manner. Nevertheless, hunger contractions occurring in the completely denervated stomach are strikingly similar in the transplanted gastric pouch and the main stomach from which it was made. This indicates the act of an unidentified humoral factor...."

The death knell to the stomach as a peripheral sensing organ seems to have been sounded by Grossman et al. (1947) who found that insulin augments food intake both in normal dogs and in dogs with extrinsically denervated stomachs and suggested that insulin hypoglycaemia acts directly on the brain to excite food taking behaviour; and by Grossman and Stein (1948) who found: "The sensations of hunger induced by insulin continue to occur after complete vagotomy in man. These sensations include feelings of emptiness and weakness. In those persons in whom epigastric pangs of distress associated with individual gastric contractions are a part of the sensation complex of hunger, vagotomy, by abolishing the contractions, eliminates this

particular kind of sensation. The removal of this component of the sensation complex of hunger is recognised by the subject, but it does not cause a significant change in the general affective response to hunger.

"In most of the subjects, the gastric component of hunger sensations is absent or negligible both before and after vagotomy. In these subjects, vagotomy caused no detectable change in the hunger response to insulin.

"Both gastric and extra-gastric stimuli contribute to hunger sensations in man. In most individuals, the extra-gastric components predominate in the sensation complex of hunger. Elimination by vagotomy of the gastric component of the hunger sensation complex (hunger pangs) has no significant effect on the manifestation of the extra-gastric component (feelings of weakness and emptiness associated with the desire for food). Vagotomy abolishes the gastric pangs by abolishing the gastric hunger contractions, not by interrupting the sensory pathway."

It appears, then, that the whole controversy was sparked off by the fact that both Cannon and Carlson found themselves subjects who, quite by chance, were among the minority who experience hunger pangs.

THE ROLE OF THE HYPOTHALAMUS.

In 1840, Mohr reported the case of an obese woman with some genital dystrophy. It appears that she died under circumstances

which permitted an autopsy, and it was found that she had a tumour in the region of the pituitary. In 1901, Fröhlich reported several cases of a similar nature and attributed the syndrome to a dysfunction of the pituitary. The syndrome came to be variously called the Fröhlich syndrome, the adiposogenital syndrome, and dystrophia adiposogenitalis. Its description and the fact that Fröhlich attributed it to pituitary dysfunction led to a controversy which extended over a long period of years.

Erdheim, in 1904, was the first to question Fröhlich's conclusion as to the cause of the syndrome. He argued that since the hypophysis was relatively undamaged in some cases and no particular type of tumour was responsible, and since the one factor common to all cases was compression of the base of the brain, it was to this last common factor that one must look for the cause of the syndrome. He therefore suggested that a neural lesion was responsible.

A curious feature of the controversy that followed was that no one seems to have thought of the possibility that the obesity characteristic of the syndrome was the result of excessive eating. It is not clear from the literature whether or not the concept of energy balance had arisen at that time. If it had, then in this controversy the input side of the balance was entirely ignored and attention was directed to the use of energy. Since the hypothalamus is the part of the brain most vulnerable to the tumours in Fröhlich's syndrome, it was natural that a great deal of attention should have

been devoted to possible hypothalamic control of metabolism, starting with Karplus and Kreidl in 1909. Interesting and profitable though this off-shoot of the controversy turned out to be, it is not proposed to follow it here since it is irrelevant to the matter in hand.

The controversy therefore narrows down to two questions. If the pituitary is removed without damage to the brain, will the Fröhlich syndrome develop? Is it possible to produce the Fröhlich syndrome by hypothalamic lesions without damage to the pituitary?

As reported by Erobeck et al. (1943), Aschner, in 1912, produced adiposity in dogs subjected to hypophyseal operations in which the infundibulum was damaged. This paper is not available to the writer and comment is therefore not possible, but, in 1913, Camus and Roussy, in investigating the role of the hypophysis in diabetes insipidus, found that their dogs with almost complete hypophysectomy remained in good health apart from polyuria and polydipsia. In 1920 they reviewed their own work. Most of it is concerned with diabetes insipidus, but they found that in three dogs with almost complete hypophysectomy there was no genital atrophy; in two other dogs, one with partial hypophysectomy and one with an intact pituitary, they cauterized the surrounding region, which resulted in the first dog in genital atrophy and obesity and in the second in genital atrophy alone. They concluded that both were attributable to brain damage and that the genital atrophy part of the syndrome could be separated from the obesity.

More definite evidence came in the following year when Bailey and Bremmer (1912 a, b) published two papers which are of great importance in the physiology of water balance but which contain the following statement: "Lesion of the tuber cinereum has produced in two dogs a cachexia hypophyseopriva with genital atrophy, and in two other dogs an insidiously developing adiposogenital dystrophy. The integrity of the pituitary was in each case verified histologically." The very close similarity in the wording of the two papers suggests that they both concern the same experiments. This very clear implication of the hypothalamus in obesity was confirmed by Camus and Roussy in 1922.

The decisive work was that of Smith who reported in 1927 and again in 1930. His careful contrast of the effects of hypophysectomy without brain damage with those of lesions of the tuber cinereum without hypophyseal damage demonstrated beyond peradventure that the Fröhlich syndrome was hypothalamic in origin. In the interim between these two papers, Grafe and Grunthal produced similar evidence (1929).

Further confirmation of the involvement of the tuber cinereum in obesity appeared from time to time from workers in the field of diabetes insipidus, but two important questions remained unanswered: what part of the tuber cinereum was involved, and what effect were such lesions producing that could lead to obesity? In 1931, Newburgh emphasized the importance of overeating in the etiology of all forms of obesity, and in 1932 Fulton et al. drew attention to the increased

appetite which accompanies frontal lobe lesions. Keller et al. (1933) came rather nearer to the matter in hand in reporting that transient adiposity in dogs and cats with hypothalamic and pituitary lesions is associated with enhanced appetite. It thus appears that at that time, thought on the etiology of obesity was turning away from metabolism towards overeating.

Although the idea that overeating was an important factor in obesity was being mooted, there was, at this time, still a great deal of work being done on the possible relationship between the hypothalamus and metabolism. It was in this psychological climate that Hetherington, usually in collaboration with Ranson, embarked on a thorough examination of hypothalamic obesity as it was now called. In 1939 Hetherington and Ranson finally exonerated the pituitary from any involvement in the syndrome. In 1940 Hetherington and Weil made a careful examination of the chemical composition of the bodies of fat rats. They found a large increase in fat deposition with a tendency to mineral depletion, especially calcium and they attributed these results to disordered metabolism. In the same year, Hetherington and Ranson used Clark's adaptation of the Horsely-Clark stereotaxic instrument in placing lesions in the hypothalamus of the rat. Their lesions were large and they could only indicate the approximate area where those effective in producing obesity must be placed, but they took the opportunity of making a thorough examination of the endocrine status of their animals. The only glands which

showed changes as compared with those of normal rats were the gonads and their appendages. In 1942 they returned to the attack with smaller lesions and produced important results. They found three types of effective lesions, those involving the anterior two thirds of the ventromedial hypothalamic nucleus, those involving the caudal one third of the same nucleus and the premammillary area, and those involving an area dorsolateral to the mammillaries. Unilateral lesions at all levels and midline lesions were ineffective, and the fornix was exonerated.

Thereafter it was possible for other workers to place lesions accurately. In the same year Brooks and Lambert produced obesity in the monkey by placing lesions in the ventromedial nucleus and Ruch found that lesions in the posteroventral thalamus and rostral mesencephalic tegmentum were effective in the same species.

Thus 1942 was a significant year. It ended with an outline of an anatomical system starting in the ventromedial hypothalamic nucleus and descending round the mammillaries to the mesencephalic tegmentum. The integrity of this system was necessary for the prevention of obesity.

It appeared that the answer had been provided as to what part of the hypothalamus was involved. The question as to how it gave rise to obesity was finally answered in 1943 by Brobeck et al. They showed beyond doubt that hypothalamic obesity was due not to a disturbance of metabolism but to overeating, which they called hyperphagia. Hetherington and Ranson's (1942) descending pathway

now took on a new meaning. The ventromedial nucleus became the satiety centre which is in some way responsive to a stimulus state that arises when an animal has had enough to eat. The downward or caudally projecting pathway is an inhibitory one, acting, although Brobeck et al. did not say so, on those motor centres in the medulla which are responsible for eating activity.

The work of Brobeck et al. (1943) was fully confirmed by Brooks and Lambert (1946) who added the interesting fact that even if hyperphagia was prevented by limiting food intake, it none the less returned when food was provided ad libitum. These authors described the dynamic phase of obesity, the period of rapid weight gain, and the static phase, the period during which obesity acquired in the dynamic phase is maintained. Fasting during the static phase to bring the rat's weight down to that of the unoperated littermate was followed by a renewed dynamic phase during which the animal once more became obese, possibly more obese than before. There is thus no doubt that the effect of the lesions in the ventromedial hypothalamic nucleus is permanent and not some passing disruption of a normal control mechanism. Even in pair fed animals, however, those that were potentially obese showed a slight advantage in weight gain over their controls. Brooks (1946) attributed this to a slight decrease in activity. However, a similar experiment performed by Tepperman et al. (1943) yielded a somewhat different result. Here the control rat was trained to eat its daily ration of food in three hours, as

was the curstom of the pair fed operated rats. The control rat then developed a metabolic pattern similar to the operated rat. This led the authors to attribute the slight weight gaining advantage of the operated, pair fed animals to a change in feeding habits. Tepperman et al. did not measure activity and there is thus no evidence that the trained control maintained its usual activity at night. It must have made some sort of change in order to avoid committing mayhem on the laws of thermodynamics.

It is therefore accepted that the state of obesity resulting from lesions of the ventromedial hypothalamic nuclei is due in the main, if not entirely, to a large increase in food intake without any compensating increase in metabolic rate. This nucleus is called the "satiety centre" which signals the fact that an animal has had enough to eat, but a satiety centre alone is not enough. There must be something on which it can act, a mechanism responsible for the urge or willingness to eat.

Anand and Brobeck (1951 a, b) found that lesions of the lateral hypothalamic area at the same rostro-caudal level as the ventromedial nucleus resulted in cessation of eating even to the point of starvation. Effective lesions, like those of the ventromedial nucleus, had to be bilateral. Since lesions in this region in previously operated, hyperphagic rats stopped the hyperphagia and converted it into aphagia, it was concluded that the lateral hypothalamic area is the basic centre and its act vity is modulated by the ventromedial nucleus. They obtained similar results in cats, and Anand

et al. (1955) obtained confirmation in monkeys and cats. The area of such lesions includes the lateral hypothalamic nucleus, the medial forebrain bundle, and the direct amygdalo-hypothalamic fibres, but Anand and Brobeck were of the opinion that the decisive factor was the nuclear lesion.

A considerable amount of work (Morgane and Kosman 1957, 1959 a, b, 1960, and Pribram and Bagshaw 1953) indicates that the amygdala are involved in the regulation of food intake, but lesions in that region alter the behaviour pattern in the direction of increased intake, so that the result obtained by Anand and Brobeck is not likely to be due to interruption of the amygdalo-hypothalamic fibres. Morgane (1961 a) effectively showed that the medial forebrain bundle is not involved in the regulation of food intake. Thus the probability that the effective lesion in the lateral hypothalamus is indeed one of the lateral hypothalamic nucleus almost amounts to a certainty.

In 1957, Morrison and Mayer noted that, while placing lesions in the lateral hypothalamic nucleus, their full sham operated animals (that is animals in which the electrode had been inserted as far as the target but no current was passed) showed a brief period of aphagia following the operation. Morgane (1961 b), noting that the electrode track passed through the lenticular fasciculus and realising that this tract is efferent to the globus pallidus, (Hanson and Hanson 1939), Ariëns-Kappers et al. 1936, Bard and Rioch 1937, and Laruelle 1934), suspected that it was this damage to the lenticular fasciculus which

caused the phenomenon observed by Morrison and Mayer. He therefore placed lesions in the globi pallici and succeeded in reproducing the aphagia usually obtained by lateral hypothalamic lesions.

Support for the theory that coming events cast their shadows before them is to be found in the paper published by Ingram, Barris and Hanson in 1936 on the association between hypothalamic lesions and catalepsy. They state, "... During the first days and weeks after operation the cats showed no interest in food and required feeding by tube. After the somnolent stage was passed they began to indicate hunger by cries and apparent restlessness. However, even though hungry they rarely ate if left to their own devices. It was necessary to give milk by stomach tube or by introducing the fluid into the mouth from a bottle. Meat was taken only when held to the lips, and often only when forced into the mouth. Once the food was in the mouth there seemed to be no difficulty; it was masticated and swallowed readily and even voraciously. Thus there was no true dysphagia, except possibly in one case (cat 5) in which the meat was not swallowed even when placed in the mouth." In cat 5 the lesions were such that they could have involved the lateral hypothalamic area on both sides and the ventromedial hypothalamic nucleus on one, though this is doubtful. They thus almost precated by 25 years the discovery by Morgane (1961 b) that there are two parts of the lateral hypothalamic feeding area.

There is a far lateral part which gives absolute aphagia, and a mid-lateral part, somewhat lateral to the columna fornicis descendens, which gives a temporary aphagia accompanied by lack of motivation or drive. This finding confirmed the previous observations of Teitelbaum and Stellar (1954) and Williams and Teitelbaum (1959) that there can be recovery from aphagia resulting from lateral lesions. In an extension of his studies, Morgane (1961 c) produced good evidence that the medial forebrain bundle, although not involved in the basic feeding mechanism, is involved in hunger drive. While the physiology of motivation and drive is not strictly germain to this work, an important fact arises. When Morgane placed lesions in the medial forebrain bundle and then stimulated the far lateral area, the animals would still eat but lacked drive. Thus while there is an efferent neural pathway for the drive mechanism, there has been no demonstration of an efferent pathway for the basic mechanism.

In various stimulation experiments (Anand and Dua 1955, Andersson 1951, Delgado and Anand 1953, and Larsson 1954), the latency between the onset of stimulation and the feeding response in long. This has suggested to Delgado and Anand that transmission is humoral rather than neural. However, the critical evidence of the extraction of a chemical factor which, on injection, will cause feeding is yet to come. There is an interesting suggestion here, especially in view of Olds (1958), that there is a basic drive mechanism which is neural but without direction, and a basic feeding mechanism which is humoral and

adds direction but does not possess drive. Against this background, the lack of drive in the hypothalamic hyperphagic rat (Anlicker and Mayer 1955, Anlicker and Mayer 1957, Miller 1957, Miller et al. 1950) would seem to arise from lack of a cut off to the feeding mechanism, which, of its very nature, will make an internal state of need impossible and thus make drive impossible.

Thus lesions and stimulations tend to confirm that a feeding centre exists in the lateral hypothalamic area, probably in the lateral hypothalamic nucleus, and that this nucleus probably receives activation at least in part from the globus pallidus. There appears to be, at present, no evidence of an efferent neural pathway from this nucleus but a faint suggestion that it might obtain its effects by humoral means. At some convergence point, either at the hypothalamic level or lower in the brain stem, the activity of the ventromedial nucleus inhibits the effects of the lateral hypothalamic area. The evidence as to the level at which this happens is not clear, although Forssberg and Larsson (1954) found evidence of changes in metabolic activity in the lateral hypothalamic nucleus in the hungry and fed animal, and this in turn suggests that the action of the ventromedial nucleus is a direct one on the lateral hypothalamic nucleus.

The fact that the existence of this feeding and satiety mechanism may be said to have been proved raises the pertinent question in the physiologist's mind, "What are the stimuli which control it?" Nothing is known about the stimuli which activate the lateral feeding

area, but there has been some debate about those which operate the satiety mechanism. The elimination of the stomach as a peripheral receptor giving rise to sensations of hunger and satiety, already discussed, and the failure of Scott et al. (1938) to correlate periods of hunger contractions with lowered blood sugar, led Brobeck (1948) to consider the control of appetite as a factor in temperature regulation.* His position was strengthened by the previous work of Booth and Strang (1936) which gave a strong suggestion that there is a correlation between skin temperature and satiety as a high protein meal progresses. Brobeck's suggestion that animals eat to keep warm bears a strong resemblance to Adolph's (1947) that animals eat for calories, and is embodied in his thermostatic theory of the regulation of food intake which is based on the inverse relationship between environmental temperature and appetite.

Support for Brobeck's theory came from Stevenson and Rixon (1957) who investigated the increase in activity shown by rats deprived of food and water. If the animals were immobilized, the deep body temperature fell.** It appears from these experiments that, failing sufficient food to preserve the deep body temperature, the animals resort to running. The biological value of this is incidental, but none the less important, since such an increase in activity is likely to take them from an area of dense population and low food to an area of sparse population and more food, almost as if they were obeying the laws of gaseous diffusion. Brobeck stated his thermostatic

theory with great clarity in 1957.[10] Following eating there is a rise in temperature, and this is greatest when the meal has a high specific dynamic action, such as a high protein diet. Following eating there is a slight rise in central body temperature and a greater rise in skin temperature. The hypothalamus contains cells that are responsive to an increase in central temperature. Granted these premises, Brobeck suggests the following process of satiation:-

1. Sensory input from the gastro-intestinal tract signals that eating is in progress.
2. With a few minutes the rate of heat production rises.
3. The consequent rise in body temperature might act direct on the hypothalamus or by vaso dilatation through skin receptors, or both.
4. Activity other than eating is inhibited.
5. A critical temperature is reached at which eating stops.
6. Within a few hours temperature falls and eating once more becomes possible.

He states that the specific dynamic action of the food is not the only factor, but that it, together with body temperature, can account for most phenomena. Indeed, in 1960 Brobeck had the opportunity of defending his theory against all comers. He made one small change; an actual variation in body temperature is not necessary provided that there is a change in the balance of activity

between those hypothalamic mechanisms responsible for heat conservation and production and those responsible for heat loss. That there should be a linkage in the hypothalamus is reasonable enough and the defence was lively and highly successful, but the final proof is lacking. The fact that in 1961 Han and Brobeck failed to reveal any abnormality in the temperature regulation of hypothalamic hyperphagic rats is not disproof of the theory. All that is necessary for hyperphagia is a break in the linkage between the temperature regulating mechanisms and the hunger-satiety mechanism.

In apparent conflict with Brobeck is the school of though centred on Mayer. In 1951, Mayer and Bates suggested that the regulation of food intake was basically biochemical. This was followed in 1952 by a paper by the same authors describing how they could alter food intake by manipulating the blood sugar within the physiological range in normal animals. If, however, the homeostatic mechanism was disrupted by hypophysectomy and alloxan treatment, then the effects of induced hyperglycaemia were much more marked. This led them to suggest that there is a glucostatic mechanism of the regulation of food intake. Mayer's argument (1953) was that since the fat and protein contents of the body have high inertia, they cannot be involved in the hour to hour regulation of food intake. On the other hand, the glucose content of the body is limited and labile and is the most likely variable to be controlling intake. The difficulty which this theory faced was the large appetite in the

diabetic with a high blood sugar. This problem was approached by van Italie et al. (1953) who took samples of finger and venous blood in man and found a close correlation between the hunger state and what they called the arterio-venous blood sugar difference, or delta glucose. The fact that it was capillary rather than arterial blood that they used does not affect the validity of their argument.* When the difference is large, the appetite is small and vice versa. In the diabetic, where the threshold for entry of glucose into cells is high, the venous level approximates to the arterial level and gives a high reading of venous blood sugar level when utilization is low and appetite large. It is only when the arterial level becomes very much higher than usual that the entry threshold is exceeded and the glucose becomes available with a decrease in appetite.

We may take it as proven that appetite is inversely proportional to the arterio-venous blood sugar difference, but for the glucostatic-mechanism to be accepted, it is necessary to show that the ventromedial hypothalamic nucleus is sensitive to this difference. Brecher and Waxler (1949) and Anderson (1953) described the development of obesity in mice after the injection of the L.D.$_{50}$ of goldthioglucose. This obesity has been shown to be essentially similar to that resulting from hypothalamic lesions (Pregly, Marshall and Mayer 1957, Hollfield et al. 1955, Larsson and Ström 1956, Marshall and Mayer 1954 and Mayer and Higbera 1954). Marshall et al. (1955) found that the hypothalamic damage resulting from the administration of goldthioglucose

was confined to the ventral part and was most extensive in the infundibular region. Marshall and Mayer (1956) demonstrated that the gold radical was toxic and the glucose radical was necessary for entry into cells. They therefore suggested that the glucose uptake of the ventromedial nucleus, or at least that general area of the hypothalamus, is high and it might thus act as a gluco-receptor. However, Owen et al. (1953) described lesions outside the hypothalamus as a result of the action of the drug, and Larsson (1959) reported increased inactivation of insulin by the livers of goldthioglucose obese mice, suggesting, if not damage, at least altered cellular activity.

Since Mayer's theory depends upon the proof that the ventromedial hypothalamic nucleus is a gluco-receptor, it must be regarded as not proven. In any case, the theory meets with one major snag; it cannot account for the high satiety value of protein. The writer finds himself doubting if there is any real conflict between Mayer and Brobeck, since, if the arterio-venous glucose difference is high, cellular metabolism and therefore heat production will also be high and an animal might then well find itself in a thermal crisis which will, according to Brobeck, prevent its eating. Although the final proof as to what is the stimulus to the ventromedial hypothalamic nucleus is yet to come, it appears that the thermostatic theory of Brobeck meets with fewer objections than does the glucostatic theory of Mayer, and that a combination of the two meets with no objections at all.

In his discussion of the etiology of obesity, Mayer (1955) referred to the possibility of a lipostatic regulation of food intake. The existence of this possibility is clear from the behaviour of hypothalamic obese animals which reach a certain degree of obesity and then return to normal food intake. It is tempting to postulate that the fat depots are exerting some sort of influence on the hypothalamus and that the threshold of response to this influence is raised by lesions of the ventromedial nucleus. There is at present no evidence as to what the nature of this influence might be, but, because of the inertia of the fat depots, such a regulation must be a long term one.

HOW MUCH OF WHAT? OR DIFFERENTIAL APPETITE.

It is a fact that the various species of animals, including man, have not only survived over a long period of years, but have indeed thrived without any knowledge of their own nutritional requirements. It is legitimate to ask if this survival is purely a matter of chance or whether there lies behind it some regulating mechanism which guides an animal to the food it needs and thus makes homeostasis possible. The first indication that the matter is not purely a question of chance came in a paper by Osborne and Mendel in 1918 (a paper which is not available to the writer). They gave rats the choice of a balanced and an unbalanced diet and found a distinct preference for the balanced one.

Although this appears to have been the first controlled experiment, there have been from time to time numerous reports,

often in obscure journals, of the tendency of various sorts of livestock to select their diets (Evvard 1915, 1922, Evvard et al. 1927, Godden 1926, Nevins 1927, Orr 1929, Price 1929). It was clear that here was a case to investigate in controlled experiments more detailed than those of Osborne and Mendel.

Appetite for minerals.

Salt.

In 1936, Richter gave adrenalectomized rats the choice of water or 1% saline, and in another group the choice of water and 3% saline. The control rats preferred water to saline, while the operated ones showed a strong preference for saline and ingested sufficient to keep themselves alive for considerable periods. Further, if normal rats were given a salt-free diet, but access to salt solution, the amount of salt they ingested each day was approximately that provided in the standard McCollum diet (0.577 gram/ Kg body weight as opposed to 0.659 for the McCollum diet). Richter and Eckert (1938) repeated the experiment, giving the adrenalectomized rats the choice of a variety of sodium salts and a variety of chlorides. It was apparent that the increased appetite was for sodium rather than for chloride. Pregnant rats also showed an increased appetite for sodium chloride (Richter and Barelare 1938, Barelare and Richter 1938). The increased salt appetite of adrenalectomized rats is reversed by administration of desoxycorticosterone acetate (Richter 1941), a finding that was confirmed by Clark and Clausen (1943). Although Scott (1945) and Scott et al. (1948) disagreed with Richter

on many points, they were at one with him in holding that salt appetite is dictated by need and is increased in pregnancy.

We may therefore take it that an appetite for salt which varies with the animal's needs is firmly established. Indeed, the existence of such an appetite in man is established by the case reported by Wilkins and Richter (1940) and is strongly suggested by Davis (1939).

Calcium.

In three studies of the effect of parathyroidectomy on appetite, Richter and Eckert (1936, 1937, 1939) found an increased appetite for calcium [redacted]. The amounts of calcium lactate eaten were sufficient not only to keep the animals alive but even to reverse the symptoms of parathyroid deficiency. Richter and Barelare (1938) found the expected changes in appetite during pregnancy and lactation, an increase in appetite for calcium during pregnancy, a further increase during lactation, and a return to normal at weaning. There seems to be no other evidence in support of these findings or in opposition to them.

It is thus established that in so far as two of the major cations are concerned, appetite is adjusted to supply the needs of the moment, that, as Richter would have liked to put it, the organism, acting as a whole, behaves in such a way as to support its homeostatic mechanisms. There is an indication that this also applies to phosphate though not perhaps to chloride. This latter deficiency

should not cause surprise, even if it is confirmed, since sodium commonly occurs in the form of chloride and in correcting a sodium deficiency, an animal would automatically correct a chloride deficiency and the processes of natural selection would not operate in favour of an animal which had a mechanism for correcting its chloride balance by adjusting intake. Such an adjustment of sodium intake is clearly necessary for survival of terrestial animals which are subjected to continuous and varying losses. It is true that such losses are to a large extent controlled by the kidney, but the kidney can only act in the direction of conservation; it can never make good a deficiency. In this sense, thirst can be regarded as a special appetite for a mineral which results in the search for and ingestion of water in support of the conserving activities of the kidney.

Other dietary constituents, however, have nothing analagous to the kidney to act as a conservation organ, with the result that the maintenance of a proper balance between the organic constituents of the body must rest heavily on adjustments of intake.

Appetite for organic dietary constituents.

It was implicit in Osborne and Mendel's (1918) original paper that such appetites must exist, since it appears that the difference between the two diets offered to the rats lay in more than mineral imbalance. The definitive experiment to test this deduction was performed by Richter, Holt and Barelare in 1937. The choice offered to their rats consisted of purified casein, olive oil, dextrose, sodium chloride, calcium lactate, dry yeast, and cod liver oil.

The rate of growth of animals given this choice was better than that of the controls on the standard McCollum diet and oestrus cycles and activity were normal. Two types of diet were selected. Some rats preferred a high fat diet and other a high carbohydrate, but the two groups showed no differences in cycles, activity, or growth. Withdrawal of cod liver oil led to a definite cod liver oil appetite when it was replaced, from which Richter, Holt and Barelare concluded that there was an appetite for vitamin A which was correcting a deficiency. When yeast was withdrawn, there was a definite appetite for it when it was replaced, again suggesting the correction of a vitamin deficiency.

The selection offered showed certain deficiencies, and these were corrected in another experiment, the results of which were reported in 1938 by the same authors. In the 1937 experiments, the rats efforts at breeding were not very successful, which was attributed to the lack of Vitamin E. This was corrected by the addition of wheat germ oil as a separate choice. Sodium phosphate and potassium chloride were also provided. Breeding was now successful and, although growth was as rapid as on the McCollum diet, the intake of total calories and total solids was less. Thus the ability of rats to select their diets from purified ingredients seemed to be established and in 1939 the final report of C.M. Davis on experiments with children indicated that such an ability is shared by man.

This report is a happy combination of fascination and interest. It is true that the experiment differed from those of Richter, Holt and Barelare not only in the choice of animal, but also in the fact that the infants had the choice of natural foods rather than purified ingredients. The children were weanlings and were therefore unprejudiced by parental conditioning. The foods offered to them were unmixed and in the main uncooked, and their attendants served simply to assist them and not to guide. That the children throve on the diet goes without saying, since one must presume that the experiment would have been abandoned were this not so. The interest lies in certain special responses. There was, for instance, the pause in eating in order to ingest salt which appeared to be taken as a duty rather than a pleasure. There was the child with rickets who voluntarily took cod liver oil until such time as the condition was healed. Finally, there were the changes in diet associated with infection, the changes in food intake coming hours before there was any overt sign of a change in the clinical condition of the children.

These two papers are in marked contrast to that published by Ken in 1931 in which he found that rats one week after weaning were unable to select an adequate diet on the free selection basis. They selected a very low protein diet and made poor growth, having a high calorie intake per gram of weight gain. The carbohydrate provided was sucrose, and the question arose in Ken's mind as to whether the

young rats became addicted to sweetness, as indeed many animals do. He therefore changed his carbohydrate to rice starch after the experiment had been in progress for forty-nine days. This had no effect on the protein intake, but two of the rats died shortly after the change, one from peritonitis and one from undisclosed causes, so that the final period from forty-nine to sixty-nine days was a measure of the performance of two rats only. It would have been more satisfactory to repeat the whole experiment using a different carbohydrate. The fact remains, however, that these rats failed to select sufficient protein to maintain a reasonable rate of growth. Kon's protein was "caseinogen" prepared in his laboratory, whereas Richter's protein was casein which he describes as purified. There might be something in this difference which led the rats to avoid protein unless the drive to eat it was higher than usual. However, the results of this experiment gain significance because they are supported by later work.

Scott (1946) and Scott and Quint (1946 b) found that 53 out of 80 weanling rats ate protein, and thought that the taste for protein might be quite capricious. This did not appear to be due to a dislike of casein per se, since roughly the same proportion of animals refused to eat protein if the one provided was casein, lactalbumen or fibrin. The only aversion, which appears to have been general, was for egg albumen. Indeed, they could find no evidence of an appetite for protein.

Scott and Verney (1947) found the appetite for carbohydrate was independent of that for protein but inversely proportional to that for fat, as if the animals satisfied their energy requirements from fat or carbohydrate and then took protein as a sort of supplement. However, Scott et al. (1948) found some indication of an increase in protein appetite with age. Out of 31 rats 21 days old, only 9 selected protein in their diets, but after the age of six weeks, 60 per cent selected protein. Harkentin et al. (1945) also found an increase in protein appetite with age but did not find an inability to select enough in the very young rat. It appears, then, that the very young rats might be unable to make reliable selections, and this is one important difference between the experiments of Scott (1946) and Mon (1931) on the one hand and those of Richter, Holt and Barelare (1937, 1938) on the other; Richter did not use very young rats. The curious result arises that the relatively unprotected weanling rat cannot select its own diet while the far more protected human infant can, but there is a difference between purified ingredients and natural foods. The question of flavour comes into it. Scott and Quint (1946 b) investigated this by adding flavours to the foods and found the rats indifferent to the additions. This, however, cannot be taken as conclusive evidence. Man is quite capable of distinguishing individual instruments in an orchestra; might not other animals with a more highly developed sense of smell be able to distinguish particular odours in an orchestra of smell? We do not know, and

at the moment there seems little chance of finding out. We can only conclude that on present evidence the existence of an appetite for protein is doubtful.

Not so with carbohydrate and fat. The inverse relationship between fat and carbohydrate appetites described by Scott and Verney (1947) is underlined by Richter and Schmidt (1941) who found an increased appetite for fat and a decreased appetite for carbohydrate in pancreatectomized rats. On the standard McCollum diet, these rats showed polydipsia (presumably the result of polyuria) increased appetite, and hyperglycaemia, these "diabetic" symptoms being largely removed or ameliorated on the self selection diet. One wonders what would have happened to these animals had they been kept for a longer period after operation (they were kept for 40 days) since they must inevitably have become ketotic. It is quite possible, of course, that the pancreatectomy was not complete, since the rat has a very diffuse pancreas which is difficult to remove in toto. There might, therefore, have been sufficient intermediaries from carbohydrate metabolism to make the proper use of fats possible. That the change in the selected diet was indeed associated with the insulin deficiency was demonstrated by Richter (1942) when he increase the dextrose intake of rats by giving insulin injections. Other experiments on the effects of endocrine function were performed by Warentin et al. (1943) who altered the thyroid status of rats. Here there were no differential effects on dietary constituents, but merely a general

increase or decrease in all of them.

Since it is clear from Richter and Schmidt (1941) and Richter (1942) that the m tabolic consequences of insulin deficiency or excess have an effect on the carbohydrate and fat appetites, it is pertinent to ask whether other means of altering metabolism can have their appropriate effects. It was known for a long time that a deficiency of "vitamin B" will depress appetite (Karr 1920, Cowgill 1921), but when detailed investigations were made by Richter et al. (1938), Richter and Barelare (1939) and Richter and Hawkes (1941) it became apparent that the effect of the deficiency was to depress appetites for protein and carbohydrate specifically, so that the bulk of the calorie intake was in the form of fat. It seems therefore, that the accumulation or possibly deficiency of intermediate metabolites must act as a stimulus to some unknown part of the central nervous system and alter the affective response to the taste or smell of the basic foodstuffs. The aversion which Richter's animals developed for protein is interesting. If there is no protein appetite, the protein appetite cannot be reduced, or so it would seem, and yet this aversion developed. The suspicion must therefore be aroused that there is in fact a protein appetite but that in certain circumstances, not immediately apparent, it cannot be detected.

We can conclude at this stage that appetites for carbohydrate and fat exist and that there is a disctinct possibility that a protein appetite also exists. These appetites change with changes in

metabolic patterns. In the case of a disturbed metabolic pattern due to a deficiency or excess of insulin, the changed appetite will prolong the animal's life but will not cure the condition. When the disturbed pattern is caused by a vitamin B deficiency, however, this prolongation of the animal's life might well have survival value since it will give an opportunity for the correction of the deficiency. For the survival mechanism to be effective, that rat's behaviour must be such that its chance of acquiring the missing vitamin is enhanced. In 1937, Richter, Holt and Barelare demonstrated not only an increased appetite for the B vitamins but a craving for them in rats suffering from a deficiency. This craving was so startling that it is worth quoting from the paper.

"(Vitamin B deficient) rats show an overwhelming appetite for vitamin B in pure crystaline form, either B_1 or riboflavin. Vitamin B_1 was given in the form of an aqueous solution of thiamine chloride in graduated, inverted bottles. One vitamin deficient rat drank 11 cc. or 5,500 international units in less than half an hour; another rat drank 29 cc or 14,500 international units in 24 hours. The odour of the vitamin as well as its taste arouses great interest. This is shown by the fact that the rats found the bottles at once, even when as many as 12 other containers filled with different foods or solutions were present in the cage at the same time. It was difficult to stop the animals from drinking the substance, once they had tasted it. Efforts to remove the bottle were met with fierce resistance.

The bottle was held tightly with both paws and even with the teeth. By reaching far up into the bottles the rats made every effort to obtain every remaining drop of the vitamin. Riboflavin ...., 0.05% solution elicited a similar though less marked craving. Due to the small available amount of the vitamin preparations, it was not determined how long the craving might remain evident."

Richter and Hawkes (1941) showed that such rats had a strong appetite for nicotinic acid as well as for thiamine and riboflavin. Their work was substantially confirmed by Scott and Quint (1946 c) who found that deficient rats developed appetites for thiamine, riboflavin, and pyridoxine, but not, rather surprisingly, for pantothenic acid. Indeed, Richter and Rice (1945) found that deficient rats would eat up to 3 to 5 grams of the faeces collected from normal adult rats and could thus supply themselves with the needed vitamins.

There is thus clear evidence that there are separate appetites for different dietary components, sodium, calcium, phosphate, carbohydrate, fat, possibly protein, thiamine, riboflavin, nicotinic acid, and pyridoxine. There might, indeed, be others. It is apparent that the appetites for these dietary components are related to metabolic need, but it is not apparent how this regulatory mechanism works, nor what the mechanism is. Young (1941) has it that there is no general hunger except in the sense that there is a sum of individual hungers. Neither Brobeck nor Mayer would agree, and their evidence indicates

that there is in fact a general hunger. The fact remains, however, that there are these specific hungers, and whether they sum to form total hunger, or whether they are modulators of a general hunger state is not determined. Brobeck et al. (1943) found that rats with hypothalamic hyperphagia selected the same diets as controls, which suggests strongly that the ventromedial hypothalamic nucleus, although concerned with general satiety, is not concerned with differential appetites.

Richter (1939 a) and Richter and MacLean (1939) found lowered salt taste thresholds in adrenalectomized rats. This suggested to Richter (1939 b) that taste is an important factor in guiding an animal in its choices, and he showed that de-afferenting the taste buds rendered adrenalectomized rats incapable of making the choice between salt and water. That this is a central mechanism rather than a change in the thresholds of the buds themselves was demonstrated by Pfaffman and Bare (1950) by single fibre recording in the corda tympani.

Little or nothing is known about this central mechanism which enables an animal to select a suitable diet and it was in the hope of throwing some light on it that the present work was undertaken.

RATIONALE. hypothetical

It is clear that the carbohydrate and protein appetites are dependent on the vitamin B complex. It is further clear that, since fat and carbohydrate appetites vary inversely, there is a further interdependence here. Thus the appetites for the three main dietary constituents are dependent on vitamin B and to a certain extent on each other. We are, on other words, dealing with a set of inter-dependent variables.

A certain number of hours after a meal, an animal's thermal balance or arterio-venous blood sugar difference will change in such a way as to induce in it the urge to eat. If the dietary components are offered separately, what happens? It may be that the animal's affective response to all foods is much the same, although there is no evidence of this. Let us assume, however, for the sake of explanation, that this is so, and that the animal's first choice of food is made purely at random. It eats one food and then stops. Why? It must be the result of some stimulus state arising from the ingestion of that particular food. Is it a by product of digestion and absorbtion or is it the accumulation of a metabolic intermediate? Here the time sequence is important. If the period of this eating is short, then a metabolic change is unlikely and one must look to digestion and absorbtion for the stimulus. If the period is long, then a metabolic intermediate becomes a possibility.

The stimulus state might be specific or non-specific. In other words, the animal might feel sated and stop eating altogether,

in which case the first action of the stimulus state will be non-specific. The state of satiety would then wear off. When the state of appetite returns and the animal returns to eat, it might sample the same food or simply sniff at it and find that the smell is no longer attractive, or go immediately to another food and eat there, or undertake a process of sampling and experiment. The behaviour of the animal in changing from one food to another should give an inkling as to the effect and perhaps the general nature of the stimulus state generated by ingesting the first food.

If the stimulus state is specific, the animal might become sated with that particular food but not become fully sated. It might go straight to another food. If an animal eats food A, will the stimulus state arising therefrom lead the animal to eat food B, and will the new stimulus state lead to food C? Finally, will eating C lead to an appetite for food A? The process might, in fact, be cyclical. There might, on the other hand be some sort of error control which will lead to an oscillatory sort of behaviour.

The writer's first aim is to find out, not how much of any food the animal eats in 24 hours, although food consumptions will of course be measured, but how the eating is distributed temporally over the period. What, in fact, is the pattern of feeding behaviour exhibited by an animal offered the different ingredients of its diet separately. Should there be no pattern, should the selection be purely random, this too will be important since it will mean that the selection of diet is in no way dictated by need and that our concept of the animal's

behaviour supporting its homeostatic mechanisms will go by the board.

Assuming, which is most likely, that some sort of pattern is determined, it follows that some brain mechanism or mechanisms must be imposing that pattern. The second stage of the project will consist of an effort to uncover those mechanisms by lesions which will disrup the pattern of behaviour.

The third stage will be the attempt to identify the metabolic intermediates or digestive and absortive factors that act as stimuli to the mechanisms determined in the second stage.

The experimental work described herein presents the first run of the apparatus and an attempt to learn from the behaviour of six rats given a complete mexed diet how future experiments of a more definitive sort should be designed.

D E S I G N O F T H E A P P A R A T U S.

THE PROTOTYPE

The project demands a detailed record of a rat's feeding activity when given the opportunity of feeding at any one of several troughs. This alone makes the standard method of operant conditioning impossible, since it would involve teaching the animal what type of food would be obtained by which manoeuvre and the learning factor would become enormous. Further, the successful recording of feeding behaviour of the type required necessitates recording responses to very subtle changes in the drive level. This means that the rat would be required to do no more than put its head in the trough to get food. The aim in constructing the apparatus was to record these insertions of the rat's head without any active participation on the animal's part.

Three methods were considered: a mechanical switching device which would operate an electrical circuit, a capacitance mechanism in which the animal's capacitance was used to change the frequency of output of an oscillator, and a photo-electric sensor.

With the mechanical switching device, the lever or plate to be moved would have to be extremely light in operation and this would in turn entail fine machining of hinges and pivots. Such moving parts would have to be in or very near to the troughs with the result that fine food particles, such as rats distribute about their troughs, would inevitably find their way into the bearings and be the cause of repeated and troublesome breakdowns.

Many circuits are available for triggering a variety of mechanisms by altering the output of an oscillator on the approach of an animal's body. Such a mechanism is, in fact, a proximity switch, and as such will be apt to give false readings since it cannot differentiate between the rat inserting its head into a trough or just bringing its head near the trough. There is also the further difficulty that the rat's propensity for gnawing extraneous objects might very well lead to damage to the sensing device.

For this reason, more serious consideration was given to the photo-electric mechanism. When dealing with a small animal such as a rat, everything has to be small in proportion. All leads and other easily damaged parts must be protected by metal shielding to prevent gnawing of vital parts. These considerations imposed the use of a small electric bulb as a light source. Experiment showed that a six volt dial light bulb would fit neatly into a half inch brass tube (18 gauge) if the inside was turned out until the wall was 1/32 inch thick. A hole 1/8 inch in diameter drilled in the side of the tube, with the bulb adjusted so that the filament was opposite the hole, permitted the emission of a beam of light which, a two inches, had acquired a diameter of one and a half inches. Thus the light intensity was somewhat low and necessitated a sensitive sensor to detect the cutting of such a beam.

Further limitations were imposed on the design. Each rat was to be given the choice of fat, carbohydrate, protein, fat soluble vitamins, water soluble vitamins, minerals, and water, necessitating

a minimum of seven troughs, an eighth being added for any other food constituent which might have to be offered separately. Thus, for one rat, a recorder having eight independent channels would have to be provided. When the apparatus was completed and the experiments proper began, it would obviously be useless to monitor the behaviour of one rat at a time. Six rats in one experiment would form the minimum number which would give reliable results. Ultimately, then, 48 units would have to be constructed. This meant that all units would have to be simple so that expenses would be low, and compact so that the apparatus would not become ridiculously large. It was decided therefore that the electronic circuits should be based on transistors rather than valves.

In considering the type of sensor to be used, selenium cells were eliminated without trial. The light source was dim, and, in order to have a trough of reasonable size, the minimum length of beam was two inches (the diameter of one trough). In order to obtain a reasonable response from the cell, the whole of the spread beam at that distance, one and a half inches diameter, would have to be used. This alone would introduce constructional difficulties and would result in a hole one and a half inches in diameter through which a rat would most certainly interfere with the selenium cell. With a wide effective beam, there was the danger of partial occlusion with a graded response from the cell, whereas a clean on/off response was required. Lastly, there was the probable expense of 48 selenium cells.

The small neon tube was attractive both in size and price, but the type of illumination to be used did not produce sufficient ionization to have a significant effect on the threshold. Thus the decision to use a transistor as the sensor was reached by a process of elimination.

In the prototype, OC 71 transistors with the paint removed were used. Their advantage immediately became apparent. The sensitive part of the transistor, the emitter-base junction, could be positioned behind a 1/8 inch hole "looking at" the 1/8 inch hole in the tube carrying the bulb. Thus the effective width of beam became small and a clean cut off of the light was possible.

The electronic unit.

Since the rat spends little of its time eating, it was decided that the recording apparatus should be off when the light beam across the trough was not interrupted and should only come on when the beam was cut by the rat putting its head into the trough. The circuit devised for this purpose by Mr. W. V. McBride, Electronics Technician in the Department of Physiology is illustrated in Figure 1.

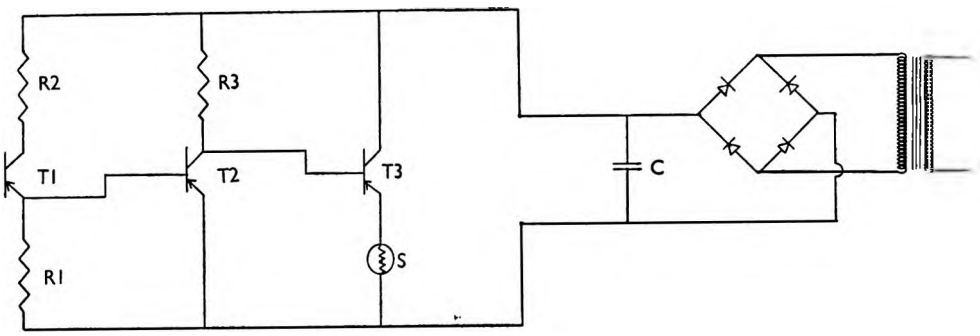

Figure 1. Diagrams of photo-electric, amplifier, and power supply circuits.

C 1,000 micro farads.
T1 OC 71 photo transistor.
T2 OC 71 transistor.
T3 OC 26 transistor.
R1 1,800 ohms.
R2 6,700 ohms.
R3 200 ohms.
S Stylus.

Figure 1.

(From de Caire 1961)

In order to explain the working of this circuit the following symbols will be used.

V voltage difference

I current

a the positive rail

b the base of the transistor

c the collector of the transistor

d the negative rail

e the emitter of the transistor

1, 2, 3. Transistors 1, 2 and 3

Thus Ve2b2 is the voltage difference between emitter and base of transistor 2, while Ib2 is the current flowing in the base of

transistor 2.

The resistor R2 is merely a current limiter and plays no active part in the operation of the circuit.

When the transistor T1 is illuminated, its resistance is relatively low and the voltage drop across it relatively small. But the total voltage drop across the circuit R1, T1, R2, is always equal to the supply voltage, therefore the voltage drop across R1 is relatively high. Conversely, when the light is interrupted, the resistance of T1 is relatively high and the voltage drop across R1 is relatively small. Thus:-

	Light on	Light off
Vae1	-0.2V	-0.1V

But since the base of T2 is tied to the emitter of T1 and the emitter of T2 to the positive rail, Vae1 is in fact the same as Ve2b2. This means that when the light is on, the base current of T2 is high and when the light is off it is low.

	Light on	Light off
Ib2	-200 μ A	-10 μ A

Consequently, when the light is on, the resistance of T2 is low and the voltage drop across it is small, while when the light is off its resistance is high as is the voltage drop. In both cases, the residual voltage drop is across T3.

	Light on	Light off
Ve2c2	-1.4V	-2.5V

One cannot say that Ve2c2 is the same as Ve3b3 because of the

presence of the recorder (s) in series with e3, but Ve2c2 does in fact control the base current of T3 and results in a phase reversal.

	Light on	Light off
Ib3	-5mA	-10mA
Vb3e3	-0.45V	-0.5V

This, of course, alters the resistance of T3 and the current flow through S and T3.

	Light on	Light off
Ie3	0.2A	0.4A
Vc3e3	-3.7V	-2.5V

Thus, with the light off, the current flowing through the recorder is doubled. It is true that a change of 0.2A is not large, but in the prototype, when the recorder consisted of a heated stylus, it was sufficient to make the difference between a hot stylus and a cold one.

The power supply for each unit of eight channels was by transformer and bridge rectifier with a smoothing condenser. The D.C. voltage was theoretically 6, but actually swung between 5 and 6 with changes in the mains voltage. There was considerable ripple, despite the condenser, but this seemed to be quite harmless.

The recording system.

The element wire used as a recorder was shaped into a small hairpin and mounted on the end of a spring loaded arm which wrote on a single channel electrocardiograph paper. It thus acted as a hot

stylus on heat sensitive paper, writing under low pressure. The actual heat of the stylus was not measured, but it was well below red heat. Since recording was done on a very slow moving paper (0.13 mm/second) neither great heat nor great pressure was needed. With this paper speed, half millimeter represented a time lapse of approximately four seconds, which was adequate for the type of work to be undertaken.

The type of record produced consisted of a black mark (stylus heated) when the rat was feeding, and no mark (stylus cold) when the rat was not eating (Figure 2).

A Palmer kymograph as used in student experiments formed the paper drive. On test, the drum speed proved to be somewhat in excess of 0.13 mm/second in bottom gear with the brake on. The brake was therefore adjusted to give the minimum possible speed. It was hoped to attain a speed of 0.10 mm/second in bottom gear with the brake on, but such tension on the brake proved to be too much for the motor, which stopped. It was impossible, in fact, to get a lower speed than 0.13 mm/second. This rather awkward speed made interpretation of the records a slow business. Faster speeds had to be avoided on the grounds of the cost of the paper and the bulk of a twenty-four hour record.

The kymograph had to be equipped with a paper supply, a paper drive, and some means of winding up the paper. In order to form a sufficiently large and stable platform on which to mount these appliances, a piece of Fermale 12 inches square and quarter inch thick was obtained.

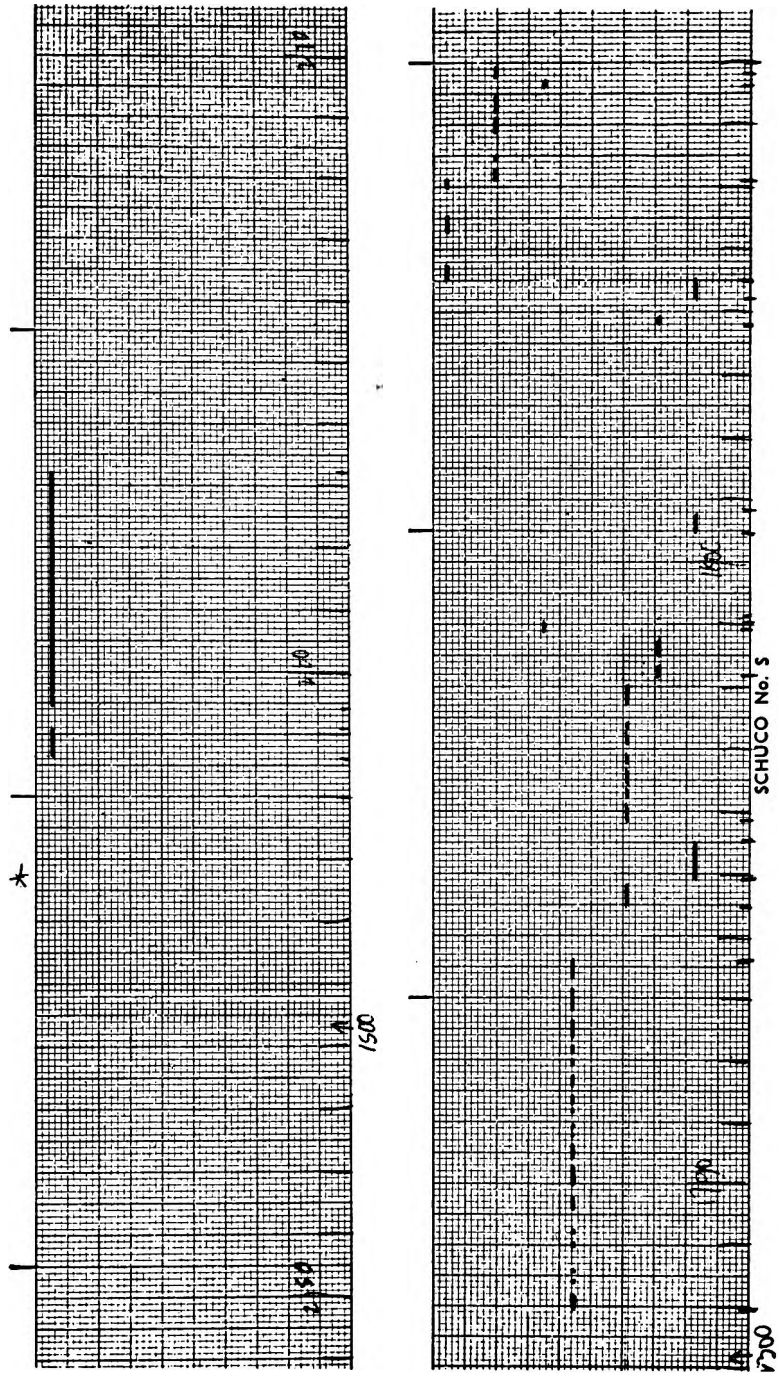

Figure 2. Examples of records. Upper trace, a rat places its head in a trough but does not eat; note the tendency to produce an unbroken line. Lower trace, a typical meal at eleven o'clock at night: broken lines and dots were produced from several channels, but in the lower one which represents visits to the water trough, where there is no chewing, the tendency is to produce unbroken lines of short duration. Marks and figures on the bottoms of the graphs represent centimetres of paper used since the start of the experiment. Figures below the graphs represent the time. Lines on the graphs have been retouched for clarity of reproduction.

Figure 2.

(From de Caire 1961)

A hole was cut in the centre, so shaped that the Permale could fit snugly on top of the motor housing of the kymograph while passing round all the excrescences that have been placed there. Screw holes, counter sunk, and corresponding with the holes for the retaining screws in the top of the motor housing were drilled in the Permale, with the result that the existing retaining screws could beused for fastening the Permale firmly to the top of the housing. A view of the layout of the top of the permale platform is shown in Figure 3.

The paper supply consisted of a vertical shaft, turned to be a free fit in the cardboard centre of Schuco No. 5, single channel, electrocardiograph paper. The bottom consisted of two perspex discs, one on top of the other visible in Figure 6. The bottom disc was pressed on to the centre shaft and the top surface was roughened with fine sand paper. The second disc was a free fit on the centre shaft, and had both sides roughened with sand paper. When a roll of paper was in position on top of the perspex discs and traction was applied to the free end, the friction between the paper and the perspex, and between the two perspex discs was sufficient to keep the paper under slight tension without imparting a breaking strain to it. The free end of the paper was led round the standard six inch myograph drum, against which it was pinched by the paper drive.

The paper drive consisted of small rubber rollers, usually used for pinching the paper against the platten of a typewriter. The rubber on these rollers proved to be rather hard and considerable pressure

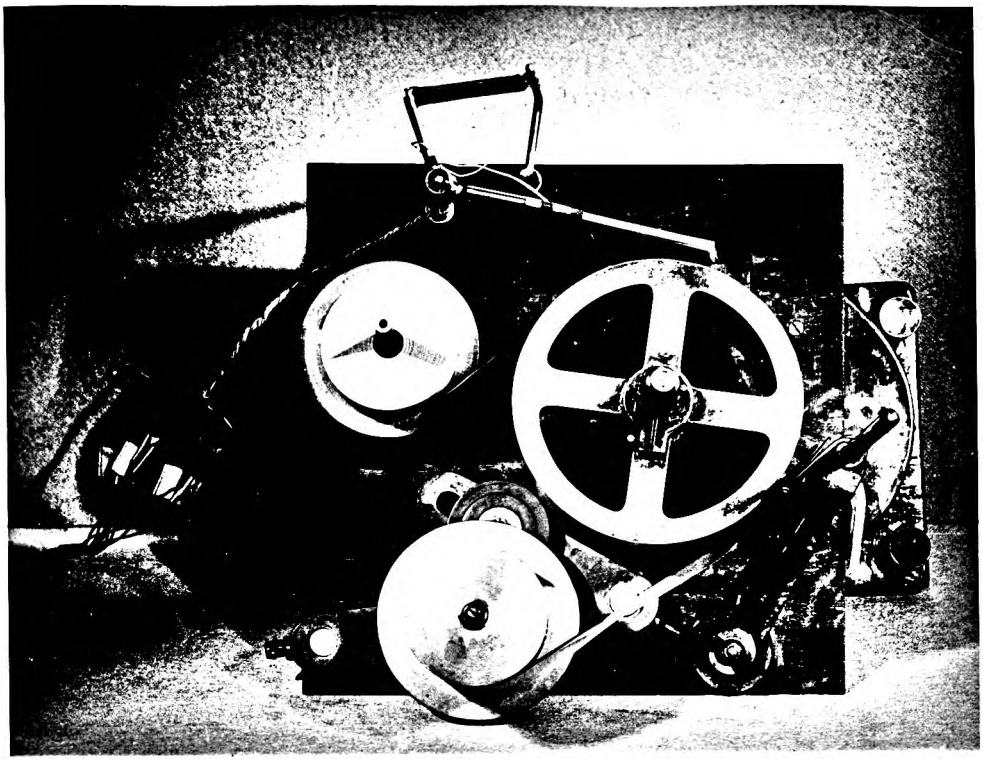

Figure 3

A view of the top of the prototype recorder. Above the myograph drum are the writing levers and the springs which hold them against the drum (the nine leads to the electronic unit are visible). To the left of the myograph drum, from above downwards, are 1, the paper supply, 2, the rubber drive wheel for the paper collector and 3, the paper collector. To the right of the myograph drum are the paper drive rollers.

had to be applied against the drum to obtain a positive drive. This was achieved by mounting the rollers on a vertical rod at the end of an arm. The rollers were then pressed into position and the arm locked by means of a wing nut. The drive obtained in this way was unfailingly efficient.

The principle involved in winding up the paper was that of a slipping drive operating a drum on which the paper could be wound at a speed rather faster than that at which it was delivered by the paper drive. The slipping drive was provided by the rubber driving wheel from a Grundig record player (Figure 3). This was mounted at the end of a free swinging arm in such a position that it could be pressed against the bottom of the myograph drum. The drum on which the paper was wound was made from the idler of a mammalian kymograph (Figure 4). A flange for carrying the paper was pressed on to it and a brass ring was fitted to the bottom. The diameter of the ring (1 7/8 inches) was less than the diameter of the collecting drum (3 inches). The collecting drum was mounted at the end of a swinging arm which could be locked in position by means of a wing nut. By slackening off the wing nut and swinging the arm over, one could pinch the rubber driving wheel lightly between the brass ring on the collecting drum and the bottom of the myograph drum. While no paper was being collected, the drive was positive. But because of this positive drive, the linear speed of the circumference of the brass ring was the same as that of th circumference of the myograph drum. The smaller dimensions of the brass ring, however, mean that the revolutions per minute were greater. The collecting drum was being rotated at the same number of revolutions per minute as the ring, but the diameter was greater, and this meant that the circumference was in fact moving faster than that of the myograph drum. When the paper

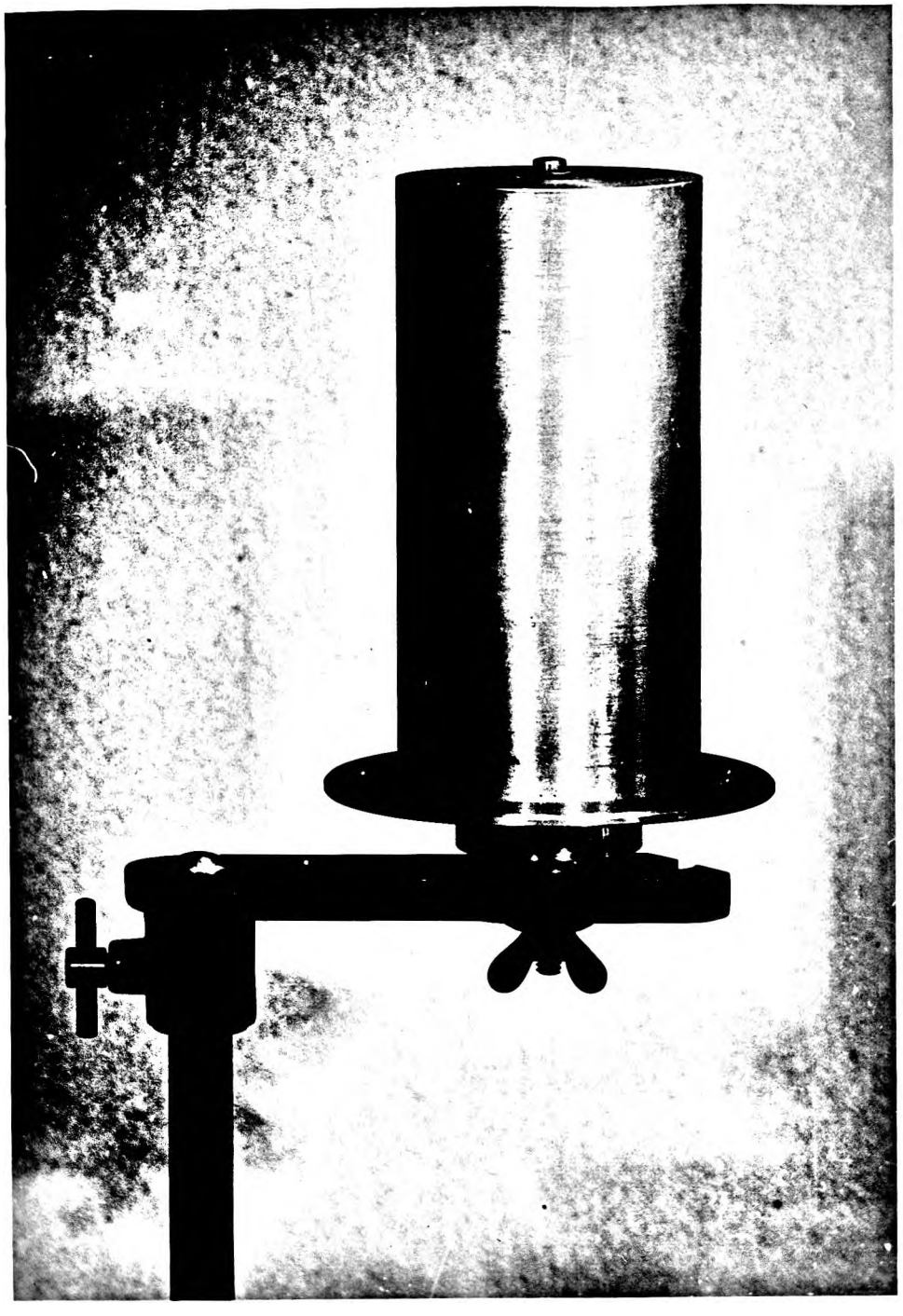

Figure 4.

The paper collector. The roller has been removed from the recorder and mounted on the top of a retort stand. From above downwards, 1, the collecting roller, 2, the flange on which the paper rests, and 3, the ring which is normally in contact with the rubber drive wheel.

fed out by the paper drive was connected to the collecting drum by a small piece of cellotape, any slack initially present was taken up. The paper then came under slight tension and the rubber driving wheel started to slip on the myograph drum. The drive was no longer positive, but a constant tension was maintained and the paper was wound firmly on to the collecting drum.

Provided that the kymograph spindle and the vertical shafts of the paper supply and the collecting drum are truly vertical to the Fermale platform, and provided also that the upper surface of the top perspex disc on the paper supply and the upper surface of the flange on the collecting drum are accurately at right angles to the shafts and at the same level, such a system works well and reliably.

The writing levers were made of several parts (Figure 5). The distal part, farthest from the pivot, consisted of three inches of copper clad laminate with copper on both sides. To the distal end of this was soldered the element wire, bent into a suitable shape and crimped for greater strength. Near the proximal end, the copper on one side was cut through, and proximal again to this cut, a brass sleeve was fitted to the laminate and soldered to the copper strips. Thus on one side, as a result of the cut, the stylus was insulated from the brass, while on the other side it was in electrical continuity with it. Proximal to the end of the laminate, the sleeve was shaped round and pinched to form a tight fit on a 1/8 inch brass rod. The proximal end of the rod was fitted and silver soldered into a brass

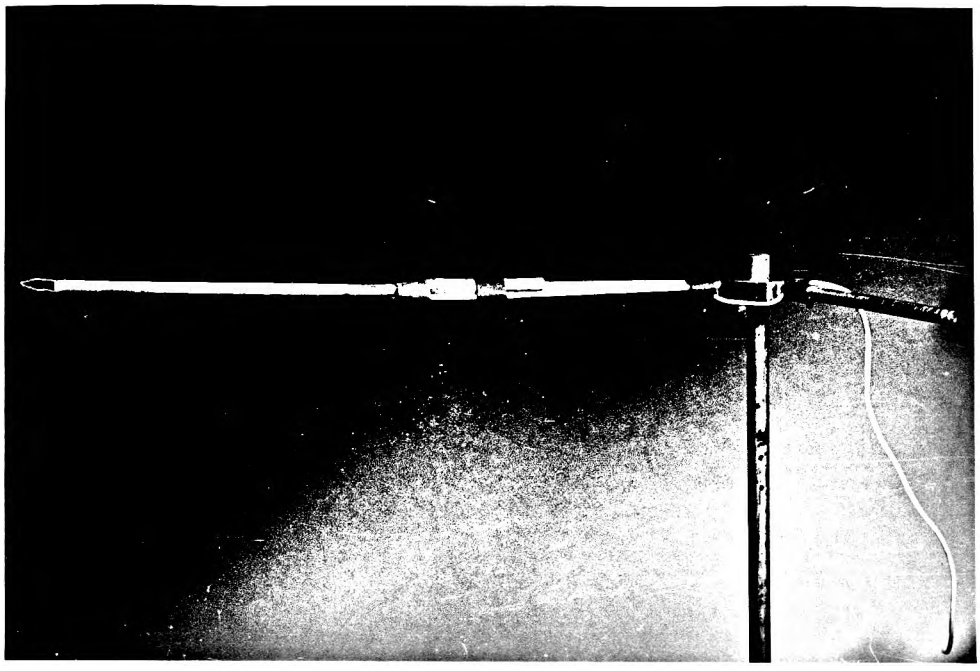

Figure 5

A writing lever from the prototype. The lever has been removed from the apparatus and mounted on a rod for photography. At the left is seen the element wire which acts as a writing point and which has been coated with chalk to render it visible. This wire is soldered onto the two copper strips of the copper-clad laminate, at its right hand end, under the first piece of insulation tape, the laminate is soldered to the brass sleeve. The upper strip is in electrical continuity with the sleeve. The light flex is soldered to the lower strip which is cut through between the point of attachment of the wire and the sleeve (the cut is not visible). At the level of the second piece of insulation tape, the brass sleeve is shaped round and fitted to the brass rod which is in turn fitted into and silver soldered onto the brass hexagon. On the next "flat" but one to the lever, is the rod for the attachment of the spring.

hexagonal section ¼ inch thick. The object of the brass sleeve fitted to the rod was to form a means of adjusting the length of the levers individually and thus of aligning the styluses. On the next

flat but one of the brass hexagon to which the lever was fitted, another 1/8 inch brass rod, 1½ inches long, was fitted. This formed the attachment for a light spring to maintain writing pressure.

The eight levers representing the eight troughs were mounted on a 3/16 vertical rod which was firmly bolted to the Permale base (Figure 6). The hexagonal brass portions of the levers were drilled so that they fitted snugly but freely over the 3/16 rod. A brass collar with a set screw was placed over the vertical rod, and the hexagonal portions of the levers placed on top of it, all rotating surfaces being separated by thin washers. Another brass collar with a set screw was placed on top of the levers, being carefully adjusted as to pressure so that free rotation was possible without rocking movement. Thus the levers could rotate on the upright independently and there was no vertical movement at the lever tips. Another vertical rod bolted to the Permale base formed the attachment for the eight springs which maintained writing pressure (Figures 3 and 6).

In all eight levers, there was electrical continuity through the brass proximal ends and the uprights with one side of the copper clad laminate. From these uprights a lead was taken to the positive earth of the electronic units. (The eight units were, of course, parallelled across the power supply.) On the other side of the copper clad laminate, the side with the cut in it, a light, single flex was soldered distal to the cut on each lever. These eight leads were connected to the emitters of the CC 26 transistors (T3 in Figure 1).

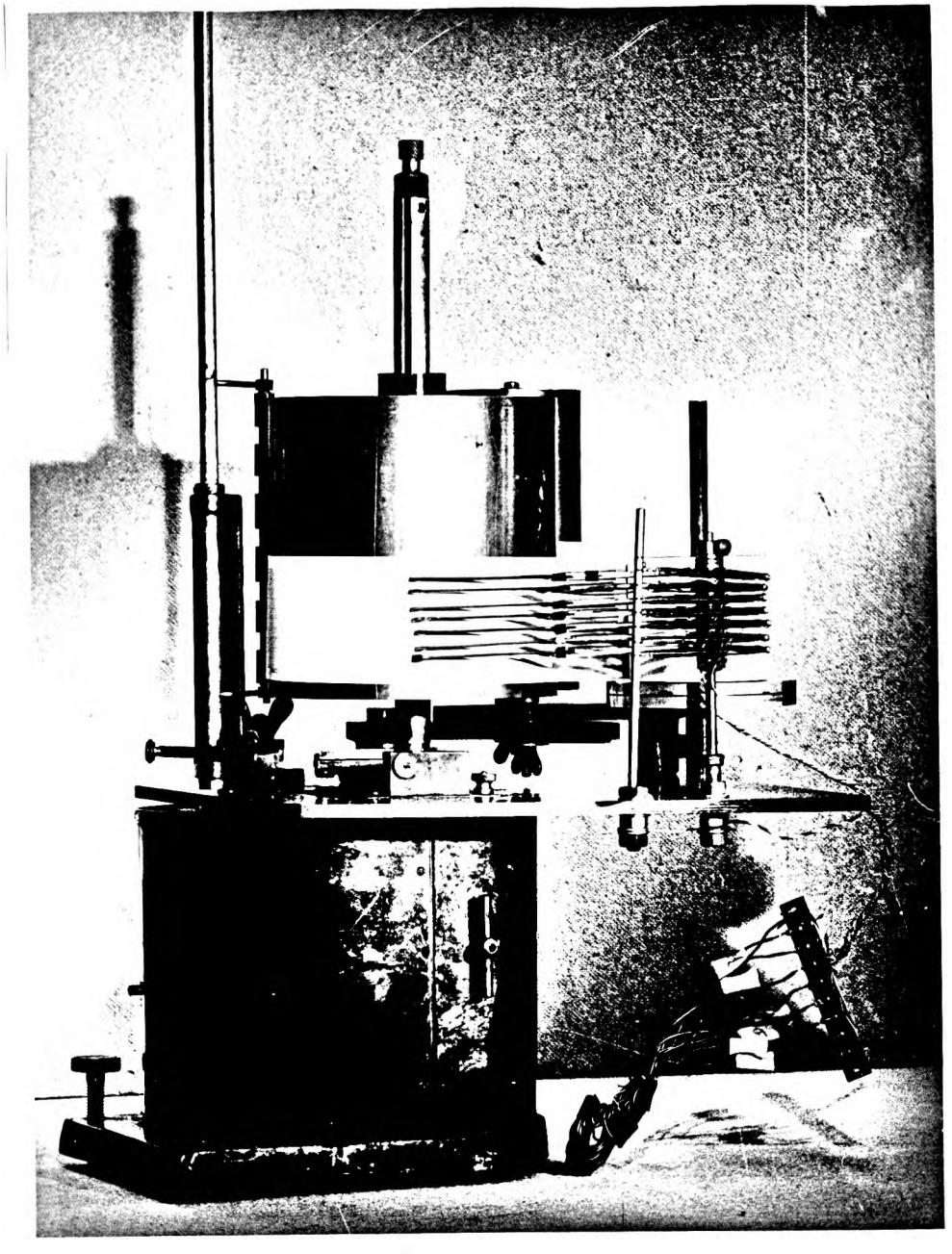

Figure 6.

A side view of the prototype recording unit. At right front are 7 or the 8 levers (one was "borrowed" to form part of another piece of apparatus). The springs and their attachment are clearly visible. Behind the lever mounting is the paper supply showing the two perspex friction discs. The paper collector is almost concealed by the myograph drum. The paper drive rollers are on the left.

The troughs

In constructing the feeding troughs, the first consideration had to be size. Limited funds prevented the construction of a cage to fit the troughs, and it was therefore necessary to construct the troughs to fit the existing cages. Certain parameters were, however, fixed. The most important of these was the size of the hole through which the rat would have to put its head to obtain food. Measurement on several rats indicated that this hole had to be not less than 1¼ inches in diameter. Such a hole could be conveniently cut in a tube of 2 inches outside diameter (1 7/8 inches inside diameter). The headhole was cut with its top edge ¼ inch from the top of the tube, which, being 2¾ inches deep, allowed a trough depth of 1½ inches. These trough dimensions were critical. The result was that an eight-trough unit would occupy a minimum length of 16 inches. Since the width of the cage was 17 inches inside, it was not possible to space out the individual troughs so that the photo beam could pass straight across the front of the head hole. It was necessary, therefore, to position the light and transistor tubes as in Figure 7. In the pair of troughs illustrated, the light tube is at the back and the transistor tubes at the front, such that the beam shone across the trough at an angle of 45° to the straight edge of the base. In the pair of troughs adjacent to those illustrated, the light tube was placed at the front, between the , and the transistor tubes at the back. Although this arrangement was not ideal, it worked well enough in the event.

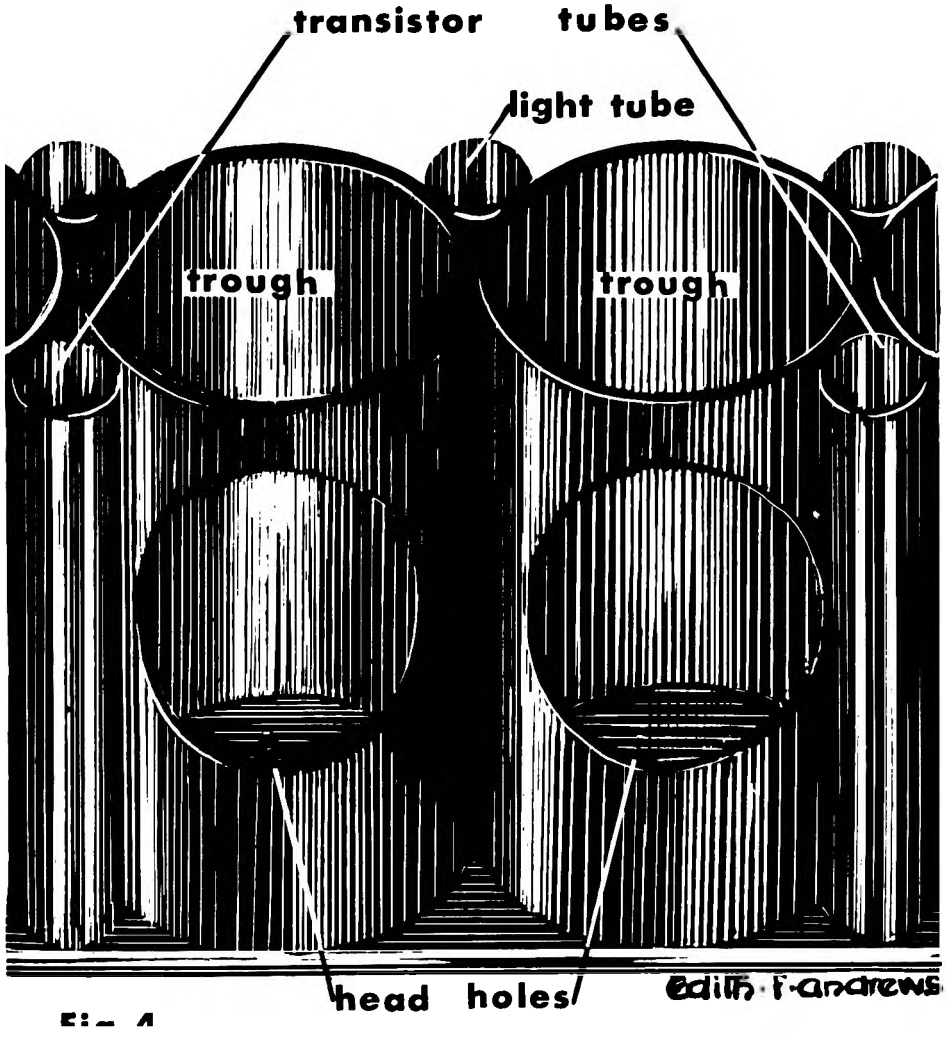

Figure 7.

A drawing of two of the eight troughs showing the positions of the light and transistor tubes. The two pairs of troughs adjacent to those illustrated have the transistor tubes at the back and the light tubes at the front. The drawing is by Miss Edith F. Andrews of the South African Institute of Medical Research.

The base of the troughs consisted of a brass plate 1/8 inch thick, and to this were soldered all tubes. The tubes were also soldered together and thus gave rigidity to the apparatus.

The top of the troughs was formed from sheet metal bent to fit snugly over the tops of the troughs and drilled with holes which registered accurately with the tops of the lamp and transistor tubes. All bulbs and transistors were mounted on this plate. In order to keep the number of wires leading from the troughs to the electronic unit to a minimum, all components of the circuit (Figure 1) R1, T1, R2, were mounted on top of the trough. They were provided with a common power supply (2 wires); there was a power supply to the lamps (2 wires); and one lead from each R1 to the base of the corresponding T2 in the unit (8 wires), making a total of 12 wires. This wiring was protected by an additional lid fitted with a half inch brass tube through which the wires were led from the cage. Four brass rods (1/8 inch diameter) were soldered to the base of the troughs and passed through holes in the top of the troughs and protecting lid. These rods were threaded at the top and fitted with nuts, so that the protecting lid could be fastened down and, pressing on the top of the troughs, held that down as well. Thus all wires, transistors and lamps were protected from the unwanted attentions of the rat.

THE FINAL MODEL

The electronic unit

The original electronic unit, having proved reasonably satisfactory,

remained unchanged except in details despite the fact that the element wire was to be replaced with a coil. This raised two difficulties which caused some trouble in operation. The first was that the cut-off in the light on position was incomplete. This meant that the coils were always energised to a small extent and involved very careful adjustment of the mechanical components of the recording system to counteract the effect. The second arose from the small difference between the light on and light off positions. The consequence of this was that the power available was small and in some channels the deflection was also small. Although this did not affect the interpretation of the records, it meant that safety margins were small and led to a certain lack of reliability.

The remedy for these difficulties would have been either to interpose relays between the electronic units and the writing coils, shunting the excess current which flowed in the light on position, or to redesign the circuit. The first would have been expensive and the second was not possible under the conditions then existing. The circuit has in fact been redesigned since the conclusion of the work described herein by Mr. Morris Milner of the Department of Electrical Engineering.

In view of the large number of rejects necessary to obtain eight OC 71 photocells, and in view of the fact that the final model required 48 such cells, the writer decided to purchase OCP 70 photo-transistors. This had no material effect on the circuit. It is a moot point whether

the change actually resulted in an economy or not. For greater simplicity of construction, the negative supply was earthed instead of the positive.

The recording system

The recording system used in the prototype suffered from two faults. First, no matter how carefully the writing points were aligned at the beginning of a 24 hour recording period, they were badly out of alignment at the end of it. The cause of this was mainly the nature of the heated stylus recording system. When the stylus is hot, it actually melts the wax or plastic surface of the paper. However, when the stylus goes cold, it tends to stick to half melted material which imparts a slight pull to it, ultimately spoiling the alignment. No doubt this fault could have been overcome, but was far surpassed by the second one.

The second fault arose not so much in the construction of the recorder itself as in the price of the recording paper. Even at the slow paper speed used in the trial period, the cost per day for one rat amounted to 50 cents. Recording continues for seven days a week, and it was envisaged that the ultimate recording periods would run for several weeks or even months. Recording from six rats simultaneously would clearly make the total cost of paper prohibitive. It was this consideration which led to the abandonment of hot stylus recording. As it turned out, the hot stylus would not have recorded all the information now obtainable because of the time it takes to heat up to writing temperature. This was not realised at the time.

There are two ways of recording on ordinary paper with some common writing instrument - intermittent and continuous. The intermittent method was tried first. In this, a pencil is held just off the paper by a weight or spring and is pulled on to the paper by a solonoid when the signal arrives. The first attempt worked satisfactorily from the mechanical point of view, but the pencil made a mark so faint that it could easily be missed. Ball point pens were next tried, but they are not immediate writers, usually requiring half a revolution of the ball before ink reaches the paper. Capillary writers proved unsatisfactory because, if off the paper for more than a few minutes, they tend to block. The intermittent system was therefore abandoned in favour of continuous writing.

Here, the writer is in contact with the paper continuously but is deflected on arrival or the signal. At once it seemed that the ball point pen had come into its own. Later experiments, however, showed that they were too unreliable and they had to be abandoned.

A further examination of pencils was made. HB and coloured pencils recorded quite will for an hour or so, but after that the points appeared to become polished and smooth and the mark became so faint as to be almost illegible. All grades of pencil up to 6B were tried. The softer grade of pencil wrote quite well, but the point soon became blunt and the line proportionately woolley. The tendency for such records to smudge means that they are not permanent.

A search for suitable writing instruments was started. It is true that the use of capillary writers would have been possible, but in the view of the Instrument Maker who was responsible for most of the mechanical work and design, the provision of feed pipes and reservoirs at exactly the right height above the writing points would have served further to complicate an already complex piece of machinery. It was therefore decided not to use them. At this stage, the author found two types of draughtman's stylographic pens which exactly fitted the apparatus and these were adopted. Their adoption immediately raised two problems.

The first was ink. Several types of ink, including Nigrosine ink as recommended for the Grass R.E.G., were tried, but all tended to block. Finally, Esterline ink proved to be the only one to approach

100 per cent reliability, and even that gives trouble sometimes.

The second problem was one of suitable paper. Up to that time, rolls of cheap paper as used in Reuter's ticker machines were employed. This paper is easily obtainable, and has a sufficient length per roll for changing of paper not to become a troublesome business. But as soon as a liquid ink was used, it was found that the paper was absorbent and the line became blurred. The type of paper used in cash registers was somewhat better but still not satisfactory. There are numerous charts for recording machines available, but all are expensive and none is very long, and the machine, as finally constructed, turns out 12 meters of paper per drum per day. Finally, and at the last moment, the problem was solved by a member of the staff of National Cash Registers who found a thin, glazed paper which was specially prepared at short notice in 5" rolls of the correct diameter. The cost of this paper for six rats is 10 cents per day. Each roll lasts five or six days.

The general arrangement of the recording drums was controlled by two considerations. In the first place, the ultimate failure of the student kymograph in the prototype, suggested that we needed a motor with a large excess of power, and that this one motor should drive all six drums. It seemed to be much easier to arrange this drive on to a horizontal shaft. Secondly, when the decision was taken, it was believed that ball point pens would ultimately be used, and these run best in a vertical position. In the event, the decision to use a horizontal shaft proved a fortunate one, since, although it involved

considerable redesign, it rendered possible the use of stylographic pens.

The frame on which the recording unit was to be mounted (Figure 8), consisted of a disused kymograph table which was 46 inches high, and, with the top on, 24 inches wide and 60 inches long. The top was removed (this later served as a shelf for the paper supply) and a third transverse support placed in the middle of the framework thus revealed. There were thus three transverse supports on which were mounted three ball races to carry the 60 inch horizontal shaft on which the drums and driving sprocket were fastened. The base of the kymograph table constituted a shelf, and on this, at one end, was mounted the power unit, a half horse power induction motor. This motor has a rated speed of 1750 rpm and considerable gearing down had to be adopted to obtain a reasonable paper speed. It was originally intended that the drive should be through a gear box giving bottom gear of approximately 130,000 to 1, but the lack of suitable milling machine and our inability to get our gears cut elsewhere led to the abandonment of the idea. Instead, the initial drive was through two V belt pulleys giving ratios of 3 to 1, 1 to 1, and 1 to 3. The driven pulley was mounted on a worm drive giving a ratio of 50 to 1. This drove a 40 to 1 worm drive through a rubber coupling, and this, in turn, drove a 20 to 1 worm through a similar coupling. Thus, using the 3 to 1 pulley, the reduction was 120,000 to one, but alternative ratios of 40,000 to 1 and 13,333 to 1 were also available. The 120,000 to 1 ratio gives a paper speed

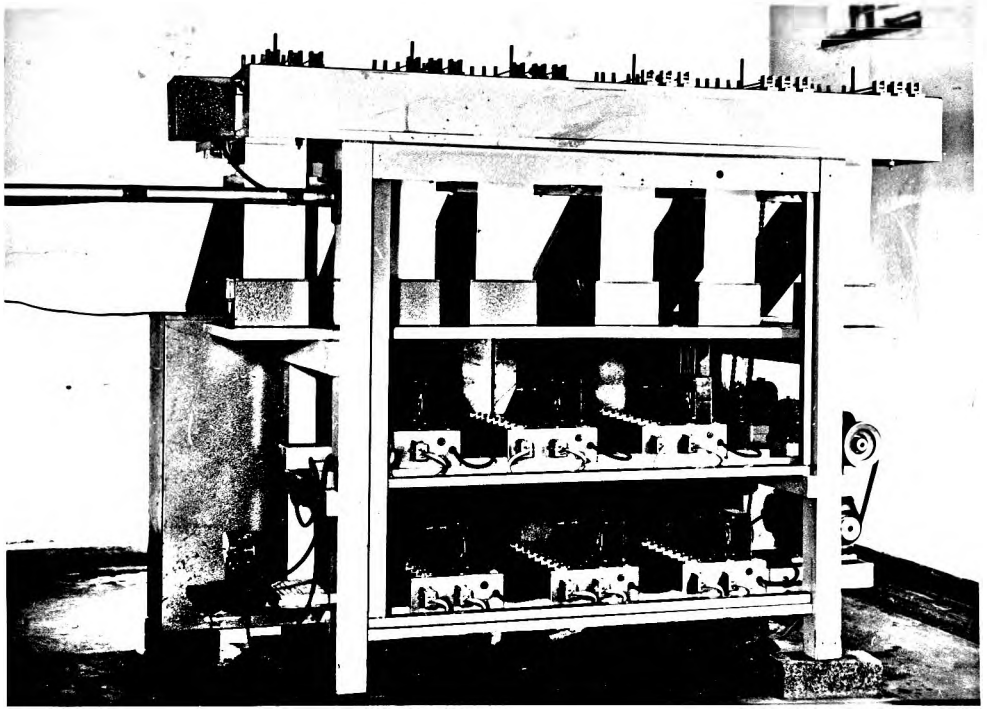

Figure 8.

The recording unit. The writing levers are on top. On the shelf below them are the paper supplies and below that the six electronic units are housed on two shelves. The power unit and reduction gears are on the right. On the left above is the pipe carrying the cables to the cages and below is the time marker. The paper collecting bins are behind.

of approximately 8 mm. per minute. The final drive from the last worm to the shaft was by means of a rear bicycle sprocket and a bicycle chain. One of the sprockets was solid, and the other a free wheel, so that the drums could be turned by hand in the forward direction when required and the need for a clutch was obviated. However, the use of these three worm drives entailed the construction of an additional small shelf to carry them. Since, with these ratios, the motor is running fairly light, its speed is constant. Its efficiency has been proved by its ability to run for several weeks non-stop.

The old system of paper drive and collection was no longer applicable. The paper supply depended on gravity to keep the supply roll on the base and thus in alignment; the drive rollers were hard and required very considerable pressure to give a positive drive; and the winding up mechanism was necessary because the paper was running horizontally and tended to kink if allowed to fall into a bin or other receptacle.

Since the supply roll now had to lie horizontally, it was decided to house it in a sheet metal box six inches long and having a cross section of 4 inches square in order to house a paper roll five inches wide and 3¼ inches in diameter (Figure 9). In each end of the box a slot was cut from the top to half way down, being wide enough to accommodate a ½ inch spindle easily. These boxes were screwed on to the shelf aforementioned (Figure 8), each one being accurately aligned with the edges of the drum above it. Eight-inch spindles (Figure 12) were turned with one inch at each end ½ inch in diameter

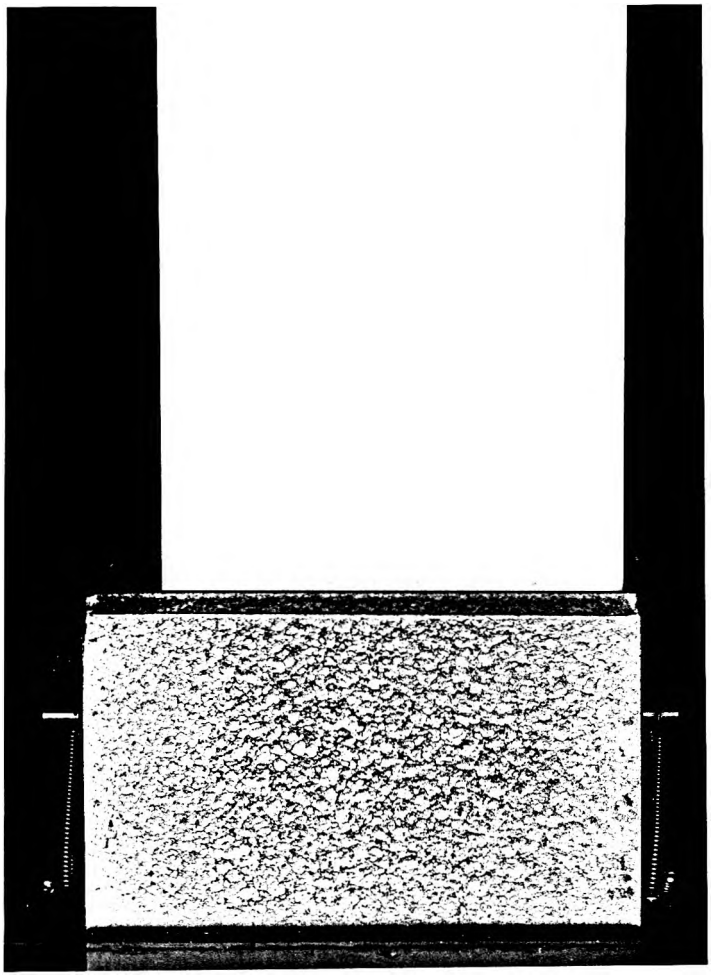

Figure 9.

A paper supply box with a roll of paper in situ. The springs preventing the lifting of the roll are seen at either side.

and the rest $\frac{1}{2}$ inch in diameter. Two perspex discs for each spindle were cut $3\frac{1}{2}$ inches in diameter with a hole in the centre large enough to be a loose fit on the spindle. Unlike the discs in the prototype, these were not roughened, but were intended as smooth surfaces to reduce friction between the roll of paper and the metal box on one side and between the paper roll and the positioning spring on the other. Since the paper roll was 5 inches wide and the box 6 inches long, there was a space between the roll and one end of the box. In this space, and forming a loose fit on the spindle, was the positioning spring which kept the paper pushed up against one end of the box. With the type of paper finally used, it was possible to drill out the wooden spool in the centre so that the roll was a loose fit on the spindle. Thus the only tension on the paper was that caused by the pressure of the positioning spring. It was originally hoped that the weight of the steel spindle would be sufficient to prevent the roll from lifting from the box. In the event, it was found that springs, screwed into the wooden shelf and clipping over the ends of the spindles, had to be added (Figures 9 and 10). Figures 9, 10, 11 and 12 show successive stages in removing a roll of paper.

From the boxes, the paper was led over the kymograph drums and under the pens which wrote vertically on top of them. Thence, the paper passed down the other side of the drum and was pinched against it by the paper-drive rollers. To obviate the very considerable pressure used in the prototype to get a positive paper drive, orton

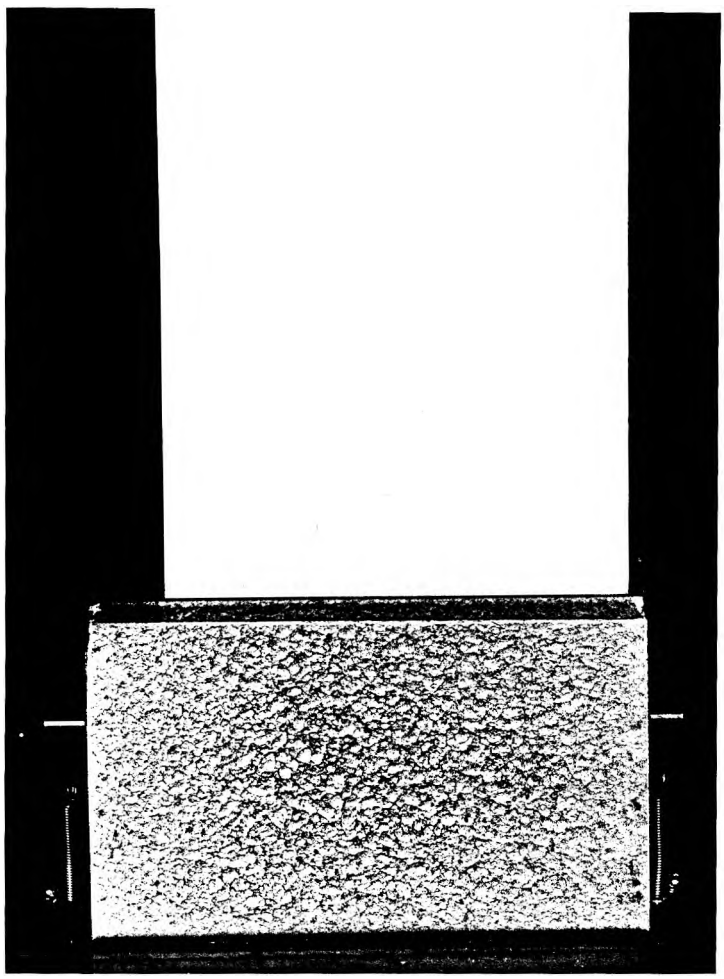

Figure 10.

A paper supply box. The first stage in removing a roll of paper. The springs are slipping off the ends of the steel spindle.

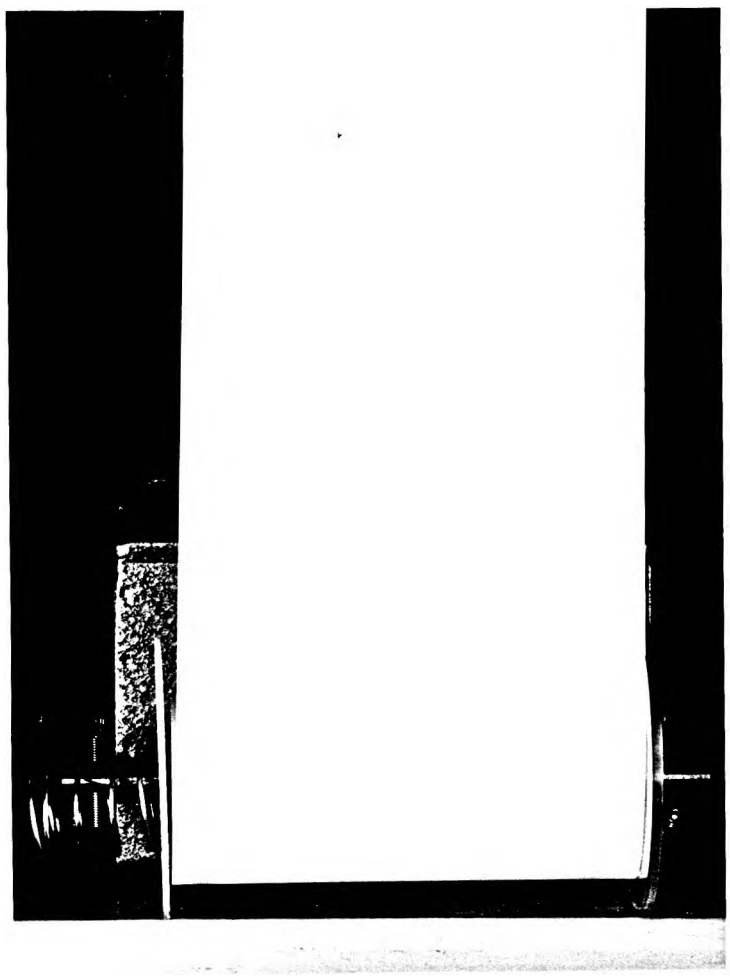

Figure 11.

A paper supply box. The second stage in removing a roll of paper. The roll and spindle are lifted out of the box. Left to right, the positioning spring, first perspex disc, roll of paper, second perspex disc.

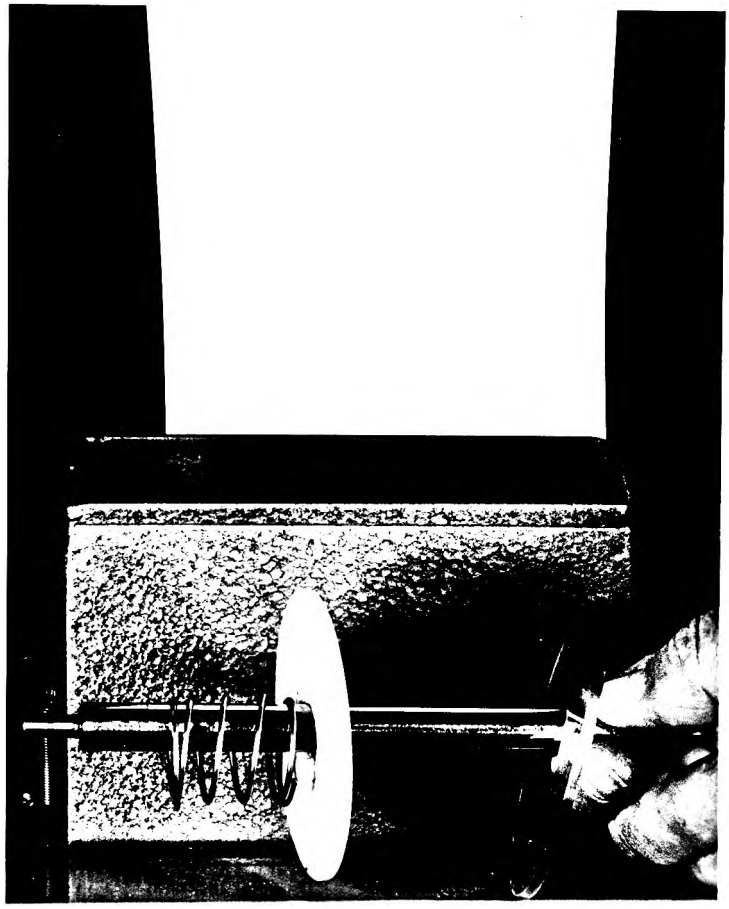

Figure 12.

A paper supply box and its component parts showing the steel spindle. On the spindle, left to right, are the positioning spring and two perspex discs.

rollers on ¼ inch steel shafts were made specially. These are soft, and, having a diameter of ¼ inch, form a good drive with comparatively little pressure. Each roller was mounted in slots cut in two brass tubes which also contained phosphor-bronze compression springs to maintain pressure against the drum. (The housing of one of the compression springs and the end of one of the rollers are visible at the right hand side of Figure 13). Although this drive is satisfactory there is a certain amount of paper slip. This is revealed by the fact that the paper speed varies between the different drums, the range being from 48 to 50 cms. per hour. Even a somewhat greater variation would not be serious, since the final model is fitted with a time marker.

When once the paper has been round the drum, it is fed down a sheet metal chute into a bin, whence it can be easily wound up by hand, (Figure 13).

It was realised from the start that with eight recording levers and one time marker, each liable to undergo a lateral deflection, there would be very little room on the paper. However, since, with the exception of the time marker, only one channel could be active at any one time, each lever could serve two channels, deflecting one way for one channel and the other for the second. This reduced the number of levers per drum to four, a fifth being inserted for the time marker. The levers (Figure 14) are made of phosphor-bronze spring wire, 14 gauge. The spring in the metal was to act as the return mechanism after deflection. A steady bearing at the hinge end was

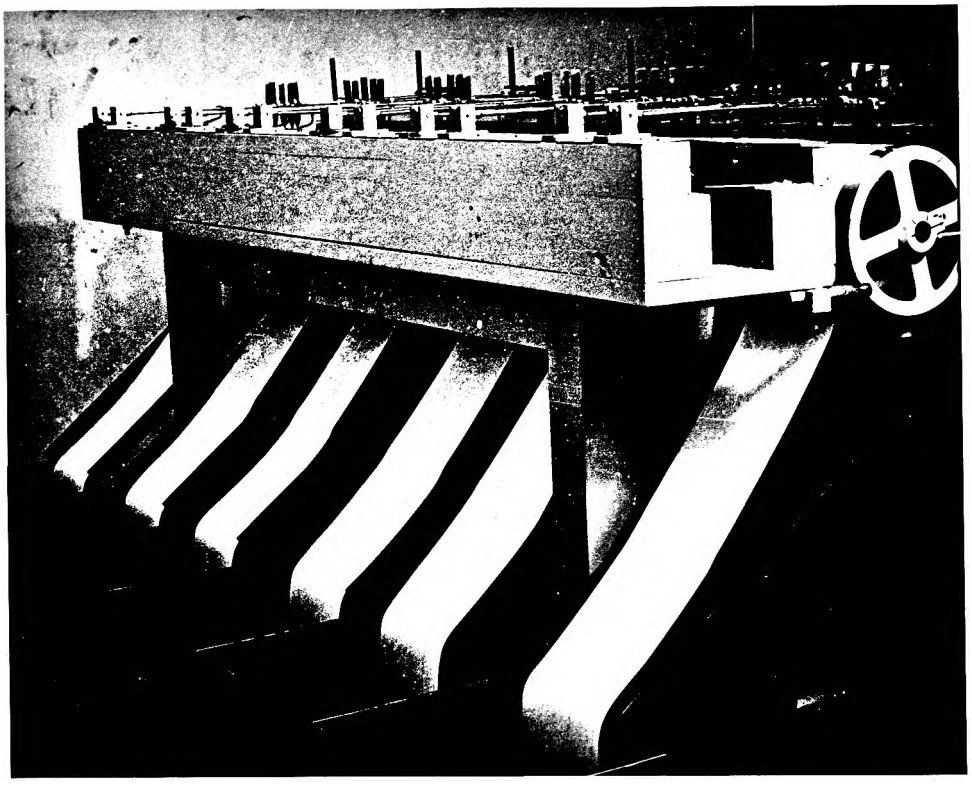

Figure 13.

The recording unit showing paper chutes and collecting bins. At about 8 o'clock on the end drum is the brass tube housing a compression spring pressing the paper drive roller against the drum. The end of the orton roller is just visible. The writing levers and pens are clearly visible on top. The six uprights projecting above the pens are part of the time marker.

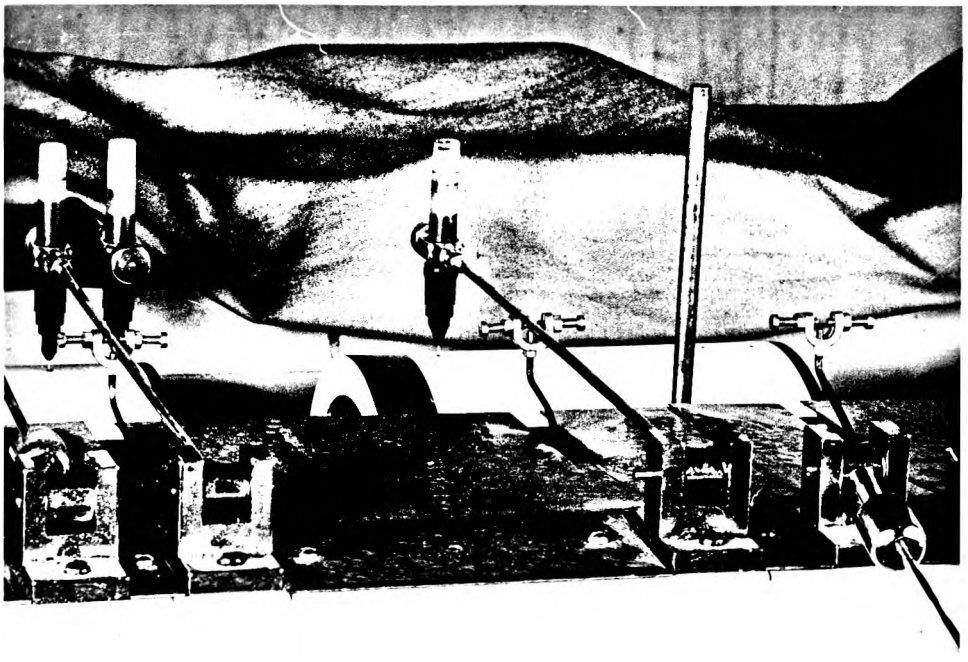

Figure 14.

A lever in position on the drum. The other levers have been turned back and those on the far side covered with a cloth. The coils which operate the levers are under the sheet metal guard. To the right is the upright of the time marker. The time marker lever, with a weight near the bearing has been turned back.

secured by the use of taper pins.

The means of deflecting the levers had to conform to simplicity and cheapness, while the amount of current to be passed by the OC 26 transistors should not be more than 2½ amps. It was decided to leave a margin of safety and draw not more than 2 amps, preferably less. (The wide discrepancy between the figures used in designing the coils and those given in the description of the electronic unit arose from the fact that both the Instrument Maker, who was responsible for this side of the design, and the writer who wound the first coils were under the impression that much greater voltages would be available than were ultimately delivered. Testing was done by tapping 4.5 volts from a 6 volt accumulator. The result of this was that the coils and electronic units were but ill matched. None-the-less, the system worked!). The first attempt at devising a suitable mechanism consisted of turning up brass bobbins on which formex coated copper wire of various gauges was wound, but the best efforts at the voltage and amperage available were indeed poor. At this stage, news came of a pile of obsolete telephones on the railway scrap yards. The A.C. bells from these, with their soft iron cores and permanent magnet boosts, when remagnetised and rewound by the Department of Electrical Engineering with S.W.G. 25 wire (800 turns per coil), proved the answer to the problem, having a resistance of 3.5 ohms and deflecting the levers adequately. (Figure 15).

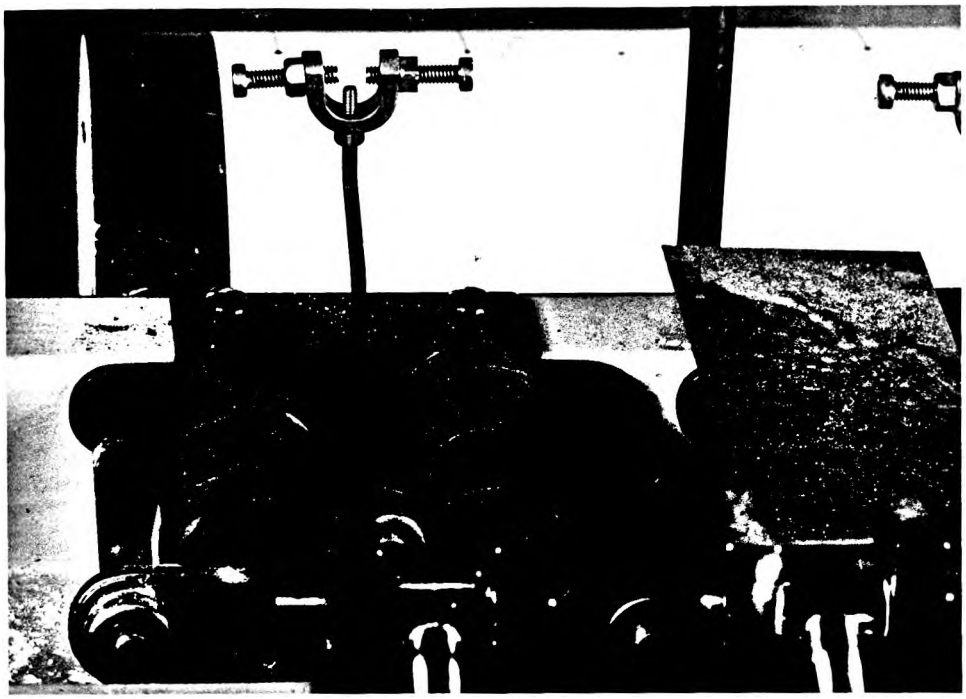

Figure 15.

The coils which operate the levers. The sheet metal guard has been removed. The white powder visible in the photograph is cockroach repellent.

It was hoped originally to use an electronic time marker, marking minutes, ten minutes and hours. In the event, the gadget never achieved a sufficient state of reliability and the Instrument Maker unearthed a synchronous motor from the store giving one revolution every five minutes. (This is visible at the bottom left of Figure 8). On this he fitted a perspex disc having five cams, one wider than the rest. These cams operated a microswitch in circuit with a relay. The time marker levers are thus deflected once a minute with a longer deflection every fifth minute.

The troughs suffered from certain defects. In the first place, it was inconvenient to have the troughs inside the cage; secondly, the fact that the photo-beam shone obliquely across the trough rendered it possible for a rat to sip liquids from a full trough without cutting the beam, although he could neither eat, nor drink from a half empty trough without doing so; thirdly it was impossible to empty or clean the troughs properly without removing the electrical components from the top, and this meant readjusting the alignment of photo cells and lamps every time, a somewhat lengthy and trying process; and fourth the writer had grave doubts about the adequacy of the water supply.

The correction of the first two faults required the construction of special cages by a wire basket maker (Figure 16). The correction of the first fault required these cages to have a two and a half inch high opening across the front equal in width to the length of the troughs. Over this opening a shelf of galvanised iron formed a platform from which to hang the troughs by means of sheet metal screws

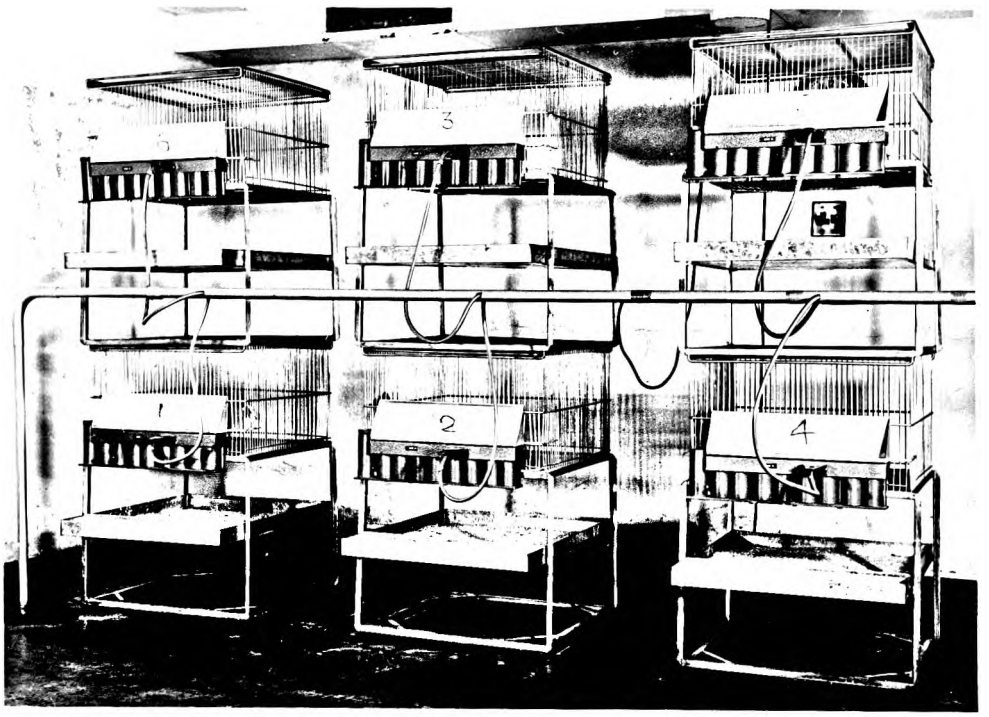

Figure 16.

The six cages with troughs in position. The curious order in which the cages are numbered is the result of crossing the cables in passing them through the pipe.

(Figure 21). The fact that the photobeams were now to pass directly across in front of the troughs meant that the length of the eight troughs would be greater than any existing cages. It also meant that there was a danger of accidental cutting of the beam. To obviate this, a half inch gap was allowed between the trough and the floor of the cage. The principle of illuminating two photo cells with one bulb was retained. Since the cut-off was never quite complete, it was essential that the two deflection coils operating one lever should be well balanced. This was easier to achieve if the two photo cells operating them were subjected to exactly the same intensity of light. The cages were made two foot square, some four inches more than necessary, in the hope of being able to add activity recording at a later date.

The third defect was rectified by making the troughs in two parts, an upper and a lower (Figures 18 and 19). The lower part consisted of the actual food containers soldered on to a ¼ inch brass plate for rigidity. The upper part of the troughs consisted of outer tubes with the head holes in them, light and transistor tubes, a top ¼ inch brass plate to which all tubes and electronic components were attached, and a sheet metal lid. All this was hung from the shelf on the cage by sheet metal screws. The new arrangement with the beam passing straight across the front of the head hole, and the resulting spacing out of the troughs, meant that there were three gaps wide enough to accommodate a quarter inch bolt. Accordingly,

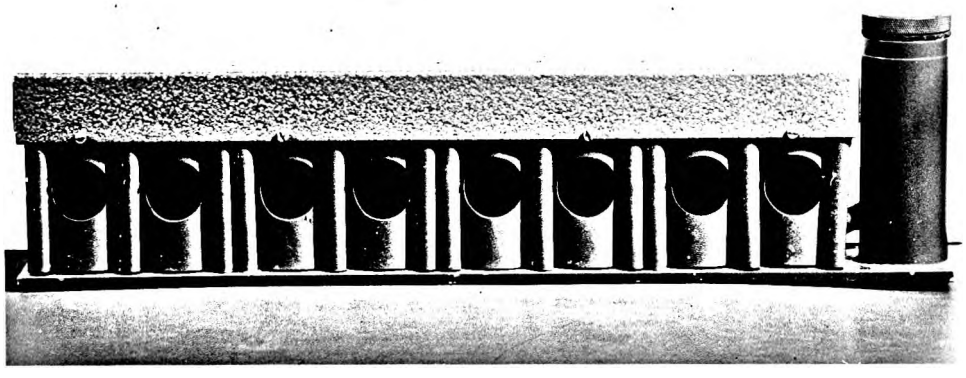

Figure 17.

The front view of a set of troughs. The water tower is on the right. On top is the sheet metal housing of the electrical components. The three pairs of tubes and the end ones contain transistors. The other four contain lights.

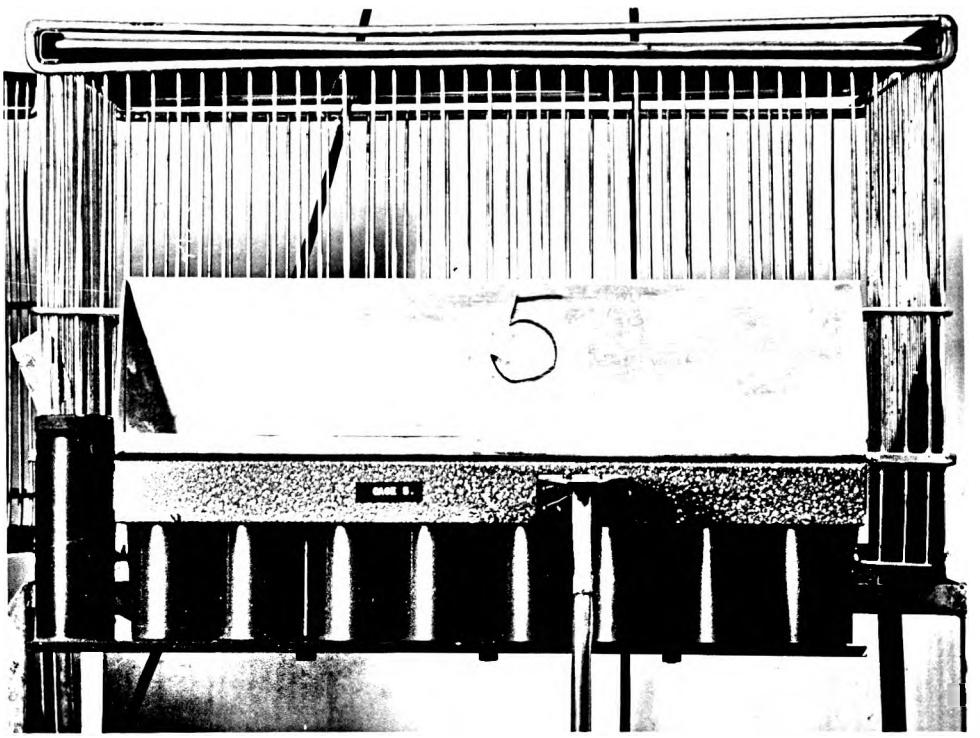

Figure 18.

The troughs in position on a cage. The water tower is at the left. The three nuts which must be removed to release the troughs can be seen under the bottom plate.

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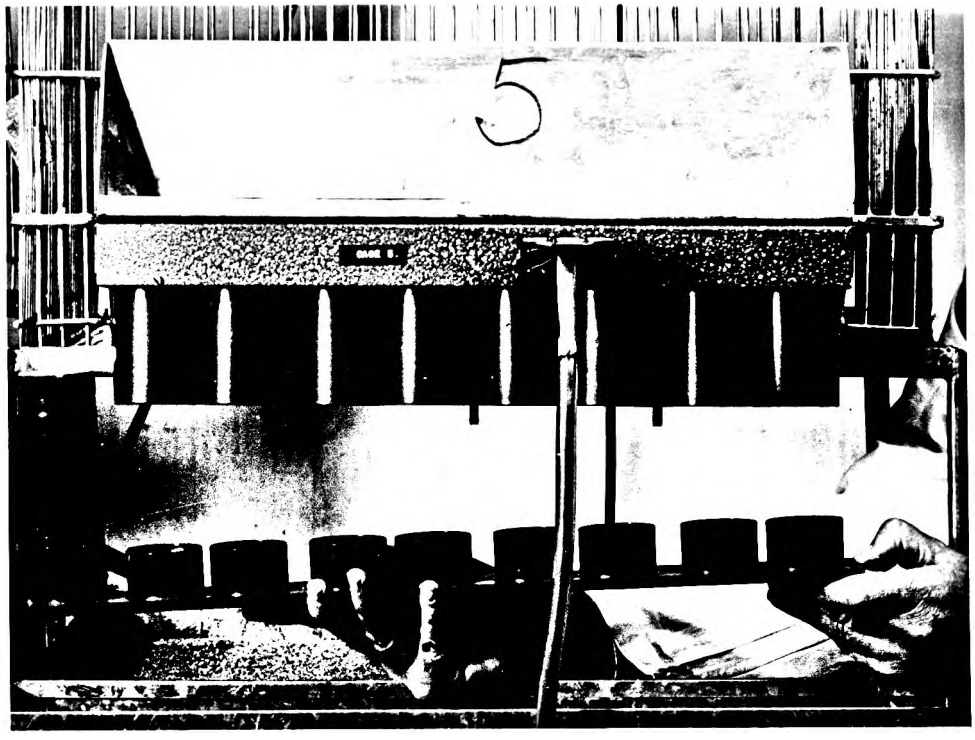


Figure 19.

The first stage in removing the troughs. When the three nuts are unscrewed the feeding troughs are easily detached from the rest. There is no need to proceed farther than this stage at feeding time.

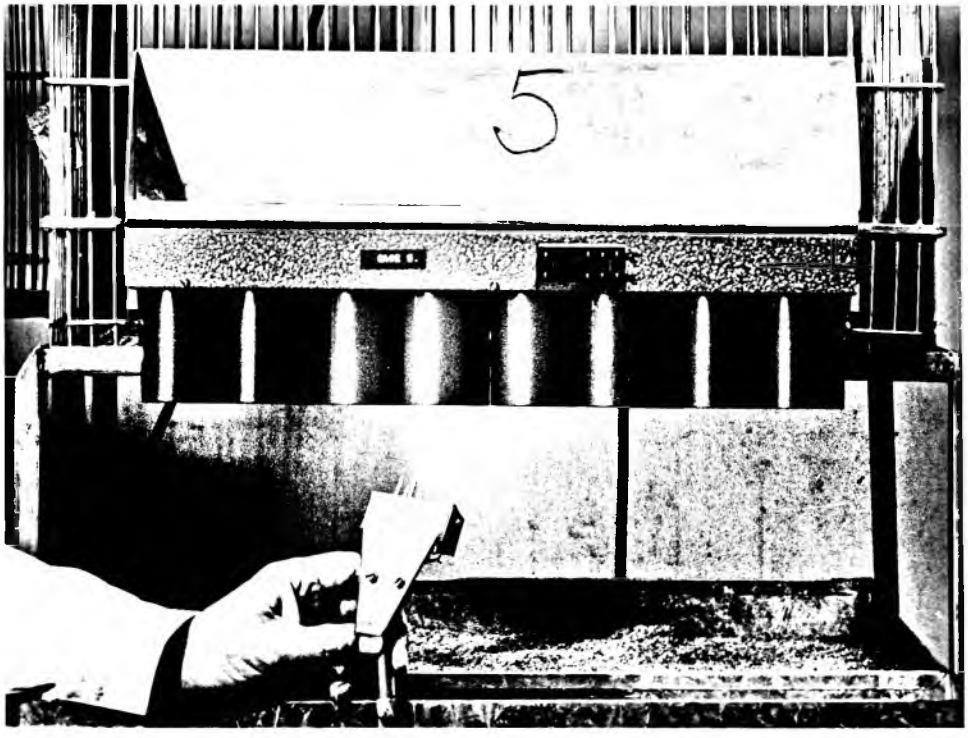


Figure 20.

The second stage in removing the troughs. The 12 point plug connecting the troughs with the electronic unit is removed.

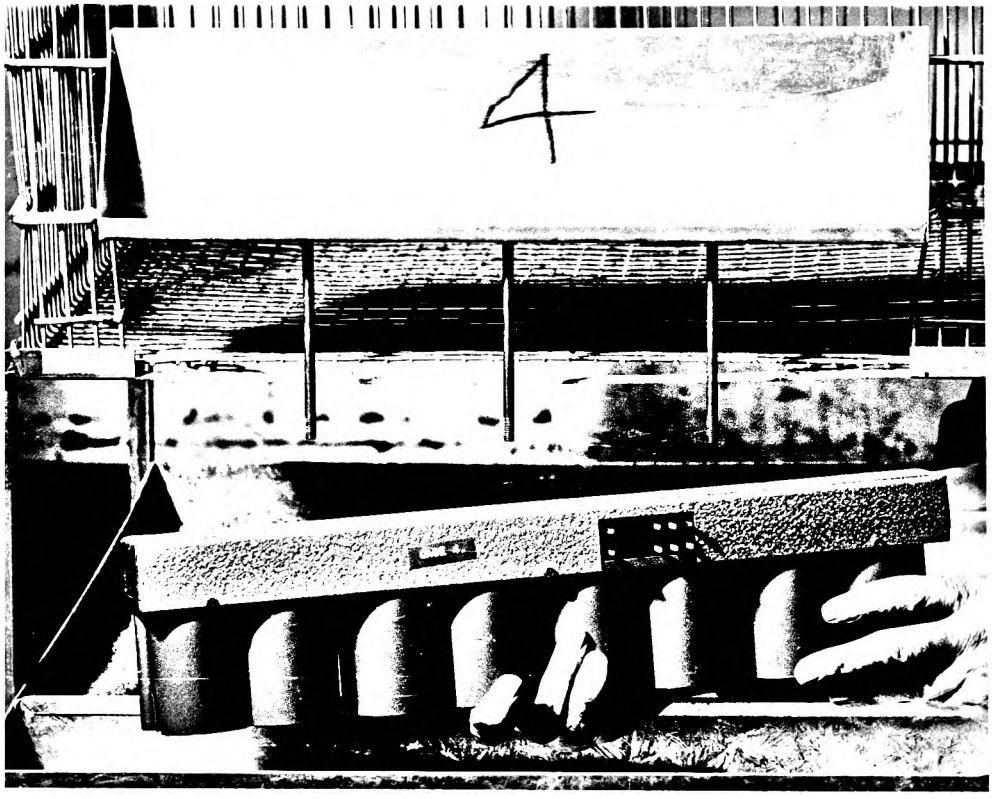


Figure 21.

The third stage in removing the troughs. The sheet metal screws have been taken out and the upper part of the trough lowered. The cage can now be taken away for cleaning and sterilising.

these spaces were drilled right through from the top of the shelf. Three long ¼ inch bolts were passed right through with the nuts at the bottom. To remove the feeding troughs, one simply has to undo the three nuts and remove the bottom plate together with the feeding troughs, the rest remaining in situ and in no way subject to distrubance (Figure 19).

The possibility of a water shortage was obviated by the provision of a water tower next to one of the end troughs (later designated trough number 1). This water tower, with a capacity of 200 ml., worked on the air-lock principle (Figures 17, 18, and 19). In the first model, a 1/8 copper tube from the bottom of the tower to the bottom of the trough was intended to act as a water supply tube, while the air lock tube consisted of copper of a similar size passing from a point ¼ of the way up the trough to the top of the tower. However, the pressure difference between the top and bottom of the tower proved insufficient to draw a drop of water up the air-lock tube, and the trough did not refill on being emptied. In the second model, a 3/8 inch tube, with the top ¼ of the way up the trough passed horizontally across to the tower, but when the level of water in the trough dropped below the bottom of the pipe, some still remained in the pipe and formed a meniscus which was a sufficient barrier to the entry of air. In the third and final model, the 3/8 inch tube passed from trough to tower at about 45°. This proved satisfactory. It meant that there was something in excess of 50 ml. of dead space in the bottom

of the tower, but there were still some 150 ml. of water available above the pipe. The top of the tower was fitted with a screw top and a cork washer impregnated with vacuum grease. This provided an air-tight seal and facilitated refilling.

Figures 18 to 21 show successive stages in the removal of the troughs.

All six electronic units were housed on the recording unit (figure 8), three on the bottom shelf, and three on the shelf between it and the one on which the paper supply boxes are fastened. This means that there are six twelve-core cables passing between the recording unit and the cages. In order to protect them and improve the general neatness of the layout, they are conveyed through a 1½ inch galvanized pipe (Figures 7 and 16). (This proved to be a mistake. It would have been better to tolerate the danger of damage and the untidy appearance and gain ease of removal.)

One further addition was made to the apparatus. Twice, during the early stages of the experiments, there were power cuts during the night. Since the whole apparatus stops and starts together, there is nothing to show when the cuts occurred, and it is impossible to form an opinion as to whether the 24-hour record should be retained or rejected. To overcome this difficulty, the University Electrician provided a synchronous electric clock which operates on the same 15 amp point as the rest of the apparatus. This clock, obtained from the former University Residence at Cottesloe, is of the old variety which is not self starting. Thus it stops at the time of the power cut and

does not start again, so that the operator knows in the morning that there has been a cut and at what time it started. A brief examination of one record tells him the time that is missing, and thus the duration of the cut. It is therefore possible to decide whether the record is so seriously curtailed that it must be discarded.

Operation

The apparatus is housed in the rat room of the Physiology Animal Laboratory. It occupies the whole of one end of the room, which is temperature controlled with a minimum of 70°F and is run strictly to routine. At 8 a.m. the cleaners enter, swill water over the floor, and brush the surplus water off with a broom. After the cleaners have finished, at about 8.30 a.m., feeding starts, being completed at about 9 a.m., depending on the number of animals in the room. Thus during this period from 8 a.m. to 9 a.m. there is considerable disturbance, and it seemed reasonable to feed the rats in this experiment at the same time, rather than introduce a further disturbance later in the day.

In view of the swilling down of the floor, all wooden apparatus, or ungalvanized metal, stands on bricks.

In front of the recording apparatus is a table on which are provided:-

The laboratory record book in which is entered,

(a) the troughs into which food is to be placed.

(b) the amount of food placed in the troughs, and the amount of water placed in the tower.

(c) the amount of food and water remaining from the previous day.

(d) once a week, the weight of each rat.

A measuring cylinder.

A baby enema syringe.

A spring balance weighing in grams.

A bottle of Esterline ink.

A Pasteur pipette.

Several pencils.

A selection of plastic spoons.

Six strips of sheet metal 2 inches wide and 24 inches long.

As the operator enters the animal house, he collects the food from the refrigerator. He then enters the rat room and glances at the clock. If it has stopped, he notes the time before resetting it. He then places the food on the table and takes the six sheet metal strips and slides one into each cage in front of the feeding troughs, thus preventing the insertion of the rats' heads. As he places the last strip in position, he notes the time on the clock to the nearest minute. At the same time, he listens to the time-marker and notes the click which comes nearest to the time. He then takes a pencil and marks the "blip" appropriate to that click with the time and the number of the cage.

Thus, on the record from cage 6 he writes, say, 833 6, and on the others 833 5, 833 4, 833 3, 833 2, 833 1. This marks the end of the previous day's record. He turns the drums until his time mark is well clear of the paper drive rollers and tears off all six records at the rollers, laying the ends over the sides of the collecting

bins. He is now ready to start feeding.

He starts with cage 1, undoing the three nuts and lowering the feeding troughs, which he places on the table. Now he takes the enema syringe and sucks the water out of trough number 1 with it until the trough is empty and no more water enters by the supply pipe. He squirts this water into the measuring cylinder and notes the volume in the record book. This is the volume of water left, less the dead space. He throws the water away and refills the cylinder with fresh tap-water. Then he fills the water tower from the cylinder and makes a note of the amount put in in the record book. Using a medium and then a small plastic spoon, he scoops out the food remaining in the feeding rough and weighs it, entering the weight in the record book. He makes this up to 30 grams with fresh food, and, having cleaned the trough of spillage, he refills it. He then replaces the number 1 troughs and proceeds to number 2 cage to repeat the process, continuing until all six cages have been dealt with.

If the pens and paper require no attention, he now removes the six metal strips, noting the time as before and entering it, the cage number, and the date on each of the new records. This marks the beginning of a new 24 hour period. It usually takes 45 minutes to replenish all six cages, so that the 24 hour record is in fact 23¼ hours long.

When pens or paper require attention, the blank period is lengthened. However, the effect of this is mitigated to some extent

by pressing into service an African laboratory assistant. This man, who operates four days a week, is fully capable of dealing with the feeding and watering, leaving the writer free to attend to pens and paper as necessary. It is always possible so to arrange the filling of the pens that they require no attention on days when the writer works alone, but it occasionally happens that the paper has to be replenished on those days.

Rolling up the previous day's records can, of course, be done at leisure after the feeding and watering is complete, but, until this process has been mechanised, it is done by an African cleaner on six days a week, being done by the writer on the seventh (Sunday).

Although the aim has been regularity in the management of the apparatus, there have been occasional lapses. A technician offered to relieve the writer on alternate Sundays. Since this was an act of kindness on the part of a very willing young man, the writer did not feel that he could impose the degree of punctuality that he imposes on himself. There were further irregularities at examination time.

In view of the complexity of the apparatus, and thus the possibility of faults developing, the writer has found it necessary to undertake a rigid routine of inspection three times a day (except on Sundays when two inspections have to suffice). In this way it has often been possible to spot and correct an airlocked or blocked pen which would otherwise have resulted in a serious loss of information.

The first act in each inspection is a glance at the clock to see that it is still going. One then counts the number of lines being drawn on each drum (a glance is not sufficient) and notes if any of the lines is abnormally thin, which indicates a pen about to stop writing. Standing by the recording machine, one glances along the cages, counting the lights in each (there should be four). It is undesirable to stand near the cages unless necessary since this elicits an alerting response in the rats who usually feed shortly after such an event before returning to sleep.

It is occasionally necessary to test a channel or adjust a pen. This results in a deflection or series of deflections. These are marked with an X to indicate that they were not done by the rat.

THE RECORDS AND THEIR INTERPRETATION.

As stated in the description of the apparatus, four levers are used to record the events taking place in the eight troughs. The left hand side in Figure 22 shows the order in which the troughs are represented on the tracings. There is an inversion of the trough order on each lever due to a fault in wiring. As this rather trifling mistake is common to all six records, the writer decided to tolerate it. In fact, it caused no difficulty in interpretation. The centre trace is the time marker's record. There is an upward deflection of the lever every minute, every fifth deflection of the lever being of longer duration than the rest.

This record, which reads from right to left, illustrates the difficulties mentioned at the beginning of the Design of the Apparatus, and the Final Model. In order to avoid the expense of 48 relays, the output from the OC 26 transistors is fed straight to the deflection coils. Since the transistors are never without current flow, there is sometimes a certain amount of imbalance even when the electronic adjustments have been performed with the greatest care, or an imbalance might appear in the middle of an experiment due to the collection of dust on a photo cell. There is thus a tendency for the lever to be pulled to one side when both output transistors are off. This can be corrected by compensating with the lever. It will be remembered

that the spring in the lever acts as a return mechanism. By bending the lever a little to one side, one can induce an equal and opposite imbalance and thus centre the righting point. In the bottom trace of Figure 22 an imbalance between channels 1 and 2 is clearly visible at the right hand side - a deflection in the number 1 direction and none in number 2. This fault is corrected by bending the lever in the number 1 direction and thus leaving some movement of the deflecting mechanism in the number 2 direction. At the left side of the record, the number 2 deflection, though small, is sufficiently visible to be legible. Should the correction be continued farther, there is a danger of reaching the condition shown in the record from troughs 5 and 6. Here the lever has been bent a long way in the direction of number 6 to get a good deflection in number 5, with the result that the return from the number 6 side is slow. This slow return, which can also result from excess writing pressure on a pen, though untidy and a nuisance in some ways, does not affect the interpretation of the record, since the start of the return is clearly visible. When a rat pays a series of rapid visits the result is a ripple effect instead of the clear rectangular pattern of the efficient lever (Figure 23 contrasted with Figures 24 and 25).

When the prototype was in operation, it was housed in the writer's office, and it was therefore possible to observe the rat and correlate the result with the record. This has led to the adoption of certain conventions in the interpretation.

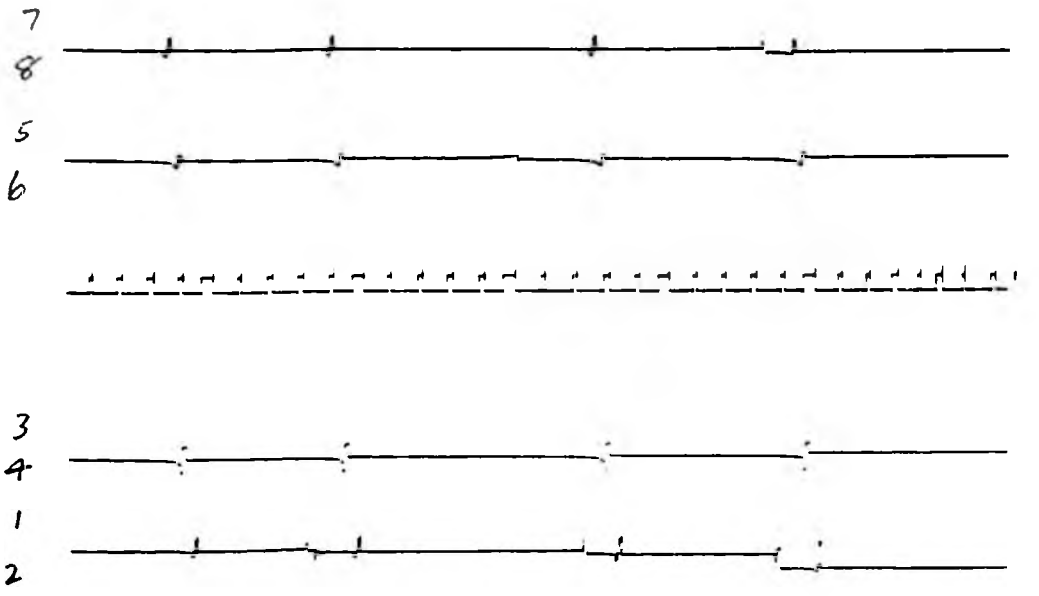


Figure 22.

A test of the recorder. The trough order is written to the left of the record which reads from right to left. In the centre trace, the time-marker gives one minute intervals. In the bottom trace the recorder was out of adjustment and is being corrected. Further details of this are given in the text.

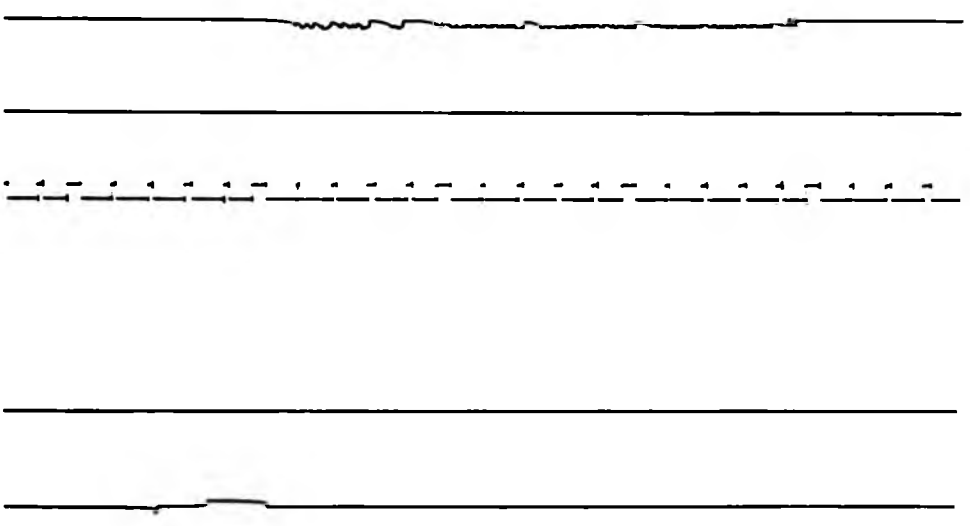


Figure 23.

Top trace. The record from a sluggish lever. This does not present any difficulty in interpretation. The record reads from right to left.

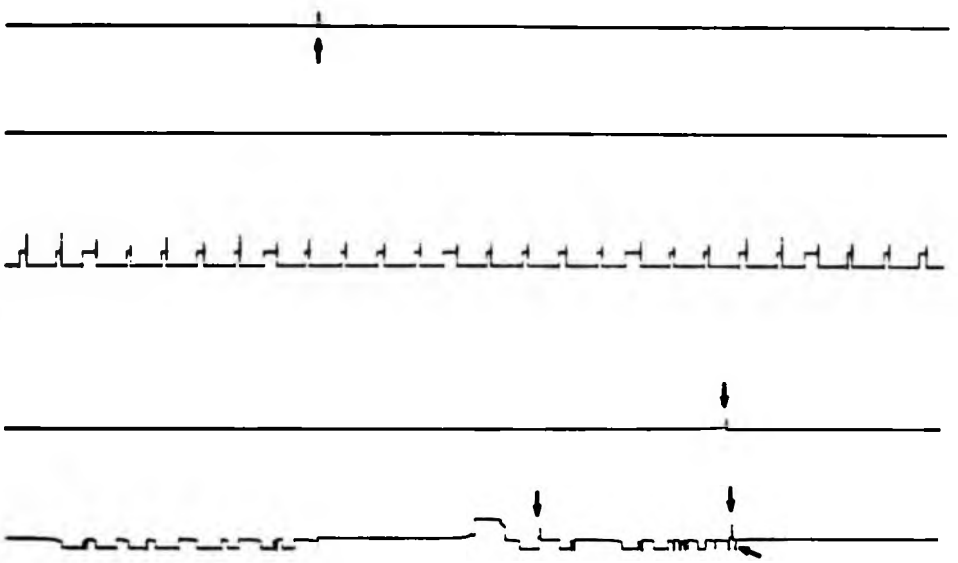


Figure 24.

A meal with the food in trough number 2. Random visits are marked with arrows. The record reads from right to left.

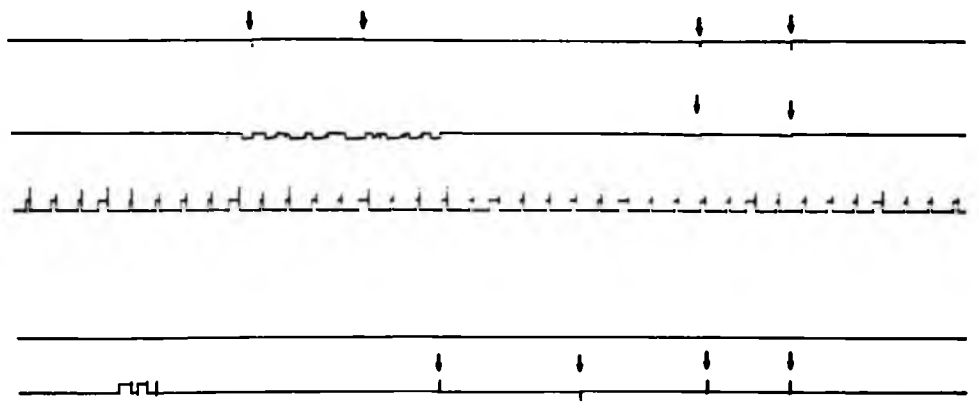


Figure 25.

A meal with the food in trough number 6. Random visits are marked with arrows. The record reads from right to left.

When a rat eats at one of these troughs it does so by inserting its head and withdrawing it several times. The periods during which its head is outside the trough appear to be occupied in chewing, and might last anything up to one minute. Thus, in the record from the prototype (Figure 2) a meal is represented by a broken line. All gaps of less than one minute are included in eating time, while gaps of more than one minute are recorded as pauses. In the records from the final model, such gaps in the record appear as returns to the baseline, or towards the baseline in the case of a sluggish lever.

A rat will occasionally insert its head into a trough for purposes other than eating or drinking. Presumably this is some sort of play and involves any of the troughs whether they contain food or water or not. Such visits are extremely brief, one second or so in duration, and are termed random visits. Such random visits might occur as interruptions to a meal or between meals and might involve the food and water troughs. It is important to distinguish between random visits to the food and water troughs and visits for the purpose of eating or drinking. With a record such as this, it is not possible to estimate duration more accurately than to the nearest quarter of a minute. Thus, anything of a duration of one eighth to three eighths of a minute is termed one quarter. Any event of a duration of less than one eighth estimated time is said to occupy zero time, this means that it is random. It might be expected, and the writer did indeed expect, that there would be borderline cases between eating time and random visits. In the event, this is not so, and the vindication of this convention will appear in a consideration of the experimental results. Meals containing random activity are illustrated in Figures 24 and 25.

As we have seen, the time at which a daily record starts is entered on the record to the nearest minute. In interpreting the record, therefore, one marks off the time trace in five minute and hourly intervals, using a 24 hour clock. Much of the record is blank, but is interrupted by episodes, which are composed of events. One

can, therefore, by recording the beginning and the end of an episode to the nearest quarter of a minute, measure its duration. If one measures the duration of all eating and drinking events, and takes random visits as zero time, then by measuring the duration of pauses between events to the nearest quarter of a minute, the total of such time estimates should be equal to the duration of the episode. This forms a useful and necessary check on one's estimations. A legitimate discrepancy can arise when a rat pays a rapid series of random visits with none of the pauses approaching a quarter of a minute, and yet the whole occupying an appreciable period of time, in fact a quarter of a minute; in this case, the total is that much less than the duration of the episode. However, one is always conscious of such a discrepancy as one goes along.

There are thus four types of event which might form part of an episode:-

Drinking time
Eating time
Random visits
Pauses

An episode which contains eating time, however short, is termed a meal. The writer wished to be able to distinguish between events that occurred at meal times and those that occurred between meals and was thus faced with the interesting question, "How long must a pause be befor it ceases to be a pause?" This is reminiscent of the problem of how long a Galvanic pulse must be before it becomes a D.C. current. However, the writer again falls back on his experience with the prototype. If a rat leaves the troughs for more than six

or seven minutes, it rarely returns to them in under half an hour. Rounding, a pause must be less than ten minutes. This somewhat arbitrary convention works well in practice. Even at the height of feeding activity, as midnight approaches, episodes are seldom less than twenty minutes apart. Out of a total of 1,183 episodes there were 81 intervals of less than 15 minutes and 57 of between 15 and 20 minutes. All other intervals lasted twenty minutes or more.

The interpretation of the record in Figure 25 as it would appear in the journal is:-

1151½

8 -
6 -
1 -
3½
1 -
8 -
¼
6 -
4¾
2 -

1200

5¼
1 -
6 3¼
7 -
¾
6 3¾
¼
8 -
6 1¼
2¾
1 1½

1217¾

That is to say, the episode started at 115½ and continuted until 1217¾. The figures in the first column are the trough numbers, those

in the second column being the duration in minutes. Where there is no figure in the first column, the time represents a pause. A dash in the second column represents zero duration or a random visit. Here feeding time is 8¼ and drinking time 1½ minutes.

There remains the question of artefacts. At infrequent intervals, a record might contain a slow deflection, taking a minute or more, instead of the usual sharp one (Figure 26). This usually occurred at one of the end troughs and could be of long duration broken, occasionally, by slow returns to the baseline. The only way to find the cause of this was to station oneself in the rat room and watch. Each rat was provided with a round enamel bowl in which to sleep, but sometimes one of them would climb out of the bowl and lie down to sleep in front of the troughs with its back towards the lights. During its movements in sleep, the long hairs of its back would move into and partly obscure the beam, causing this slow, partial deflection. These deflections are easily distinguished from the more usual kind and, when once their cause was known, they were ignored. When there was any doubt at all, the deflections were recorded.

The other type of activity, even more rare, consisted of a sharp deflection which was maintained for a long period. This proved to be of similar origin, but in this case the rat inserted its caudal end into the beam and went to sleep. When such events are of ridiculously long duration they can be written off as artefacts and ignored. However, one does not know how often short events of this

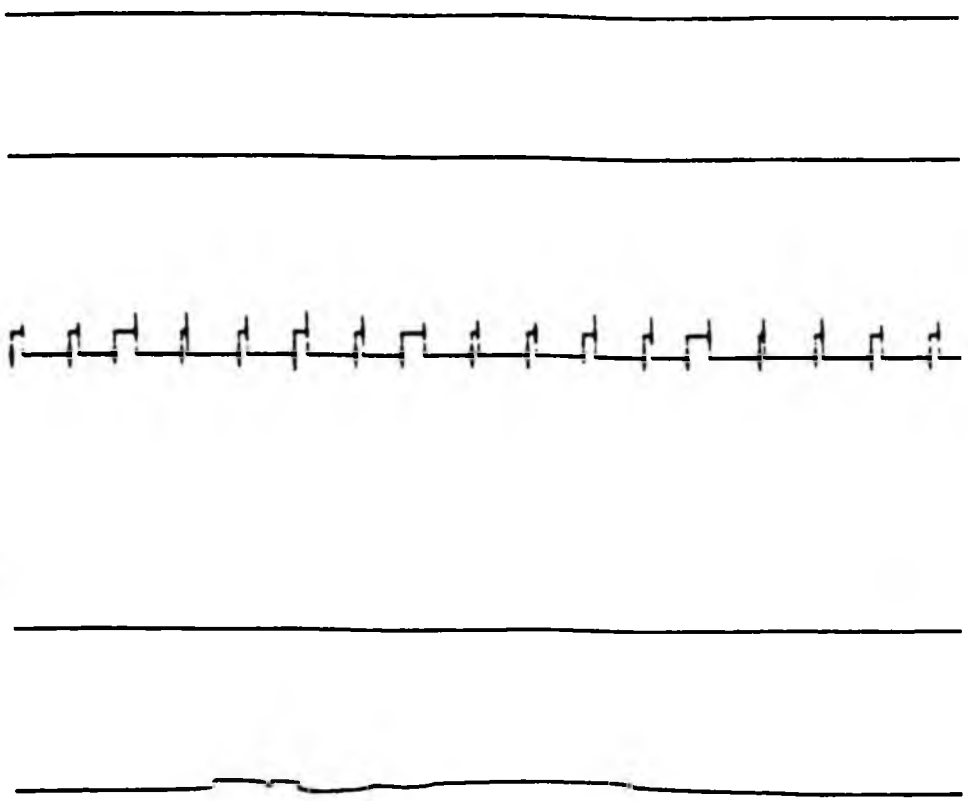


Figure 26.

An artefact. In the bottom trace there is drift in number one channel. This is followed by a clean deflection which was counted as drinking time between meals.

sort occurred. That they are frequent is suggested by an examination of between meal activity. Much as the writer would wish to avoid it, it seems necessary to further protect the troughs by making the rat enter a short passage to get to each one.

THE EXPERIMENTS.

As already indicated, the object of the experiments described herein is two fold. In the first instance, it is necessary to know as much as possible about the rat's eating behaviour under the conditions presented by the experiment. The importance of this lies in the slowness of the procedures and the fact that only six rats can be accommodated at one time. Thus, to obtain results with, say, a hundred rats, the experiment in question would have to be repeated seventeen times in order to accommodate them. Supposing an experiment lasts three weeks with each group of rats, the whole will take about a year, assuming continuous running, which is unlikely. To reduce this, one must somehow increase the efficiency of the experiments so that smaller numbers can be used. This involves knowing where the main sources of variation lie in a uniformity trial and thereafter so arranging or grouping the rats that this variation is eliminated in the statistical treatment of the results. Alternatively, some fault in the management of the rats might be revealed which will lead to a better procedure in future. Thus, preliminary, exploratory experiments are essential.

In the second place, when one comes to the definitive experiments, some of the ingredients offered to the rats will be taken in very small quantities, the B vitamins for example, and thus visits to these troughs will be of very short duration. One can, it is true, dilute those constituents and thus make the rat spend longer obtaining his requirements, but there is a limit to this; one must not dilute below

the taste and smell thresholds. It is therefore necessary to make a study of the random visits to troughs and see, if possible, how they can be reduced or eliminated. If they cannot be eliminated, one must know whether trough position is in any way correlated with the number of random visits, whether, for example, the end troughs are more subject to random visits than the middle ones. Armed with such knowledge, one can arrange things so that the dietary ingredients which are taken in only small quantities are placed in troughs least subject to such random visits. There is, of course, an essential fallacy in this reasoning. We have made the assumption that a rat's behaviour towards a trough which presents no gustatory or osmic stimulus will be the same when a definite stimulus is provided. The writer does not believe this but admits that one must take due precautions in case he is wrong.

No special arrangements are necessary for investigating the rat's general feeding behaviour. It matters not which trough the food is placed in nor even if it is placed in all of them, since one must assume that the quantity it eats and the time intervals at which it eats are determined by the factors discussed in the first part of the introduction. When it comes to random activity, however, the position of the trough must be of importance. Since it was possible to put a little more than a day's supply of food in one trough, the writer decided to use one trough only and supply a complete mixed diet.

It was the writer's intention to use the McCollum Diet described by Richter and Schmidt (1939).

Graham Flour	725 gm.
Skim Milk Powder	100 gm.
Casein	100 gm.
Butter	50 gm.
Sodium Chloride	10 gm.
Calcium Carbonate	15 gm.

Which amounts to:-

Carbohydrate	29.2%
Protein	26.7%
Fat	14.1%

However, it proved impossible to obtain Graham Flour and this was replaced with unsifted wheat flour. No doubt this resulted in a small dilution by reason of the cellulose content, but this should not have affected the rats' intake of nutriments (Adolph 1947).

The food was moved to a different trough at the beginning of each week, and, since there were seven trough positions for each rat, this imposed a seven week period on the experiment. The troughs were used in random order so that the rats did not form habits and were unable to predict what the next change would be. This was, perhaps, being rather pernickety and led the writer into an act of thoughtlessness. He randomised the order for each rat independently. This needlessly complicated feeding and led one of his assistants to make a mistake. Had all the rats used the same trough each week, no information would have been lost and the mistake would not have occurred. The only alternative to this arrangement, strictly speaking, is one in which

no two rats had the same trough position in the same week. The only additional information to be obtained by such a complex arrangement would be an unbiassed estimate of the performance at each trough, and, as it turned out, this would not have been worth while. As it is, trough position is confounded with time and is biassed. Ieccavi!

The rats were albino laboratory rats which have been bred in the Department of Physiology for twelve years. Three of them, those in cages 2, 3, and 4, were littermates, while the other three are unrelated to them or each other. The term "unrelated" is, of course, relative in an inbred colony. All were males, 4 months old in October 1963 when the first run of the experiment, later abandoned, began and their weights were:-

Cage number	Rat weight at start (gms)	Weight at end (gms)
1	208	230
2	244	342
3	222	324
4	220	290
5	230	268
6	218	310

Details of their weights throughout the second run of the experiment are given in Appendix I.

On the 14th of October 1963, the first experiment began [redacted]

[redacted]

It soon became apparent that it was impossible to interpret the records at the speed at which they were produced, and the writer decided to concentrate on one rat and keep the interpretation up to date at all cost. Any spare time which became available was to be devoted to bringing another rat up to date. The reasons for this decision were partly that it was the more interesting way of doing a monotonous job, and partly as a check on the apparatus. It was hoped that, should anything subtle go wrong, it would be spotted and action could be taken before too much was lost. In this way it was noticed, during the fourth week, that the random activity between meals increased rapidly, with the rat hardly getting one hour's consecutive sleep. Then a record appeared in which, while the rat was feeding, another channel became active, so that it appeared as if the rat were visiting two troughs simultaneously. [redacted]

[redacted] Investigation showed that there were undoubted signs of cockroach infestation in the rat room. It was, of course, impossible to take heroic measures since there were other experiments in progress. Efforts to save the experiment by surrounding the cages with a cockroach repellent with a boric acid base and by placing cockroach poison at strategic points proved unavailing. On the fifteenth of December the experiment was abandoned.

One important lession was learnt in this abortive run, and that is that it is essential to keep up to date with the interpretation of records while it is impossible to do so if all the records are interpreted. It was therefore necessary to resort to a system of sampling, the nature of which will be described in the section of the statistical treatment of results.

By the time the necessary steps had been taken in the rat room and time had been allowed for the rats to settle down, it was 7th January 1964 before it was possible to start again. The first week went smoothly, but during the second week, [redacted]

a power failure occurred [redacted] at 10.55 p.m., the cut extending for over an hour. Coming at a time of maximum

feeding activity, this was a serious break. It was impossible to repeat that 24-hour record without repeating the whole week since the day to day variations which are important in certain contexts would be lost. The writer therefore decided to abandon the whole week and repeat it at the end of the experiment. Thus, the experimental period was eight weeks instead of seven, week 2 being absent.

In week 3, the rat in cage number one developed diarrhoea. Since the flour supplies appeared to be developing fungal growth, they were replaced and the condition cleared up.

On February the 3rd, trouble developed in the electronic components in the troughs in cage number 2. When the troughs were removed for repair, it was found that the rat had been gouging copper off them. Careful note was taken of the size, shape, and depth of the tooth marks. These had not changed at the end of the experiment, so presumably the rat did not repeat the exercise.

On the 20th February, the pen in channels 1 and 2 in cage 5 stopped writing at 7.30 p.m., the food being in trough 2. Cage 5 was one of the two cages to be sampled that night, the other cage having a good record. To repeat would mean repeating the whole week. This raised a special problem. It was the last week of the experiment and thus another week would have to be inserted so that there would be a trough change at the beginning. In fact, the experiment would run for ten weeks altogether and extend well into the beginning of the next term when the writer would be heavily engaged in other ways.

He therefore decided not to repeat but to accept the loss of information and to treat that record as if it were a missing value.

There were various electronic troubles and several mechanical adjustments were required to the levers, but these happened to develop in cages which were not appearing in the samples. However, they pointed to a certain lack of reliability in the apparatus.

It was only when the writer was collating the results that he discovered that on one day the food had been placed in the wrong trough for one of the rats that appeared in the sample. He felt that this would not affect the feeding and drinking behaviour and therefore accepted the result as it stood for that part of the results. However, with random activity it was quite another matter. Observation showed that a change of troughs initiated an increase in random activity for one day at least, so that this mistake would lead to a false reading in midweek. The change back to the correct trough next day did not matter since that day did not appear in a sample and was thus not recorded. This is dealt with further in the section on statistical treatment.

THE STATISTICAL TREATMENT OF THE RESULTS.

When it became apparent that he would be unable to interpret the records as fast as they were produced, the writer decided to resort to a system of sampling. His main interest was in random activity and here he was interested especially in day to day variations. It was thus essential that each day of the week should be represented without bias. It was equally important, lest there should be interrat variations, that each rat should be equally represented. The week to week variations did not seem so important. In the matter of feeding behaviour, on the other hand, the day to day variations were not likely to be of any importance, while the longer term changes might be. If it is true that the day to day variations are not important, the omission of a day in each week, in random order, will not bias the long term assessments.

He therefore decided to draw a sample in such a way that each rat appeared in it once in each week, each time on a different day, and no two rats appeared on the same day in the same week. Thus each day of the week would appear six times in the course of the experiment, and each time would be represented by a different rat. Randomisation was done by means of a table of random numbers and the following table resulted (Table 1).

Table 1.

The distribution of the days of the week within a sample.

	Rat Number					
Weeks	1	2	3	4	5	6
1	1	5	4	6	7	2
2	7	4	3	5	2	6
3	3	2	1	7	6	4
4	6	3	2	4	5	1
5	2	1	7	3	4	5
6	5	7	6	2	1	3
7	4	6	5	1	3	7

This meant that there were only 42 records to interpret in 49 days. The writer felt he could do better than that, and therefore decided to draw a second sample in the same way but independent of the first. Tables of this sort proved inconvenient for working, and the two samples were reconstructed showing the distribution of rats within days and weeks (Table 2).

Table 2.

The distribution of rats by days and weeks.
Sample 1 is above sample 2.

	Days						
Weeks	1	2	3	4	5	6	7
1	1	6		3	2	4	5
	3	2	4	6	1		5
2		5	3	2	4	6	1
	5	3	1	2	6	4	
3	3	2	1	6		5	4
	1	4		3	5	2	6
4	6	3	2	4	5	1	
		1	6	5	2	3	4
5	2	1	4	5	6		3
	2	6	3		4	5	1
6	5	4	6		1	3	2
	4	5	2	1		6	3
7	4		5	1	3	2	6
	6		5	4	3	1	2

Thus 84 records were interpreted out of a possible 294, or nearly one third. While the writer admits that it would be preferable to interpret all the records, the system of sampling is imposed on him by practical considerations.

It appears to the writer in retrospect that it was a mistake to make the two samples independent, because it is possible for the same rat to appear in both samples on the same day. This in fact happened four times. In these cases the same readings were treated as if they were two independent ones. It was also possible for a day to pass with no samples taken. This, fortunately, only happened once. The writer was at fault here. The samples should not have been independent.

Week 2 is actually omitted and placed at the end, so that the weeks are 1, 3, 4, 5, 6, 7, and 8.

FEEDING AND DRINKING BEHAVIOUR.

The missing value.

In order to estimate the probable missing value, the following method was applied. The results were set out in a 6 x 14 table. That is, six rats (columns) by two lots of seven weeks. The rows and columns were totalled and the missing value estimated by applying the following formula:-

$$M = \frac{rR + cC - T}{(r-1)(c-1)}$$

where

r is the number of rows

c is the number of columns

R is the total of the row which contained the missing value.

C is the total of the column which contained the missing value.

T is the grand total.

The estimation of this missing value entails the loss of one degree of freedom for both total and error sums of squares. Had this happened in a randomised block experiment, a correction would have been applied to the sum of squares for treatment[5]. In fact, there are no treatments here and the correction therefore has no application. In any case, the factor is normally very small and unlikely to affect estimates of probability. This estimation was applied to all those readings taken direct from the records. When other parameters were calculated from them, the estimated values were treated as if they were real values. These calculations were divisions and thus errors are not multiplied.

Parameters.

The following parameters were determined by direct measurement:-

Food consumption (grms per day) Appendix II.

Feeding time (minutes per day) Appendix III.

The number of meals per day, Appendix V.

Water consumption (mls. per day) Appendix VII.

Total drinking time (minutes per day) Appendix VIII.

Time spent drinking at meals (minutes per day) Appendix IX.

Time spent drinking between meals (minutes per day) Appendix X.

In addition, all rats were weighed weekly (Appendix I). This is rat room routine and the weights were entered in the record book. On one day the routine failed to function, so that there is no record for week 7. In week 2 the rat weigher was sick and the weighing omitted without the writer being informed. However, as there are no other readings for that week, this is in fact not a loss.

In addition, the following parameters were calculated:-

Rate of eating (mgms. per minute. Food consumption/ feeding time) Appendix IV.

Average duration of meals. (Minutes. Feeding time/number of meals per day) Appendix VI.

The statistical analysis.

The writer's first approach to the analysis of results was wrong and his error was pointed out by Professor J. E. Kerrich of the Department of Mathematical Statistics. The final analyses went according to Professor Kerrich's suggestion, but the wrong approach had a useful result and it is therefore proposed to give a little attention to it.

It appeared that, since there was a table of 84 figures, there were 83 degrees of freedom in total, less one for the missing plot, 82. Within the table there were six columns and fourteen rows, giving 5 x 13 - 1, or 64 degrees of freedom for error. There were

thus 18 degrees of freedom left for the various comparisons. These were apportioned as follows:-

Between samples	1
Between rats	5
Between weeks	6
Between days	6
	18

The writer had completed the analysis of all parameters before consulting Professor Kerrich, obtaining his sum of squares for error by difference. In one case, as a check, he computed the error sum of squares directly. For each cell in the table, he computed the ideal value by:-

$$V = m_c + m_r - m_t$$

where

m_c is the column mean

m_r is the row mean

m_t is the general mean

In each cell of the table he entered the discrepance between the ideal value and the observed value, squared them and added them. The figure obtained for error sum of squares was the same as that got by the difference. Thus, whatever else was wrong with this method of analysis, at least the error mean square was a good estimate of the population variance.

For each parameter, the variance arising from differences between the daily means was computed and was insignificant when compared with this estimate of population variance. Thus the hypothesis that there is no difference between days of the week is retained. This has three important consequences.

1. In deciding on the method of drawing samples, it was assumed that there would be no significant daily variations in feeding or drinking behaviour. This is true.

2. In the method of analysis finally adopted it is assumed that the day on which the reading was taken was the middle day of the week. Since there is no significant difference between the daily means, this introduces a negligble error.

3. In each weekly mean, the mean of both samples, two days are missed out of the possible 14. However, as there is no significant difference between the days, the weekly means are still without bias.

Plotting the readings for each rat against the weeks of the experiment revealed that there appeared to be a regression on time in some of the readings in addition to a difference between rat means. This led to the adoption of the following model for statistical analysis.

$$Y_{ij} = \mu + a_i + \beta x_{ij} + \epsilon_{ij} \quad \ldots\ldots\ldots\ldots \quad (1)$$

Here, Y_{ij} is the value of any Y. This is equal to the general mean u, plus the deviation from the mean a_i, plus the slope of the regression line times the deviation of the appropriate X from the general mean of X, plus an amount which is independent of X and Y

and drawn at random from a normally distributed population. In the analysis, this leads to the testing of two variances, that arising from the differences between the rat means which is a measure of the height of the regression lines, and that arising from slopes. These are tested against the experimental error which is an estimate of ϵ_{ij} and thus of the population variance. The error sum of squares and degrees of freedom are obtained by difference. This gives rise to the following outline of the table of analysis of variance.

Source of variance	degrees of freedom.
Between rat means	5
Rat slopes	6
Error	71
Total	82

The sums of squares for between rat means and total are computed by:-

$$\sum y^2 = \sum_{i=1}^{k} \frac{\sum_{i=1}^{j} Y^2}{j} - \frac{\left(\sum_{i=1}^{k}\sum_{i=1}^{j} Y\right)^2}{kj} \quad \ldots\ldots\ldots (2)$$

In the case of feeding time (Appendix III) this becomes:-

Total $65.25^2 + 84.25^2 + \ldots 98.50^2 + 85.25^2 - 1/84(6,973.75^2)$

$= 602,714.6 - 578,966.5 = 23,748.1$

Between rat means $1/14(1,152.25^2 + 1,104.50^2 + \ldots 1,434.25^2)$

$- 578,966.5 = 14,537.1$

In computing the sums of squares appropriate to slopes, the writer first computed b (the regression coefficient) for each rat, since that figure will be required later, according to the formula:-

$$b = \frac{\sum_{i=1}^{k} \frac{\sum_{j} XY}{j} - \frac{\left(\sum_{i=1}^{k} \sum_{j} X\right)\left(\sum_{i=1}^{k} \sum_{j} Y\right)}{kj}}{\sum_{i=1}^{k} \frac{\sum_{j} X^2}{j} - \frac{\left(\sum_{i=1}^{k} \sum_{j} X\right)^2}{kj}} \quad \ldots\ldots\ldots\ldots (3)$$

which is

$$\frac{\sum xy}{\sum x^2} \quad \ldots\ldots\ldots\ldots (4)$$

The sum of squares is then obtained by multiplying b by its own numerator, giving:-

$$\frac{\left(\sum xy\right)^2}{\sum x^2} \quad \ldots\ldots\ldots\ldots (5)$$

The computation of the sum of X and the sum of X^2 requires caution. First there is no X = 2. Then the X's are 1, 3, 4, 5, 6, 7, 8 repeated. The sum is 68, and the sum of squares of deviations from the mean 69.71.

The computation of the numerator of b presents no problem since k is 14 and j is 1. Having computed b for each rat, one multiplies each by its own $\sum xy$, thus obtaining the six sums of squares. These are totalled giving the sum of squares for all rats.

Designating the first part of the numerator in equation (3) as XY, and the second part as Kxy, and the sums of squares for b as SSb, the computation goes as follows, again for feeding time.

	Rats						
	1	2	3	4	5	6	Total
XY	5721.50	5607.75	4761.50	6861.50	4656.25	6861.50	
Kxy	5596.64	5364.71	4667.71	6643.36	4633.71	6966.36	
xy	124.86	243.04	93.79	218.14	23.54	-54.86	
b	1.791	3.486	1.345	3.129	0.338	-0.787	
SSb	223.63	847.28	138.81	682.56	7.95	43.09	1,943.28

It will be remembered that of the six rats, three, numbers 2, 3, and 4 are littermates while the others, numbers 1, 5, and 6 are not. It is therefore possible, and from the writer's point of view interesting, to subdivide the variance arising from the rat means into the difference between the littermate (hereinafter LM) and non-littermate (herinafter NLM) means with one degree of freedom, and the variance between the means of the three LM rats on the one hand, and the three NLM rats on the other, each with two degrees of freedom. Apart from the information which the writer wishes to obtain from these comparisons, they form a useful check on the arithmetic since their sums of squares add up to that for between rat means. The computation of the sums of squares in these cases is strictly according to equation (2) and raises no difficulties provided one observes carefully the values of k and j.

In all cases, the first test of significance is the variance ratio F. The writer observes two levels of probability of committing

a Type I error, that is rejecting a null hypothesis which is actually true. When P is less than 0.05 but greater than 0.01, the F value is marked with one asterisk and the writer considers that, in view of the small number of rats involved, this indicates only that the null hypothesis might be rejected if the result can be repeated in a subsequent experiment. Where P is less than 0.01 the F value is marked with two asterisks and the null hypothesis can be rejected with some confidence.

The table of analysis of variance, again for feeding time, appears as follows:-

Source of variance	Degrees of freedom	Sums of squares	Mean squares	F
Between rat means	5	14,537.1	2,907.4	28.39**
LM v NLM	1	137.0	137.0	1.34
Between LM rats	2	6,079.5	3,039.8	29.69**
Between NLM rats	2	8,320.6	4,160.3	40.63**
Slopes	6	1,943.3	323.9	3.16**
Error	71	7,267.7	102.4	
Total	82	23,748.1		

It is of course of some interest to know whether there is in fact a bigger variation between non-littermate rats than between littermate rats. A value of F for this comparison can be obtained by dividing the one mean square by the other. However, with ν_1 and ν_2 both equal to 2, a P of less than 0.05 would require a ratio of 200 or more, while a P or less than 0.01 would require one of 4,999. Such values were never achieved and it is not possible to reject the null hypothesis.

Having obtained significant values for F, it is necessary to delve into where the actual variation lies. This involves the use of the t test. Before going into this, however, it is advisable to look further at the experimental error.

Were it not for the missing value, the error would have 72 degrees of freedom. We can rewrite the table in Appendix III in such a way that it has 42 cells, each containing two figures, one from each sample. Presumably the true value for each cell would lie somewhere between the two figures. The difference between each pair of such figures would thus contain two deviations from the cell mean. Thus by taking half the sum of squares of these 42 differences, one obtains an estimate of Σ_{ij} with 42 degrees of freedom, one from each cell. Having computed this sum of squares, one could then obtain the sum of squares appropriate to the remaining 30 degrees of freedom by difference. The mean square computed from this figure could be due to interactions between rat means and slopes, yielding, by way of example, the interesting information that rats with large means have steep regressions in time while rats with low means have flat ones. The writer confesses that he cannot see the physiological implications of this and cannot thus see the value of splitting the error. There is, however, another possible identity of this variance, and that is deviations from the rat regressions. There would be 5 degrees of freedom for each rat and with six rats this would give 30 degrees of freedom. The writer may be talking nonsense and confesses that he

is not mathematician enough to decide the point. It is one of some importance, for in order to use the t test he requires an estimate of both deviations about the mean and deviations about the regression lines. With the pooled error he knows where they are, while with the split error he is not sure. He has thus decided to pool the error, feeling that nothing is gained but uncertainty by splitting it. At least he is certain that his experimental error contains all he requires of it. It might be added that splitting has surprisingly little effect on the error variance.

To decide where among our rat regressions the significant ones lie, we first require s_b, the standard error of b. This is computed from the formula:-

$$s_b = \sqrt{\frac{s^2_{y.x}}{\sum x^2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

We take it that the error mean square is our $s^2_{y.x}$. In the case of feeding time s_b becomes:-

$$\sqrt{\frac{102.4}{69.71}} = 1.212$$

With

P	t(d.f.70)	significant b
0.05	1.994	2.417
0.01	2.648	3.209

Thus armed, we can examine our b's and mark them with 0, 1, or 2 asterisks as the case may be. The results of this exercise and the conclusions drawn therefrom will be given in the next section.

Here it is necessary to mention two assumptions made. First, as already mentioned, we have assumed that all samples are drawn on the middle day of the week. This is not serious because of the lack of significant differences between the days of the week. It is true that the weeks are not all of the same length, but the average length of the week is indeed seven days.

The second assumption is that the regressions are in fact linear. They might well not be. It is probable that some are exponential.

Next we come to the differences between rat means. Here we require the standard error of the difference between two means and since the number of rats in each mean is the same we use the formula:-

$$s_{\bar{x}_1-\bar{x}_2} = \sqrt{\frac{2s^2}{n}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (7)$$

In the case of feeding time, this comes to:-

$$\sqrt{\frac{2(102.4)}{14}} = 3.825$$

With

P	t (d.f. 70)	Significant difference
0.05	1.994	7.627
0.01	2.648	10.129

The question arises, "How do we apply these differences?" There is no legitimate method of pairing the rats and we must not compare the highest mean with the lowest. The writer is interested in the difference in the performance of the littermate and non-

littermate rats. He therefore first divides into these groups and then places the rats in order of magnitude of their means. He next takes the differences between adjacent means and compares them with the significant differences computed above, with the following result.

Rats	LM rats means	Differences	Rats	NLM rats means	Differences
4	97.70		6	101.45	
		23.06**			19.15**
2	74.64		1	82.30	
		6.00			13.16**
3	68.64		5	68.14	

It is, of course, understood that where F is not significant, one is not entitled to proceed farther with the analysis.

The conclusions to be drawn from such results appear in the next section. This completes the analysis of variance.

Correlations

It is of interest to know the extent to which the various parameters correlate with one another. In the first instance, the writer performed an analysis of covariance for several combinations of parameters. However, since the original concept was faulty, this had to be abandoned. The model, represented by equation (1), on which this analysis of variance is based is in fact a model of covariance. To proceed farther with this, then, would involve performing a multiple covariance, which, while perfactly possible, if laborious, would not be justified on such a small group of animals. Indeed, it is doubtful whether such elaborate methods would yield any more information than is obtainable by much simpler means.

In plotting the appropriate graphs, the writer has somewhat arbitrarily put one parameter on the ordinate and one on the abscissa, and the graphs could as well be drawn the other way, for it is impossible to say which variate is dependent and which is independent. In fact neither is independent. One could calculate two regression coefficients, and the lines drawn with their aid would cross at the point representing the means. It is not possible, therefore, to produce one regression coefficient and test its significance. Instead, the writer has thought it better, and sufficient, to compute their geometric mean, the correlation coefficient.

This coefficient will tell him if there is a correlation and how close it is. He will then know what to look for or what to explain in future experiments. The question resolves itself into one of which figures to use in computing the coefficient. One could use the six rat means or all the figures in the tables.

In his working graphs, the writer plotted 84 points and has based his conclusions and calculations on them. It thus seems reasonable to work with the total sums of squares and products. (The graphs used for illustration are simplified. In the originals there were six symbols and two colours. It is impossible to use the two colours in reproduction and their omission reduces the graphs to a state of utter confusion. Thus, in this thesis, 42 points are used, each point being the mean of two sample readings.) In inclining to this view, the writer believes that he will draw frowns

from Mathematicians unless he can show that the individual rat regressions do not differ significantly from each other. This the writer has not shown, and in some of the graphs it appears to be manifestly untrue. In other graphs there appears to be a distinct possibility that it might be true.

Against the use of rat means is the fact that with only 6 points and 4 degrees of freedom, one gets a result with very low inertia, where one divergent rat could invalidate the whole thing. In fact, such regressions are very nearly meaningless. The correlation coefficients obtained by using rat means alone are usually large, approximately twice those obtained by using all the figures, but never attain significance. The writer therefore uses all the figures in the table in calculating his correlation coefficients with the proviso that all correlations whether good or not, are mere indications of where problems lie and in which directions attention should be directed when larger numbers of rats are available.

The formula used in computing the coefficient is:-

$$r = \frac{\sum xy}{\sqrt{\sum x^2 \sum y^2}} \qquad \text{.....(8)}$$

Two assumptions are made in using this coefficient. First the distributions of both variates are normal, which, of course, has been assumed throughout. Second, the relationship between the variates is linear. This second assumption might well not be true

and this is a point which in some cases will merit future attention. It must, however, be realised that a small coefficient might be due not only to a wide scatter of points about the regression line, but a departure of that line from linearity.

The sums of squares in the formula in equation (8) have, of course, already been obtained by the use of the equation (2). The sums of products are obtained by using the numerator in the formula (3) for computing b, b being the sum of products divided by the sum of squares.

RANDOM VISITS TO TROUGHS

These random visits are immediately divisible into two groups, those visits that take place at meal times, and those that take place between meals. These are analysed separately since, not only are there important differences between them, but the implications from the writer's point of view are different.

When one has in mind the nature of the future experiments that it is intended to perform, the importance of these random visits is clear. What interests the writer is the distribution of these visits over the troughs and their regression on days. By the time he had reached this stage in his computations, it was abundantly clear that there are large differences between individual rats and he had formulated plans in his mind as to how to deal with it in the design of future experiments. He is therefore not interested in inter-rat differences. For this reason he has pooled the readings from all rats.

During the analysis of feeding and drinking behaviour, the regression on weeks was examined. In that context it is important, since it has implications in the fields of maturity and settling down time. In addition, the weekly readings were unbiassed. With random activity, on the other hand, a regression on weeks would mean only changes in the rate of learning with repetition, a problem for the Experimental Psychologist and not for the Physiologist. Not only that, but the regression on days is very highly significant in the case of random visits at meal times. Since one day is omitted from each week, this means that the weekly means are no longer unbiassed. Thus, not only is this regression irrelevant to the matter in hand, it is also unreliable and is not included in the results. (It is in any case insignificant.)

There are innumerable comparisons which could be made between the troughs. Of this number, however, there are a few which are logical and, in the writer's opinion, have a bearing on future experiments. These are as follows:-

At meal times.

Adjacent versus remote. The troughs on either side of the food trough compared with those separated from it by one trough or more. The water trough, number 1, is included.

Inter versus extra. Those troughs lying between the food trough and the water trough compared with those that do not lie between.

End versus middle. Troughs 1 and 8 compared with the rest.

Food and water versus the rest. This speaks for itself.

Food trough versus empty troughs.

Between meals.

Food and water versus the rest.

End versus middle.

8 versus middle.

1 versus middle.

1 versus 8.

Adjacent versus next but one. This is a slight modification of adjacent versus remote becase the writer suspected that this particular difference might exist. (It doesn't.)

Food versus empty.

Food versus empty omitting trough number 8 from the computations.

It will be remembered that there is one missing day and on one day the food was put in the wrong trough. The missing value now becomes eight missing values, and to this must be added another eight from the day on which the food was placed in the wrong trough. The writer felt that the simplest and safest course was to discard both of these days.

In view of the limited nature of the analysis to be undertaken in this case, the writer has not felt that it is necessary to

construct a full table of analysis of variance, but rather to take each comparison on its own, compute the standard error of the difference between the means, and thence arrive at t.

The only complication resulting from this decision is the computation of the regressions of random activity on days.

Computation of b.

Rather than indulge in a wordy explanation, the writer gives in full the computation of the regression coefficient of the number of random visits to troughs per day at meal times on days.

Days X	Y totals	n of Y	Y means	x	y
1	625	12	52.0833	-3	16.7846
2	472	11	42.9091	-2	7.6104
3	430	12	35.8333	-1	0.5346
4	365	11	33.1818	0	-2.1169
5	348	12	29.0000	1	-6.2987
6	401	12	33.4166	2	-1.8821
7	248	12	20.6667	3	-14.7320

From which we get:-

$$\Sigma X = 28$$
$$\bar{x} = 4$$
$$\Sigma x^2 = 28$$
$$\bar{y} = 35.2987$$
$$\Sigma xy = -120.0681$$
$$b = -4.2881$$

$\hat{Y} = \bar{y} + b(X - \bar{x}) = 35.2987 - 4.2881X + 17.1524 = 52.4511 - 42.2881X$

$\hat{Y}$	$Y - \hat{Y}$
48.1630	3.9203
43.8749	-0.8658
39.5868	-3.7535
35.2987	-2.1169
31.0106	-2.0106
26.7225	0.6941
22.4344	-1.7677

giving : $\sum d^2_{y.x}$ = 86.66664003 with 5 degrees of freedom.

$s_{y.x} = \sqrt{1/5(86.66664003)} = 4.163$

$s_b = s_{y.x}/\sqrt{\sum x^2} = 4.163/\sqrt{28} = 0.786$

$t = b/s_b = 4.2881/0.786 = 5.456 \quad P < 0.005$

All other regressions were treated in the same way.

The direct comparisons.

First one must extract the appropriate figures from the tables in appendices XI and XII. The method used by the writer was to divide a sheet of paper into two columns, one wider than the other. He headed the narrow column, for example, ends, and the wider one middle. This is for the comparison ends versus middle at meal times. He entered the table and starting at day 1 week 1, wrote 2, 6 under ends (i.e. the number of random visits to troughs 1 and 8) and 7, 1, 0, 3, 4, 2 under middle. He repeated this for day 2 week 1 and continued right through both samples, ending up with 164 numbers under ends and 492 under middle, including zeros. He then totalled both sets of figures, using this total as a check on the accuracy of the summing of the squares. After summing the squares, he ended up with three numbers for each column. In the case of ends versus middle at meal times this came to:-

n_1	164	n_2	492
$\sum X_1$	797	$\sum X_2$	2,115
$\sum X_1^2$	5,895	$\sum X_2^2$	19,981

This provided him with all he needed for the computation. The

next step is to draw up a table, as follows:-

Position	n	degrees of freedom	means	sums of squares
ends	164	163	4.86	2,022
middle	492	491	4.30	10,889
	sum	654 difference 0.56		sum 12,911 $=\sum x^2$

The sums of squares in the last column were obtained by subtracting the appropriate correction factors from the sums of squares of the original columns.

To obtain the pooled mean square we have:-

$$s^2 = \sum x^2/(n_1 + n_2 - 2) = 12,911/654 = 19.7$$

The standard error of the difference between the means is given by:-

$$s_{\bar{x}_1 - \bar{x}_2} = \sqrt{s^2(n_1 + n_2)/n_1 n_2}$$ (which, where $n_1 = n_2$ simplifies to $\sqrt{2s^2/n}$)

$$= \sqrt{19.7(164 + 492)/(164)(492)} = 0.4002$$

$t = \bar{x}_1 - \bar{x}_2 / s_{\bar{x}_1 - \bar{x}_2} = 0.56/0.4002 = 1.40$ which is not significant.

We thus retain the null hypothesis that $\mu_1 - \mu_2 = 0$.

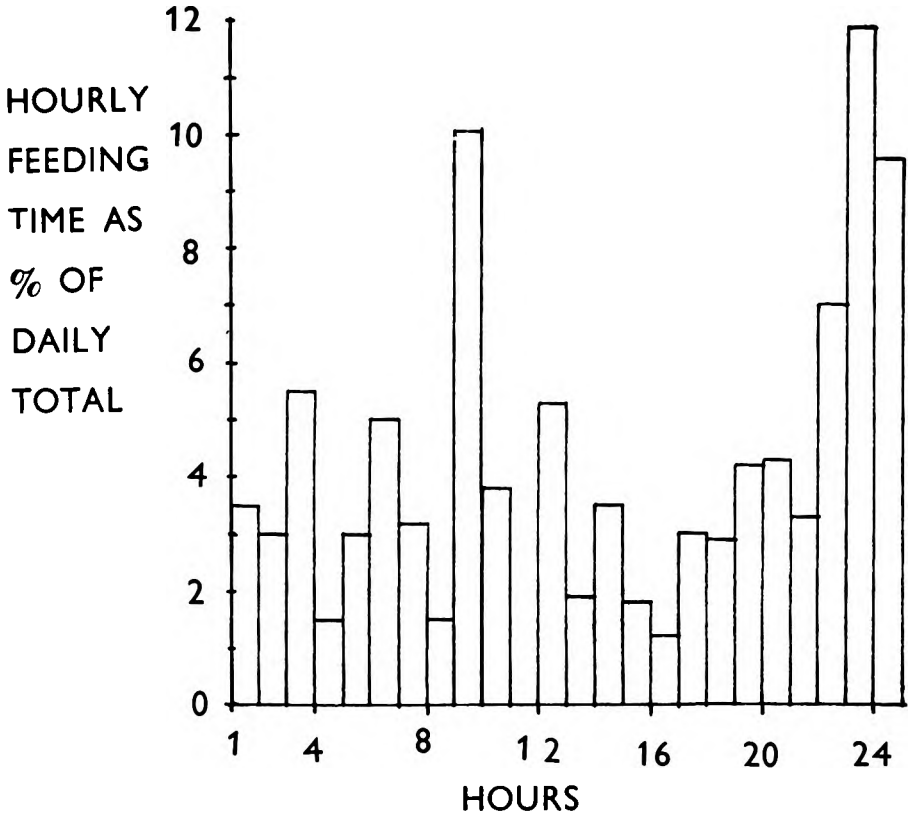
We are now in a position to proceed to a consideration of the results obtained by these methods.

THE RESULTS

THE DISTRIBUTION OF FEEDING ACTIVITY.

The histograms depicted in Figures 27 - 32, show, for each rat, number of minutes spent eating in each of the 24 hours expressed as a percentage of the total daily feeding time. The graphs should in reality, be represented as wrapped round a cylinder and not spread out on a flat surface, and the writer knows of no method of dealing with such data statistically. This does not mean, however, that one may not indulge in comment.

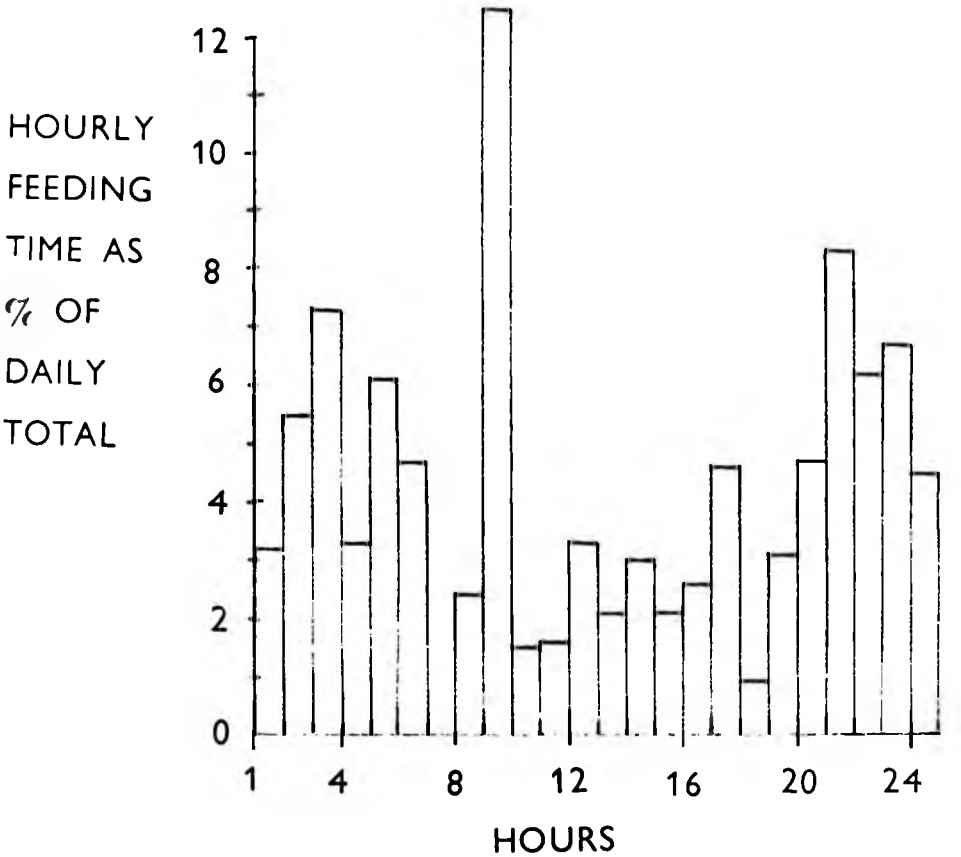
The first point that meets the eye is the heavy concentration of feeding activity in hour 9 in all rats. (Hour 9 means the hour between 9 and 10 a.m.). This is the more remarkable when one realises that hour 9 was almost invariably a broken hour in that feeding and attention to the apparatus started in hour 8 and extended into hour 9 until about 9.15 to 9.30. Thus all this feeding activity, representing from 9 to 13 per cent of the total daily time is crowded into half to three quarters of an hour. There can be no doubt that one factor involved here is the disturbance caused by the morning activity of the experimenter or his assistant. The behaviour of the rats at this time varies considerably but ends up with feeding. Rats 1, 3, and 6 usually appeared to be asleep and paid no attention as the metal strip which acted as a guard screen was placed in position preparatory to removing the troughs. As the guard screens were removed after the troughs had been replenished, the rats would approach



THE TIME SPENT FEEDING IN EACH OF THE 24 HOURS AS A PERCENTAGE OF TOTAL DAILY FEEDING TIME.

RAT NO. 1

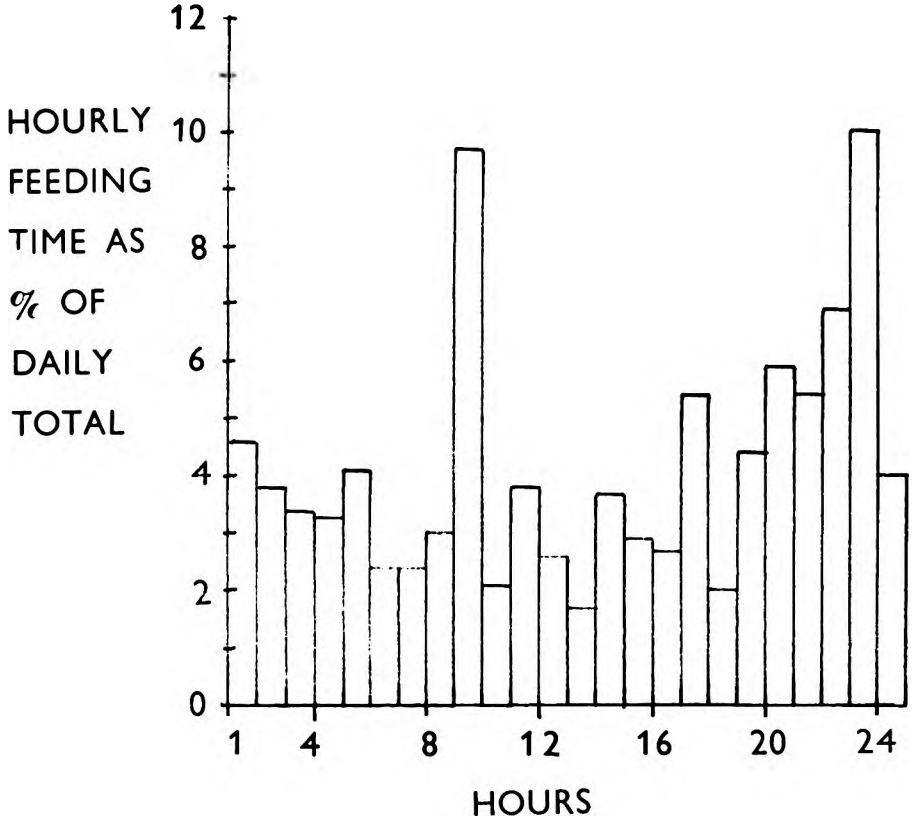
Figure 27.



THE TIME SPENT FEEDING IN EACH OF THE 24 HOURS AS A PERCENTAGE OF TOTAL DAILY FEEDING TIME.

RAT NO. 2

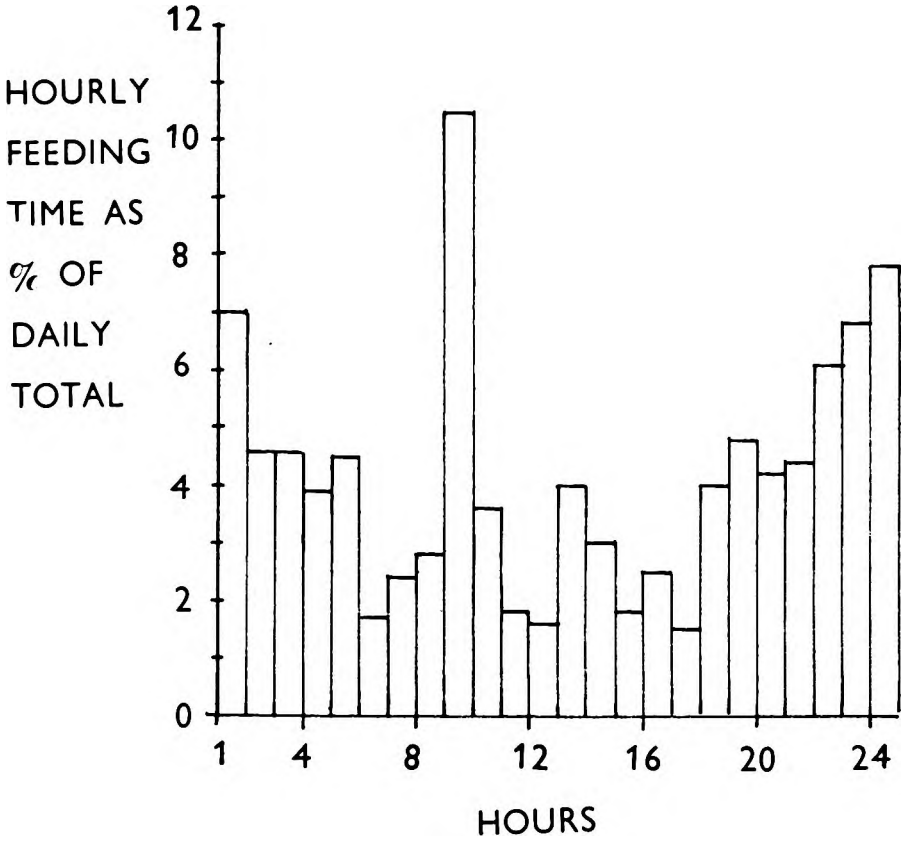
Figure 28.



THE TIME SPENT FEEDING IN EACH OF THE 24 HOURS AS A PERCENTAGE OF TOTAL DAILY FEEDING TIME.

RAT NO. 3

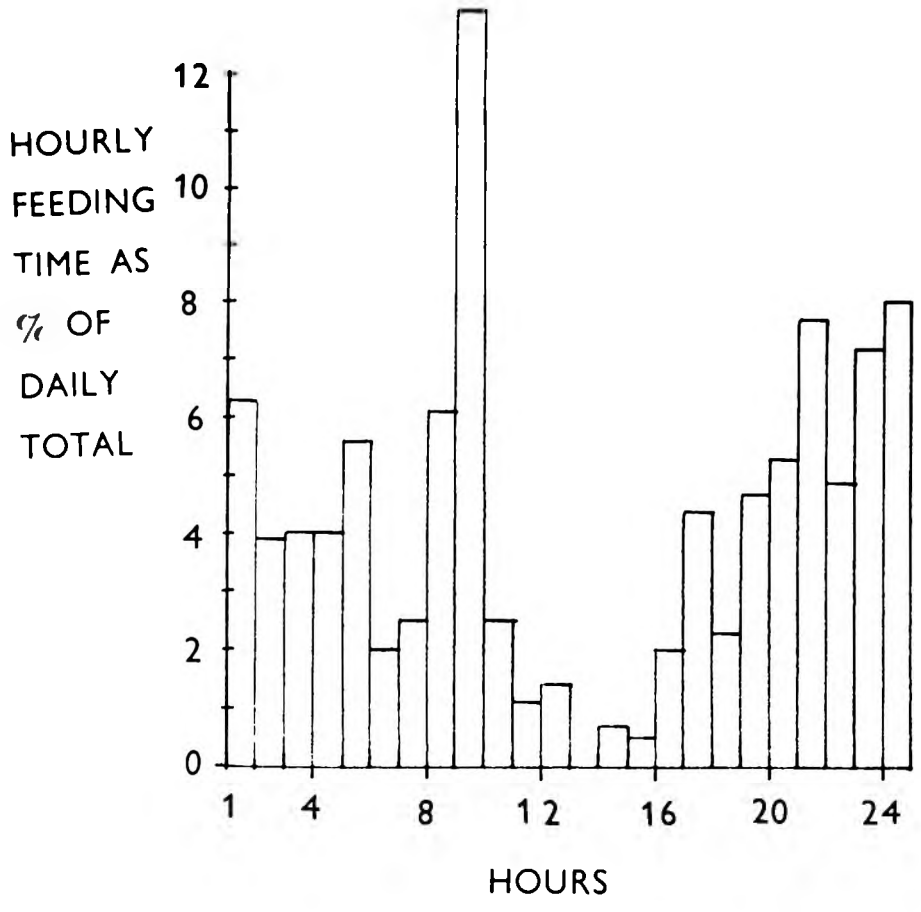
Figure 29.



THE TIME SPENT FEEDING IN EACH OF THE 24 HOURS AS A PERCENTAGE OF TOTAL DAILY FEEDING TIME.

RAT NO. 4

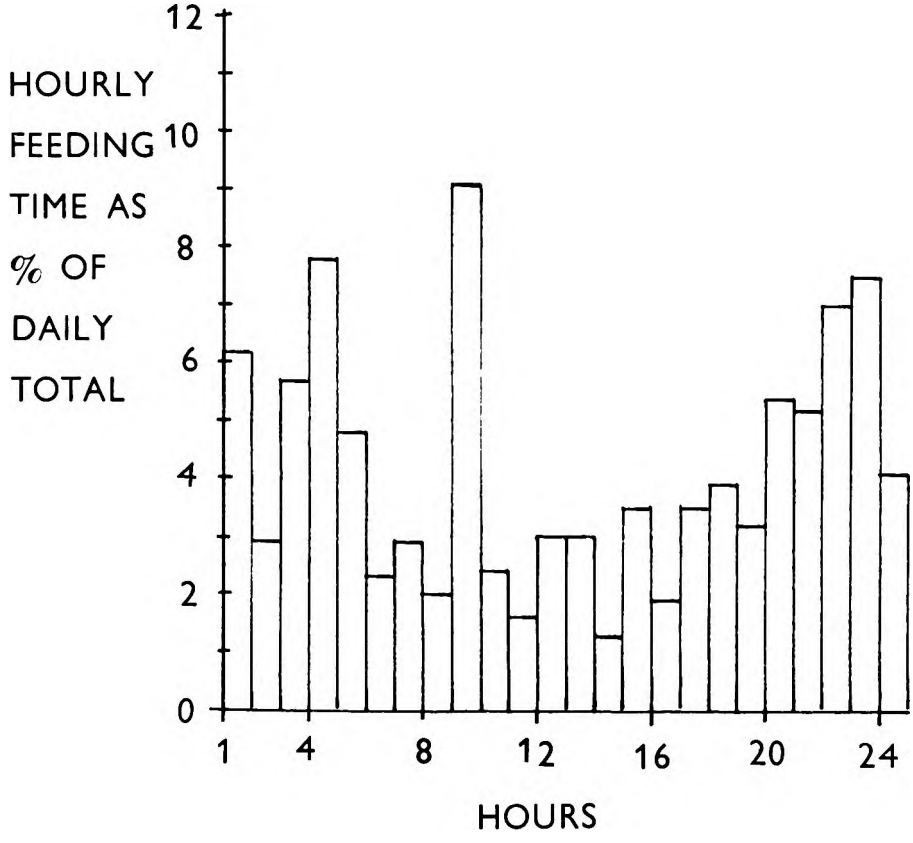
Figure 30.



THE TIME SPENT FEEDING IN EACH OF THE 24 HOURS AS A PERCENTAGE OF TOTAL DAILY FEEDING TIME.

RAT NO. 5.

Figure 31.



THE TIME SPENT FEEDING IN EACH OF THE 24 HOURS AS A PERCENTAGE OF TOTAL DAILY FEEDING TIME.

RAT NO. 6.

Figure 32.

the troughs in a leisurely manner and start feeding. Rat 2 would invariably attack the end of the screen as it was inserted into the cage, and, when it was nearly in position, redirect his attach suddenly towards the experimenter's hand. When the troughs were being removed or replaced, he would once more attempt an attack. Finally, as the screen was removed, he would once more attack the experimenter's hand and then start feeding at the trough. Rat 5 would approach the troughs as the screen was inserted, place himself in a crouching position opposite the food trough and fix his eye on it, remaining in that position until the screen was ultimately removed, when he would start feeding. Rat 4 seemed to be rather "experimenter orientated". As one placed the screen in position, he would climb up the bars of the cage and sniff at the experimenter and seem pleased if one spoke to him. As one removed the screen, however, he would try to dodge round the end of it to get at the troughs as soon as possible. Curiously, despite this keenness to get at the troughs, the day frequently, even usually, started with random visits to empty troughs. A typical start to a day is depicted in Figure 33. Here the random visits are marked with arrows.

One may, of course, question whether this time spent at the troughs is actually spent feeding. This is easy to answer in the case of rats 4 and 5. Their method of feeding was to insert their heads into the troughs, and sometimes, it seemed, one front paw as well, and then withdraw from the trough, apparently with much more food than they could comfortably chew. They would sit up on their

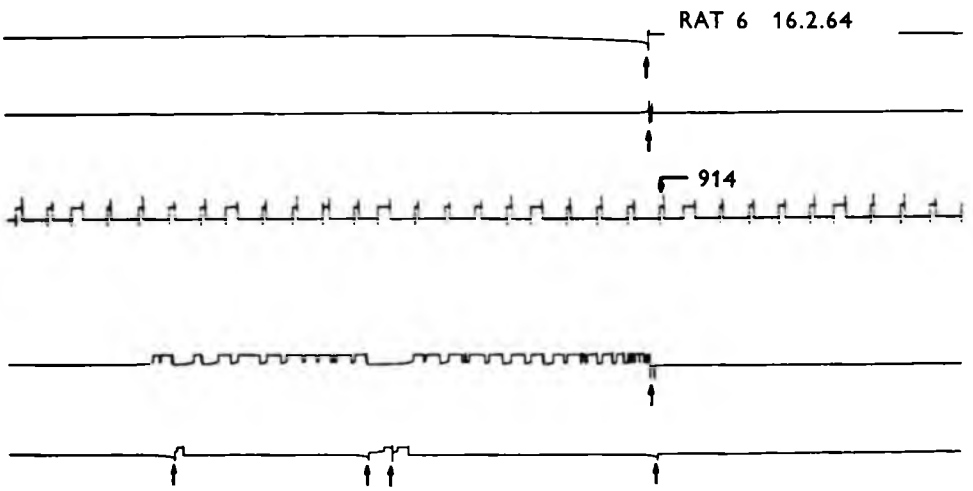


Figure 33.

The start of a day. The record reads from right to left. The bent arrow indicates the time of starting. The day invariably starts with a meal, usually preceded by random visits which are marked in this record with straight arrows.

haunches and, with both front paws held to their mouths, chew steadily until the food was finished, when they would dive into the trough again. In the case of these two rats, there is no doubt that this time is genuine feeding time. With rats 1, 2, 3 and 6, the matter is different. Their technique was to crouch before the troughs and insert their heads for a little while and then withdraw again for a short time, repeating this over and over again. It was difficult to see, without going very near to their cages, and thus introducing yet another disturbance, whether or not they were eating. Very occasionally one could actually see jaw movements, but generally the whole attitude of the rat strongly suggested eating. Sometimes this alternate insertion and withdrawal would be kept up for from 15 to 20 minutes, and it is difficult to imagine a rat spending that amount of time in meditative sniffing. It seems, then, that we can say that this time spent at the troughs is eating time.

Granted that this is genuine eating, we must consider what effect such an artefact has on the diurnal metabolic rhythm of the rat, which is expressed, in these experiments, as feeding behaviour. The general pattern of the distribution of feeding activity is with a heavy concentration at or just before midnight with, in the case of rat 2, another bout of eating in the early hours of the morning. In most of the rats there is a distinct depression of feeding activity from hour 10 until hour 17 or 18, giving the graphs a nocturnal appearance. Would this distribution of activity be so marked if this

artefact could be eliminated? We cannot speculate about this. There is no evidence that the rate of feeding in the artefact is the same as at other times, nor indeed, do we know if the rate of feeding is the same throughout the 24 hours. This measurement would involve some system of continuous automatic weighing which is not available at present.

This matter is more important than it seems. It might well be that the effect of the disturbance is to induce in the rat some sort of indiscriminate feeding activity and, with such components of the diet as the B vitamins, the rat might, during the artefact, obtain sufficient to last the rest of the day. This might completely invalidate experiments on differential appetite. The writer therefore thinks it is important to try and eliminate the artefact.

His approach will be to supply the rats with enough food and water to last several days, and then to replenish the troughs on days and at times chosen strictly at random, thus avoiding the inducement of habits in the rats. It seems important, in any case, to avoid the emphasis on regularity which has been a feature of the organisation of these experiments.

FEEDING BEHAVIOUR.

Regressions on time.

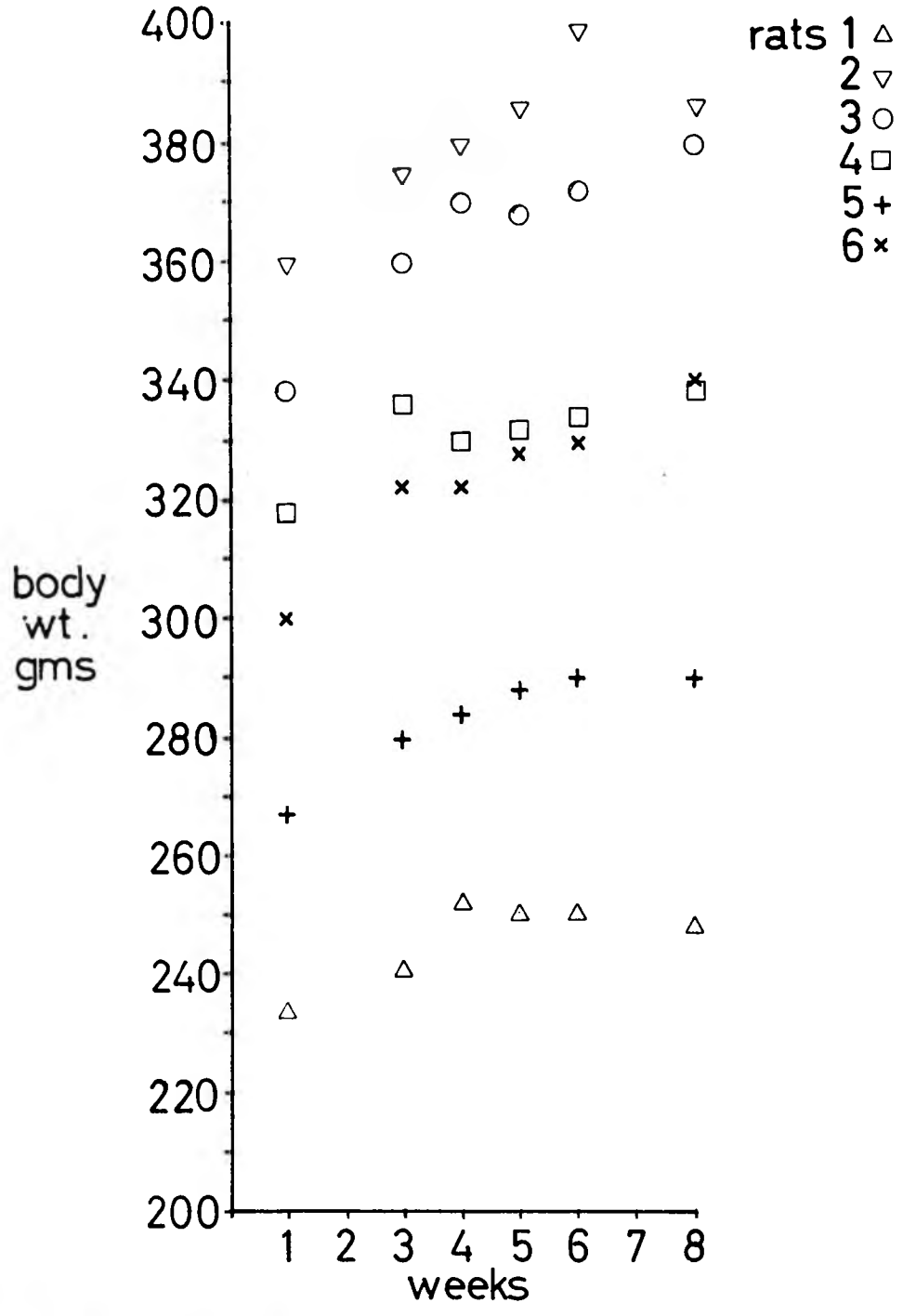
The regression coefficients calculated according to the methods already described are summarised in Table 3.

Table 3.

The regression coefficients and their significance.

Regression on weeks of	Rats					
	1	2	3	4	5	6
Body weight (gms)	* 2.254	** 3.949	** 5.359	* 2.305	** 3.305	** 5.254
Food Consumption (gms/day)	-0.296	-0.214	-0.320	0.104	0.081	-0.089
Feeding time (mns/day)	1.791	** 3.486	1.345	* 3.129	0.338	-0.787
Rate of eating (mgms/mn)	** -13.298	** -18.046	** -15.335	-5.437	1.205	-0.186
Number of meals/day	0.094	-0.111	-0.168	-0.314	-0.174	** -0.531
Average duration of meals (mns)	0.049	** 0.624	0.166	** 0.466	0.173	0.138

Let us first consider the first two lines of this table, those referring to body weight and food consumption. There is a tendency for all rats to increase in weight throughout the experiment, less marked in the cases of 1 and 4 than in the rest. If we look at the graph in Figure 34, we see that the main increase in rats 1 and 4 was in the first three or four weeks, and that their weights were stabilised thereafter. This tendency to stabilise is also visible but comes later in rats 2 and 5, while it does not appear at all in rats 3 and 6. This apparent tendency to curvilinearity is noteworthy. It is unlikely that, over a period as short as 8 weeks,



The changes in body weights with the progress of the experiment.

Figure 34.

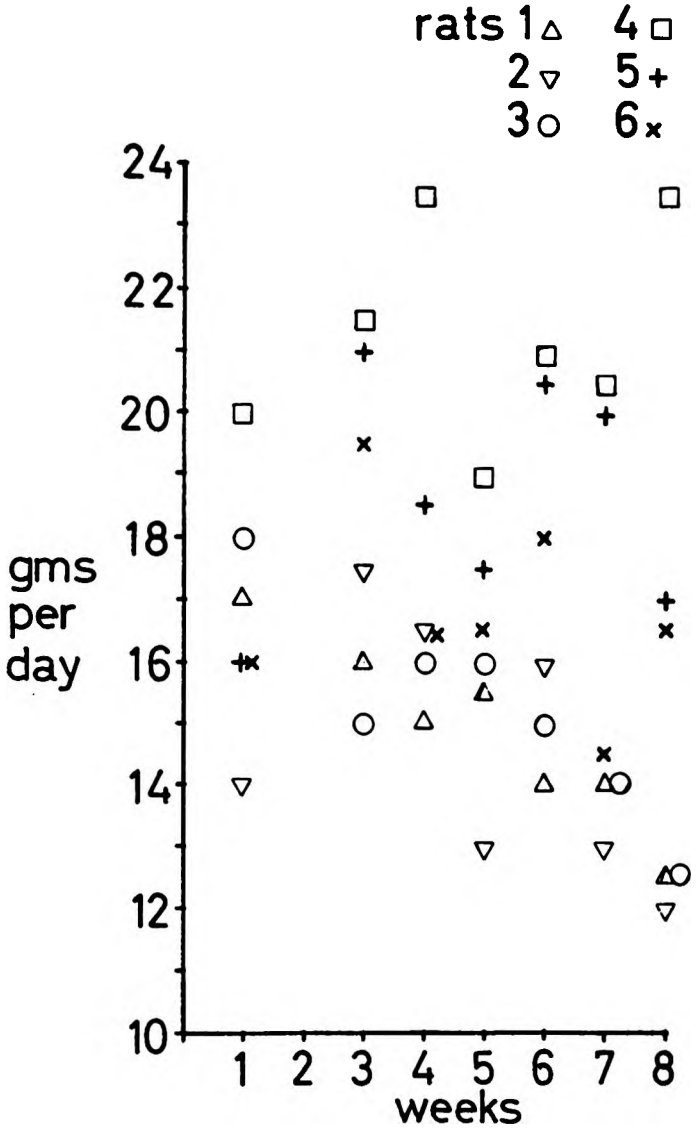
one would notice the curvilinearity due to the natural exponential growth curve, and one must suspect that this change in weight is in some way associated with the conditions of the experiment. Had these rats been younger, this effect might have been obscured, but, due to the postponement of the experiment, they were 7 months old at the beginning and 9 months at the end. At this age the growth curve would be very nearly flat. The explanation offered by the writer for this increase in body weight and its variations is that, on being transferred from the communal cage to their individual cages, the rats decreased the amount of exercise they indulged in without decreasing their food intake in proportion.

The total lack of significance of the regression coefficients for food consumption would appear to support this view, but it must be remembered that the changes in body weight are small, less than 2 per cent per week, while the scales on which the food was weighed only estimated weight to the nearest gram, and not all the food "consumed" was in fact eaten - some was spilt. So far as the regressions are concerned, the total lack of correspondence between body weight and food consumption is not surprising. This is a matter to which we will return in considering the rat means. The indications, albeit somewhat unreliable, are that as rats decrease their exercise, they do not decrease their food consumption in proportion. This is in accordance with the suggestion of Mayer (1955) that there is a level of exercise below which the appetite regulator does not compensate. It is quite

possible, therefore, that these rats were simply becoming obese.

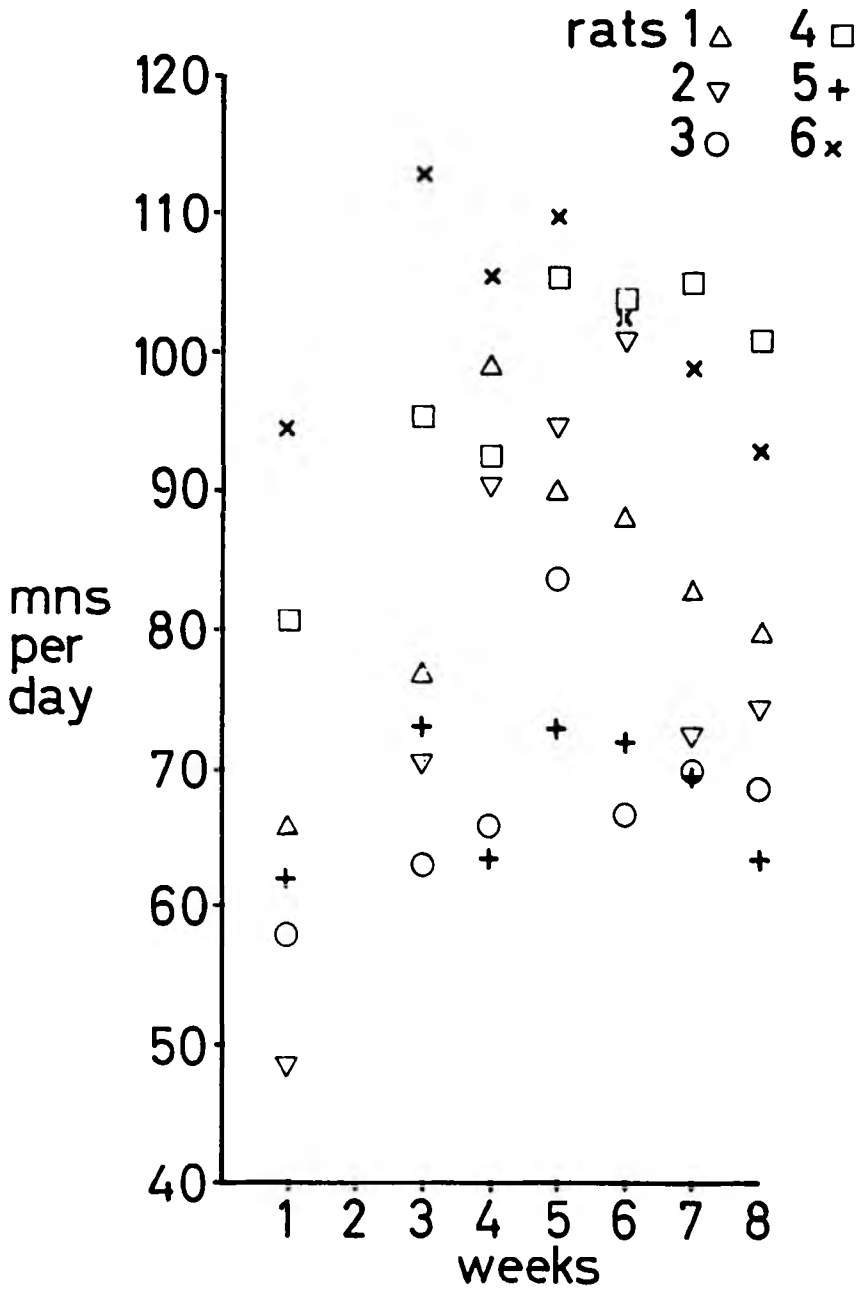
The regression of food consumption with time is depicted in Figure 35. There is an apparent regression in some rats, particularly number 3, where the tendency is for food consumption to decrease. However, as the coefficients are not significant, one should be cautious about drawing conclusions from such apparent trends.

Turning to the matter of the number of minutes spent feeding in 24 hours, that is feeding time, the table shows that all the coefficients are positive except in the case of rat 6, but only two of them reach significance, rats 2 and 4. However, on referring to the graph in Figure 36, we wee that the feeding time in rat 2 does in fact increase up to week 6, but that thereafter there is a sharp decrease to week 7. In rat 4 there is a steady increase to week 5 with a decrease or stability thereafter. This tendency for an early increase and then a decrease seems to be fairly general, except in rat 3. The writer therefore recalculated the regression coefficients omitting week 1. Now all the coefficients except that for rat 4 become negative, but only that for rat 6 achieves significance. There is a strong suggestion here of adaptation in the early part of the experiment. Once more we might be dealing with the effects of isolation. In communal life one must presume that rats compete with one another for food to a certain extent, and this might lead to fast eating. in isolation, where this element of competition is absent, the rate of eating will tend to decrease and the time spent eating increase.



The changes in daily food consumption (gms) with the progress of the experiment. Where points coincide, the symbols are placed side by side.

Figure 35.

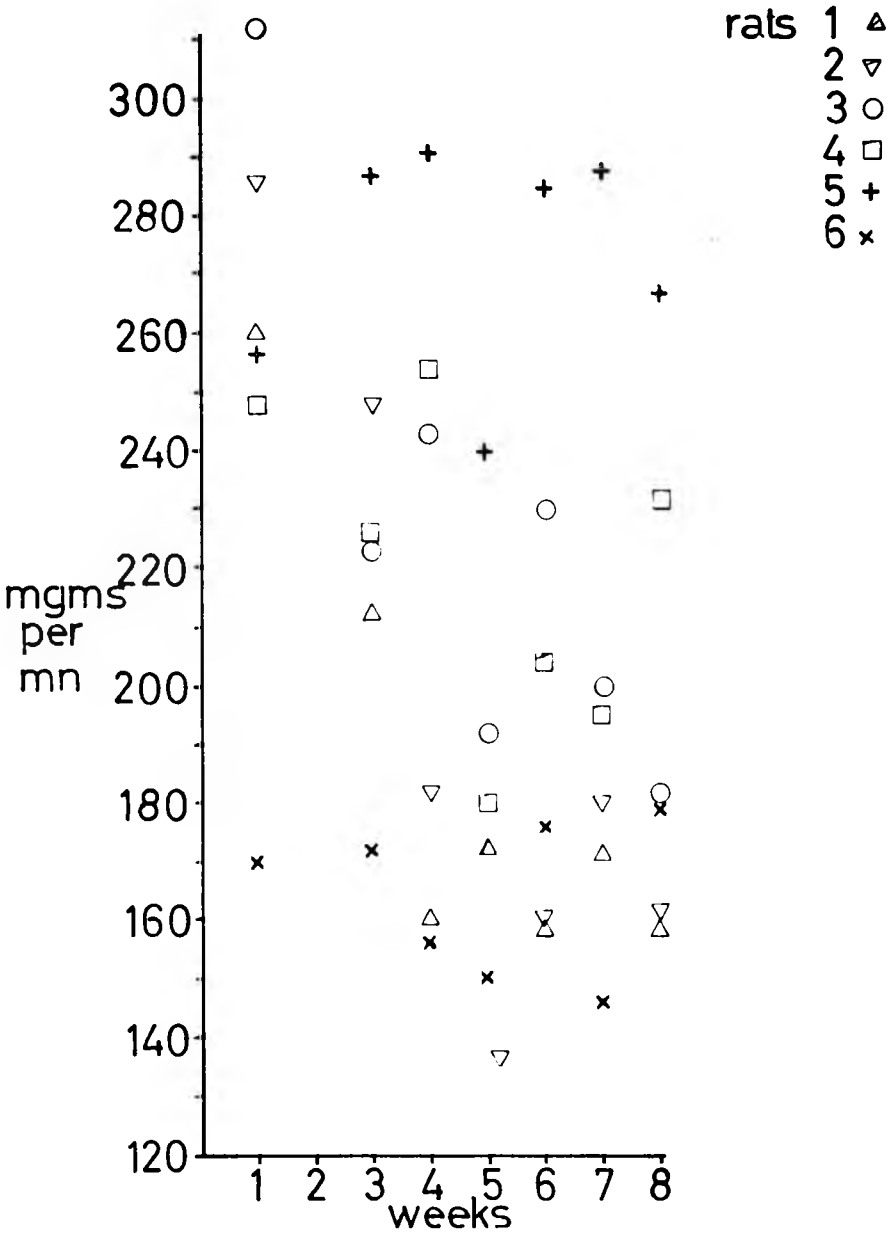


The changes in feeding time (mns/day) with the progress of the experiment.

Figure 36.

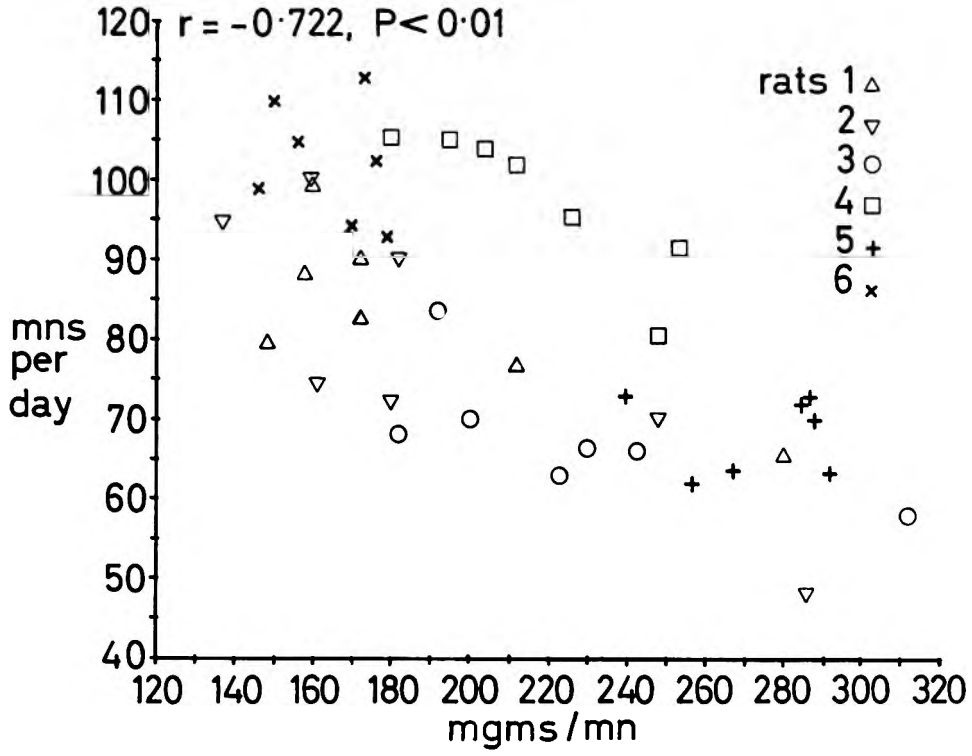
The next line of the Table and Figure 37 are concerned with this point. Here daily food consumption is divided by the feeding time and the results expressed as rate of eating in milligrams per minute. Here rats 1, 2, and 3 show strong tendencies to decrease their rate of eating, the other three showing rather wide deviations. This lends some support to the notion of adaptation, although the correspondence between feeding time and rate of eating is not as close as one would like to see it. However, if one ignores the regression in time and plots a scatter diagram with feeding time as a function of the rate of eating (Figure 38) one finds that the correspondence is closer than expected, the correlation coefficient of -0.722 being high by biological standards and having a low uncertainty. Certain interesting features are illustrated in this graph. In the first place, the individual rat readings tend to be scattered along the regression line. This effect varies in degree from rat to rat, being most marked in rat 4 and almost absent in rat 6. However, the suggestion is there and strong and obviously merits further investigation. In the second place, if one removes rat 4 from consideration, there is a strong suggestion of curvilinearity in this relationship. At present, it is idle to speculate on the underlying physiology.

It will be possible to make some further reference to this matter later. In the meantime, some consideration must be given to the other correlations of feeding time. In view of this close correlation one would expect the relationship between feeding time and food



The changes in the rate of eating (mgms per mn) with the progress of the experiment.

Figure 37.



Feeding time (mns/day) as a function of the rate of eating (mgms/mn).

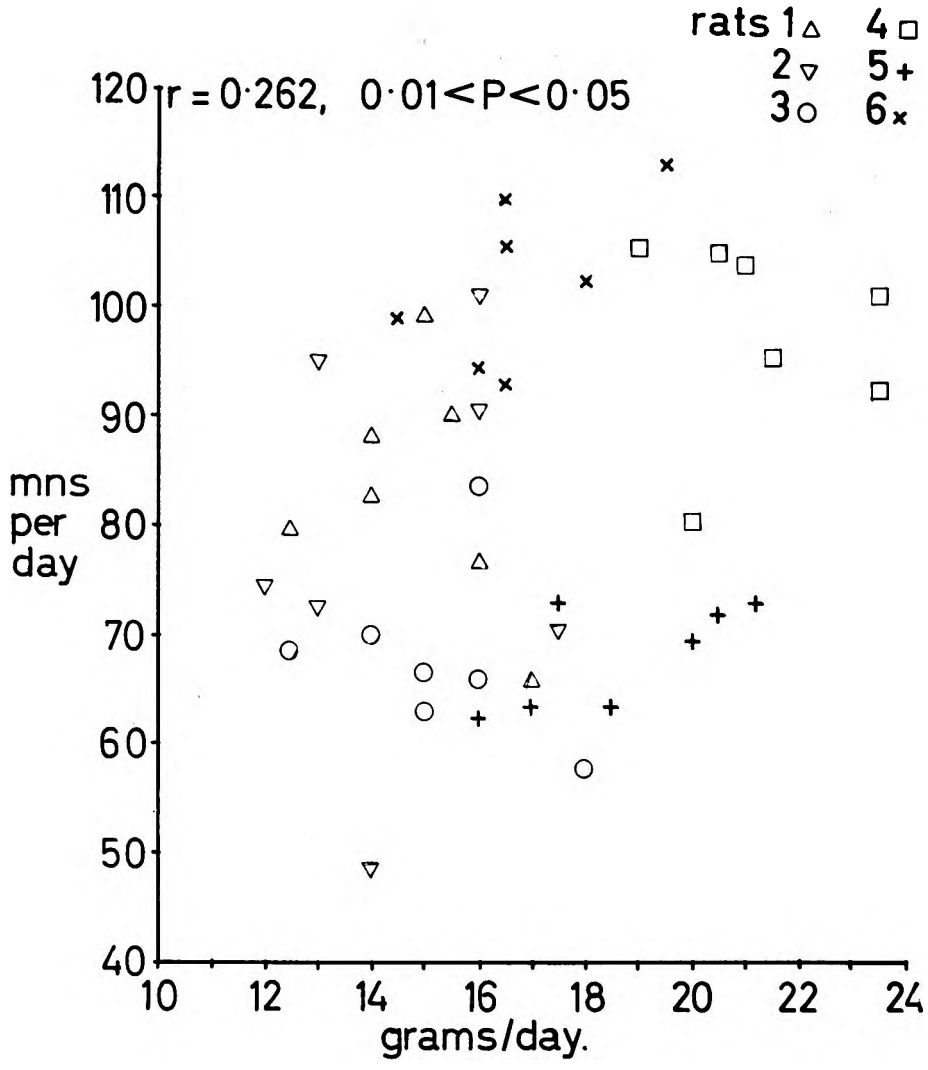
Figure 38.

consumption to be weak or obscured. Figure 39 shows that this is indeed so, and makes one wonder whether food consumption alone is a sufficient measure of appetite, or whether one should take the rate of eating into account.

In looking at feeding time as a function of body weight, (Figure 40), one is impressed with two things. First, there is little or no correlation when considering rat means alone, but when one looks at each individual rat, one gains the distinct impression that in some of them at least there is a tendency to eat for a longer time as body weight increases, an effect which is most obvious in rats 1, 2, 3 and 4 but not noticeable in rats 5 or 6. This appearance cannot be tested under the conditions of this experiment and, indeed, it must be repeated with many more rats before one can make a reliable statement about it.

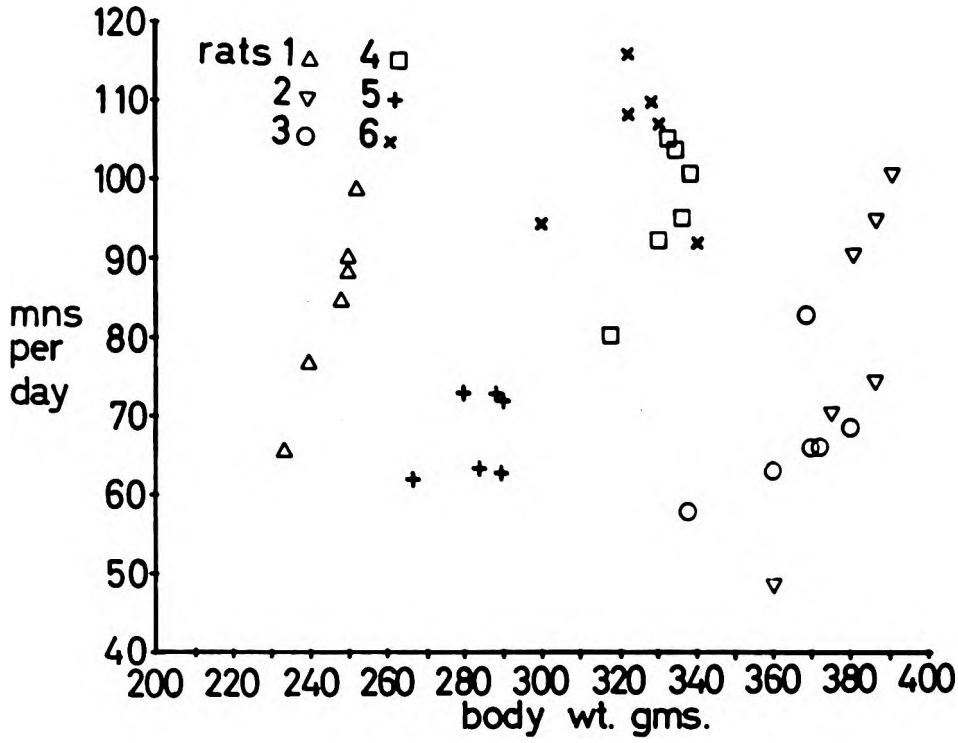
The question arises, "When a rat changes the time it spends eating in a day, does it do so by changing the number of meals per day or by changing their duration?"

One would expect the number of meals per day and therefore the interval between meals to be controlled by the amount of glycogen stored and the rate of its utilisation. Indeed a glance at the regression coefficients in the Table shows that the number of meals per day does not change except in the case of rat number 6. This rat was decreasing the number of meals per day, and, as is clear from Figure 41, the rate of decrease was linear with only two rather wide deviations. It will be remembered that this is the rat, which, when



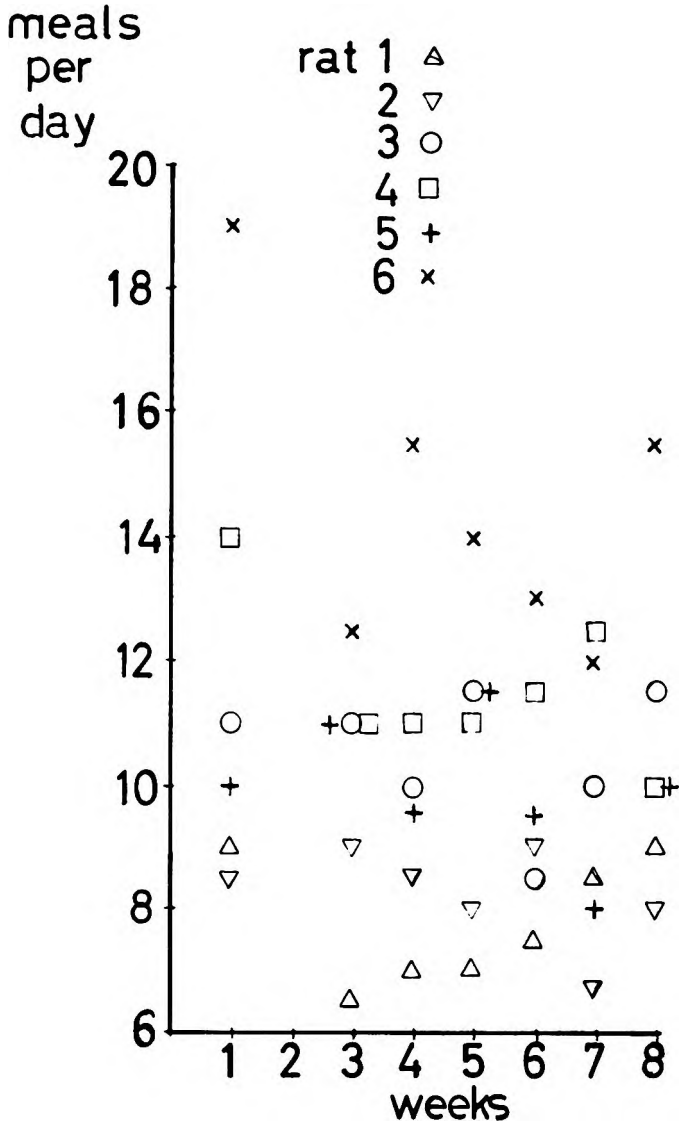
Feeding time (mns/day) as a function of daily food consumption.

Figure 39.



Feeding time (mns/day) as a function of body weight.

Figure 40.

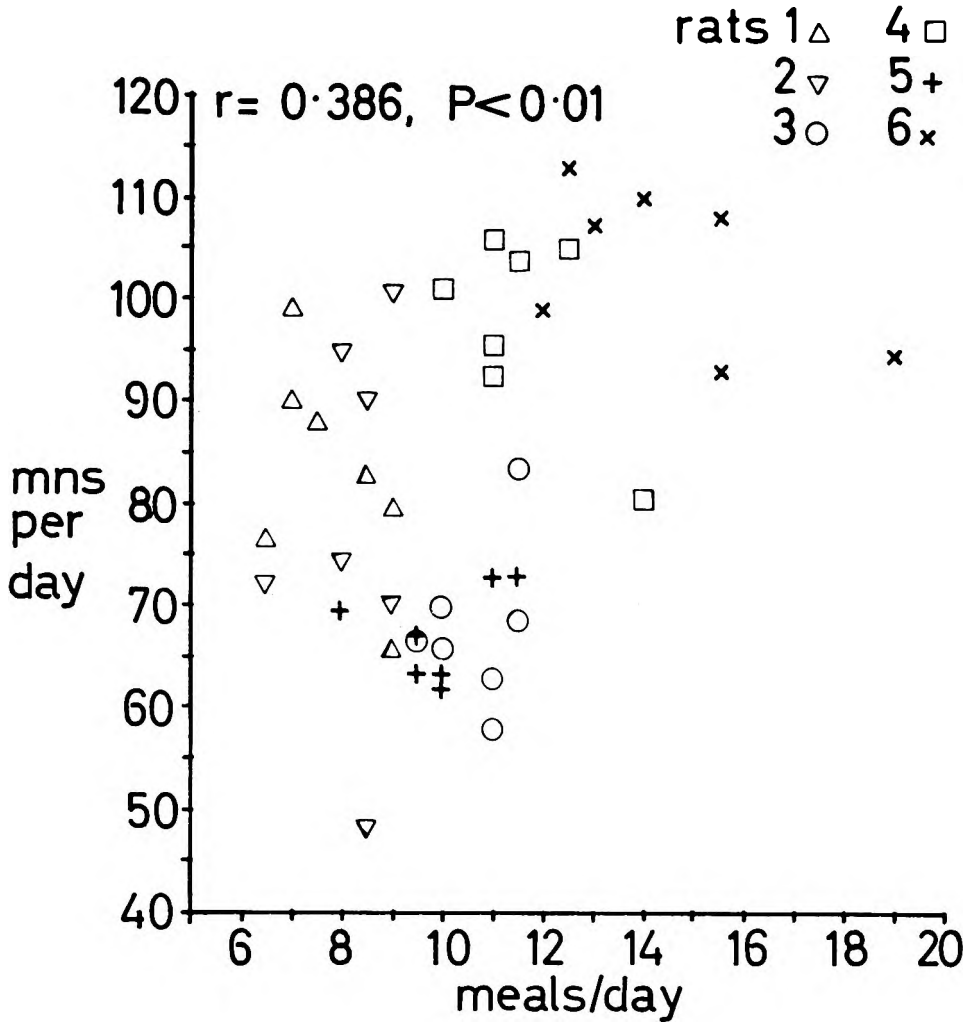


The changes in the number of meals per day with the progress of the experiment. Where points coincide, the symbols are placed side by side.

Figure 41.

week 1 was omitted from consideration, had a significant negative regression coefficient for feeding time. It seems, then, that this rat was reducing its feeding time by reducing the number of meals in a day which is apparent, but not marked, on the scatter diagram in Figure 42, where feeding time is plotted as a function of the number of meals per day. If, as seems reasonable, we accept that the interval between meals, which must determine their number, is controlled by the amount of glycogen stored and the rate at which it is used, then this rat was undergoing a far more persistent and marked decrease in activity than the others, unless it was suffering from some metabolic change which led to an increase in glycogen storage. It remains a fact that this rat alone of the six decreased the number of meals per day over the eight weeks of the experiment. Six constitutes a small sample and it might well be that, were the sample larger, more rats of this sort might appear. In that case, one would have to reconsider one's interpretations.

This relative fixity of the number of meals in a day would lead one to expect a poor correlation with feeding time. Indeed (Figure 42) the correlation coefficient is small (0.386) although its probability is good. The graph gives an impression of curvilinearity which might have contributed to the low coefficient, but this impression is largely due to the position of rat number 6 on the graph, in the top right hand corner. Without this rat, the correlation might well have approximated to zero.

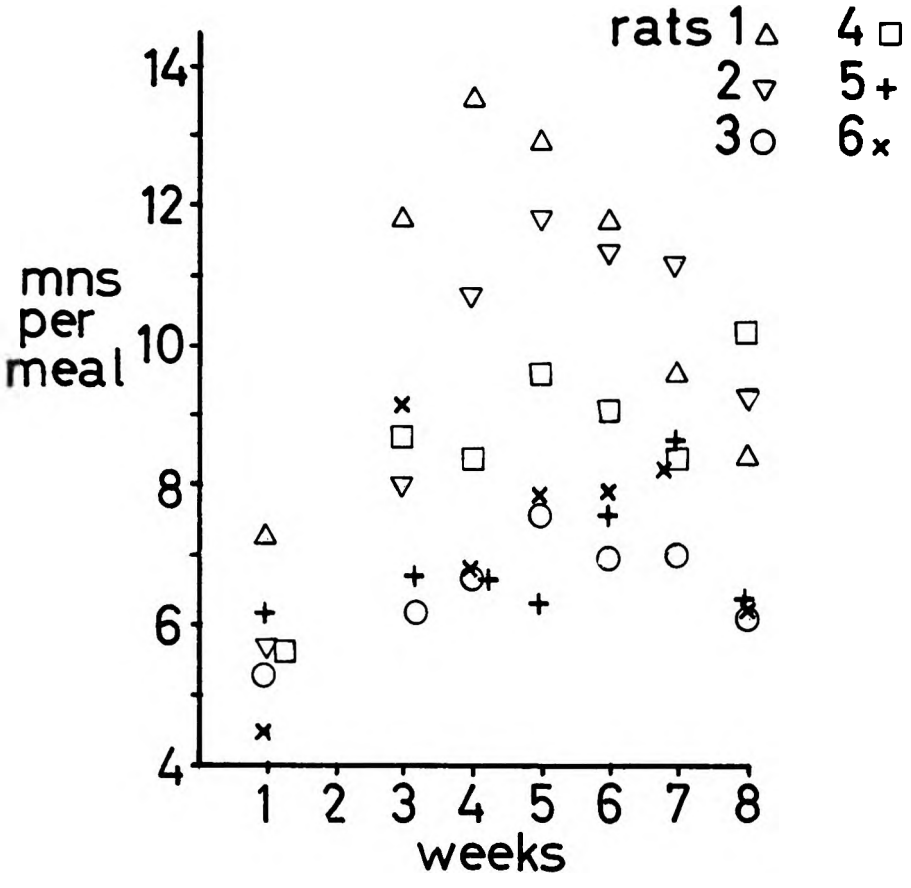


Feeding time (mns/day) as a function of the number of meals a day.

Figure 42.

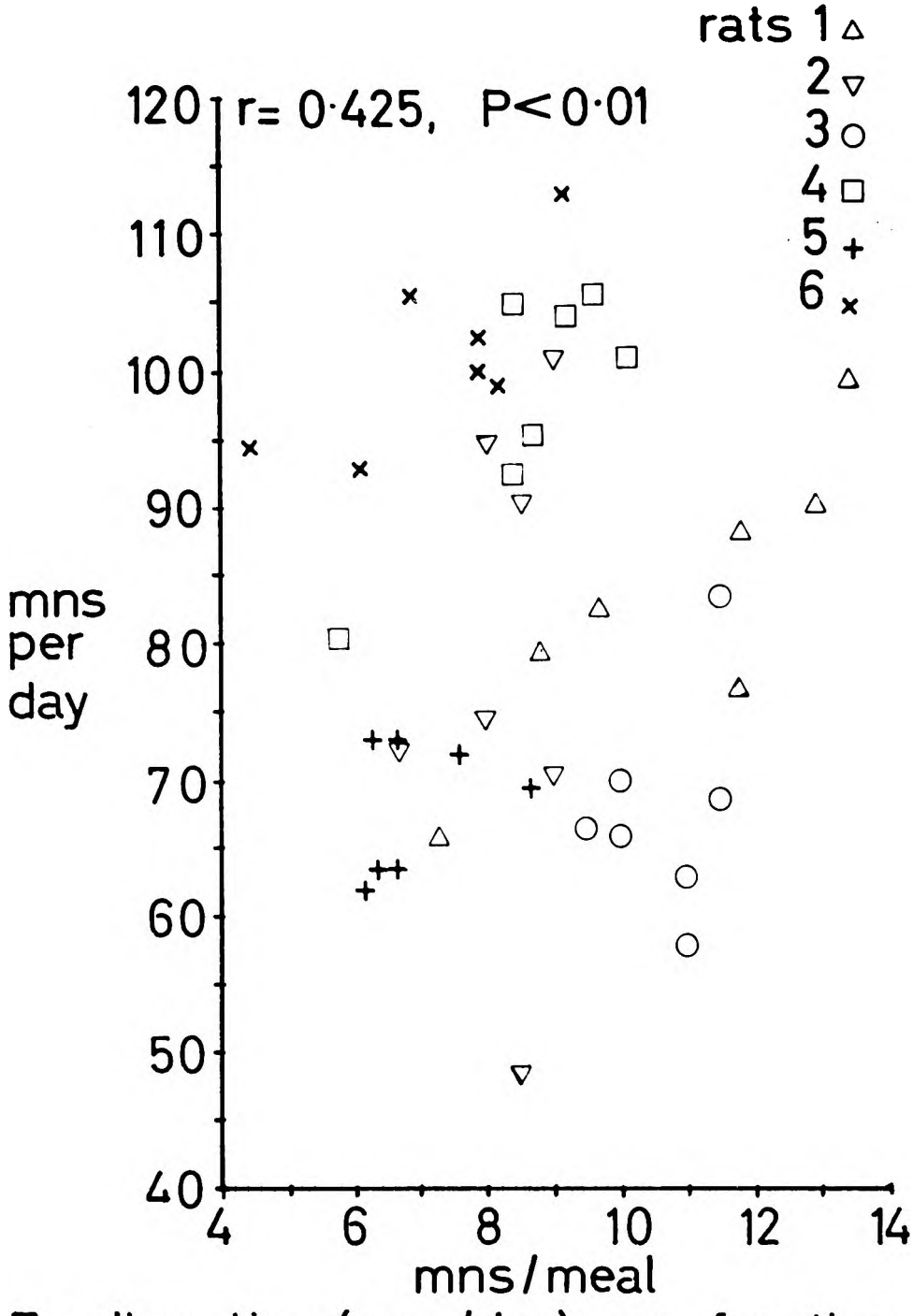
What, one may ask, of rats 2 and 4 which were increasing their feeding time but maintaining the number of meals per day constant? The rather obvious answer is that they must have been increasing the duration of their meals. This is in fact so, as shown in the last line of the Table. Reference to Figure 43 and its comparison with Figure 36 reveals a remarkable correspondence between feeding time and the duration of meals. Rat number one increased its feeding time up to week four and thereafter decreased it, while the duration of its meals followed the same pattern. Rat number 2 increased both up to the fifth week and then decreased. With rat number 3, both curves are rather flat. Rat 4 increased both up to the fifth week with a tendency to stabilise thereafter. Number 5 shows little trend in either. Rat 6 has a sharp increase between week 1 and week 3 with a steady decline thereafter, though the deviations from this decline appear to be rather large in the duration of meals.

In view of this close correspondence, one would expect a close correlation, but Figure 44 is disappointing, the coefficient being 0.425 although the significance is good. Closer examination of the graph, however, shows that in rats 1, 2, 4 and 6 the general line of the individual regressions appears to be in the direction of the general trend, but that in rats 3 and 5, where there was little change in feeding time, there is no trend. The correlation is spoilt by the fact that the individual rats have their own characteristic positions on the graph. One can say, then, that when a rat changes



The changes in the average duration (mns) of meals with the progress of the experiment.

Figure 43.



Feeding time(mns/day)as a function of the average duration of meals (mns).

Figure 44.

its feeding time, it does so by changing the duration of its meals, but one cannot say that rats which eat for a long time have longer meals than rats which only eat for a short time. This is an expression of the fact that the number of meals per day, and consequently the inter-meal period, is highly characteristic for each rat.

If one may attempt to summarise the findings of this examination of the regressions and correlations, they would be thus.

1. Throughout the period of this experiment, all the rats increased in weight. It is doubtful whether this is just a matter of growth, and a tendency towards obesity is suspected.

2. None of the rats showed any tendency to change its daily food intake, but here there are considerable inaccuracies in measurement.

3. Five of the rats showed a net tendency to increase the time they spent eating although there appeared to be a change in trend in the middle of the experiment. Rat 6 is a deviationist, showing a net tendency to decrease feeding time.

4. There is a general tendency, absent in rat 5, to decrease the rate of eating, three of the regressions being highly significant.

5. The number of meals a rat eats in a day is highly characteristic for each rat and does not tend to change. Rat 6 is exceptional. It follows from this that when a rat changes the time it spends eating in a day, it does so by changing the duration of its meals.

What conclusions or speculations arise from this summary?

1. Rat 6 is apparently aberrant, but in fact might be less so than it seems. Most of the rats were decreasing their feeding time towards the end of the experiment, and might ultimately have conformed to the pattern set by number 6. Presumably they would all have stabilised eventually. Here one feels accutely the need for some means of measuring activity.

2. The stability of the daily food intake, ignoring inaccuracies, appears to represent the efficient working of the appetite mechanisms. However, underlying that stability there seems to be some other, more subtle mechanism at work which is expressed in a tendency to reduce the rate of eating. One is reminded of the finding of Tepperman et al. (1943) that a rat which is induced to eat quickly tends to gain weight faster than expected, and also that of Brooks and Lambert (1943) which suggests that the fat depots exert some influence on appetite in the hypothalamic obese animal. Might it not be, in the intact animal, that these depots are affecting the pattern of eating? Some investigation of the changes in the rate of eating with changes in the state of obesity is clearly indicated.

3. The rats might be taking a very long time to adapt to the apparatus, a fact which might be of importance in the design of future experiments.

<u>The differences between the means.</u>

The writer's main interest in the differences between means was to find some method of grouping rats so that he can adopt some

more efficient method of designing future experiments than a completely randomised arrangement. It was for this reason that he divided his rats into littermates and non-littermates.

Throughout the experiment and all the readings and measurements arising from it, there were no significant differences between littermate and non-littermate rats, with the exception of body weight. It would almost seem as if the person responsible for pairing the breeding stock was influenced, consciously or unconsciously, entirely by even matching as to size and nothing else. How great are the similarities is shown by the figures in Table 4. Here, the means of the littermates are given in the first column, and those of the non-littermates in the second column. The third column gives column 1 as a percentage of column 2.

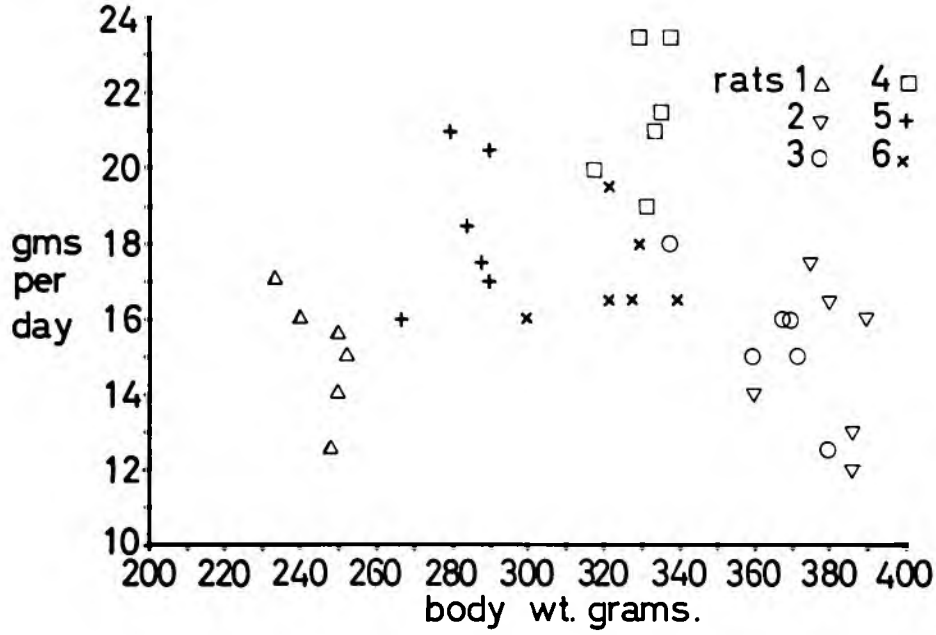
Table 4.

The means of the littermate and non-littermate rats, with the littermate means expressed as a percentage of the non-littermate means. The significant difference is marked with asterisks.

	LM	NLM	$\frac{LM}{NLM} \times 100$
Body weight (gms)	358.5	284.1	126.2**
Food consumption (gms)	17.0	16.8	101.2
Feeding time (mns/day)	81.7	84.3	96.9
Rate of eating (mgms/mn)	213.2	207.4	102.8
Number of meals per day	10.7	10.1	105.9
Average duration of meals (mns)	8.3	8.3	100.0

At first appearance, these results suggest that no efficiency would be gained by dividing the rats into groups of littermates. This, however, is not necessarily true. It might be that, by a trick of chance in selecting the rats, the non-littermate rats averaged out to the same mean as the littermate rats. Let us immediately note that this is not true of the body weight, the littermate rats being the three heaviest. The chances of sampling did not operate there. It would have been convenient if one could have grouped the rats according to body weight, but we have already seen that body weight and feeding time do not correlate (Figure 40). It is also evident in Figure 45 that there is no correlation even between body weight and food consumption. It is true that there are serious inaccuracies in the measurement of food consumption, but even so, one ought to be able to detect the difference between rat 1 with a mean weight of 245.5 grams and rat 2 with a mean weight of 379.5 grams.

This division of the rats into littermates and non-littermates is not ideal, but was forced on the writer by the combination of the heavy demands of the teaching laboratories on the rat pool and a concomitant crisis in the breeding stock. This is unlikely to be repeated, and a further testing of differences between litters is indicated. The lack of correspondence between weight and food consumption is not strictly germain to the matter in hand, and the writer will say no more than a further investigation would be interesting.



Food consumption (grams / day) as a function of body weight.

Figure 45.

Granted that there are no significant differences between littermate and non-littermate rats, and granted too that this is the result of an accident of sampling, one would expect a greater variance from the non-littermates than from the littermates. Tested by the variance ratio of littermates to non-littermates, this was never significant, but the writer has already referred to the difficulty arising here from the small number of degrees of freedom available. He has therefore examined the significant differences between the means of the individuals within the two groups. The results of this are in Tables 5 and 6.

Table 5.

The differences between the means of individuals within the littermate group. The rats are placed in the order of magnitude of their means and significant differences are marked with asterisks.

Body weight (gms)	Rat order	2		3		4
	Means	379.5		364.7		331.3
	Differences		14.8**		33.4**	
Food consumption (gms/day)	Rat order	4		3		2
	Means	21.3		15.2		14.6
	Differences		6.1**		0.6	
Feeding time (mns per day)	Rat order	4		2		3
	Means	97.70		74.64		68.64
	Differences		23.06**		6.00	
Rate of eating (mgms/mn)	Rat order	3		4		2
	Means	226.0		220.0		193.6
	Difference		6.0		26.4**	
Number of meals per day	Rat order	4		3		2
	Means	11.57		10.64		8.21
	Differences		0.93		2.43**	
Duration of meals	Rat order	2		4		3
	Means	9.74		8.59		6.55
	Differences		1.15		2.04**	

Table 6.

The differences between the means of individuals within the non-littermate group. The rats are placed in the order of magnitude of their means and significant differences are marked with asterisks.

Body weight (gms)	Rat order	6		5		2
	Means	323.7		279.8		245.5
	Differences		43.9**		34.3**	
Food consumption (gms/day)	Rat order	5		6		1
	Means	18.6		16.8		14.9
	Differences		1.8**		1.9**	
Feeding time (mns per day)	Rat order	6		1		5
	Means	101.45		82.30		68.14
	Differences		19.15**		13.16**	
Rate of eating (mgms/mn)	Rat order	5		1		6
	Means	273.7		184.3		164.1
	Differences		89.4**		20.2	
Number of meals per day	Rat order	6		5		1
	Means	14.50		9.93		7.79
	Differences		4.57**		2.14**	
Duration of meals (mns)	Rat order	1		6		5
	Means	10.80		7.30		6.94
	Differences		3.50**		0.36	

An examination of these two tables reveals that there are seven significant differences between the means of the littermate rats and ten between those of the non-littermates. The writer does not feel that this is sufficient evidence that, when we retain the null hypothesis that there is no difference between the variance of littermate and non-littermate rats, we are committing a type II error.

On the basis of this experiment, therefore, there is no evidence to suggest that dividing the rats on the basis of litters would increase the efficiency of future experiments. However, the division

of the rats into littermates and non-littermates is not ideal and further investigation should be undertaken.

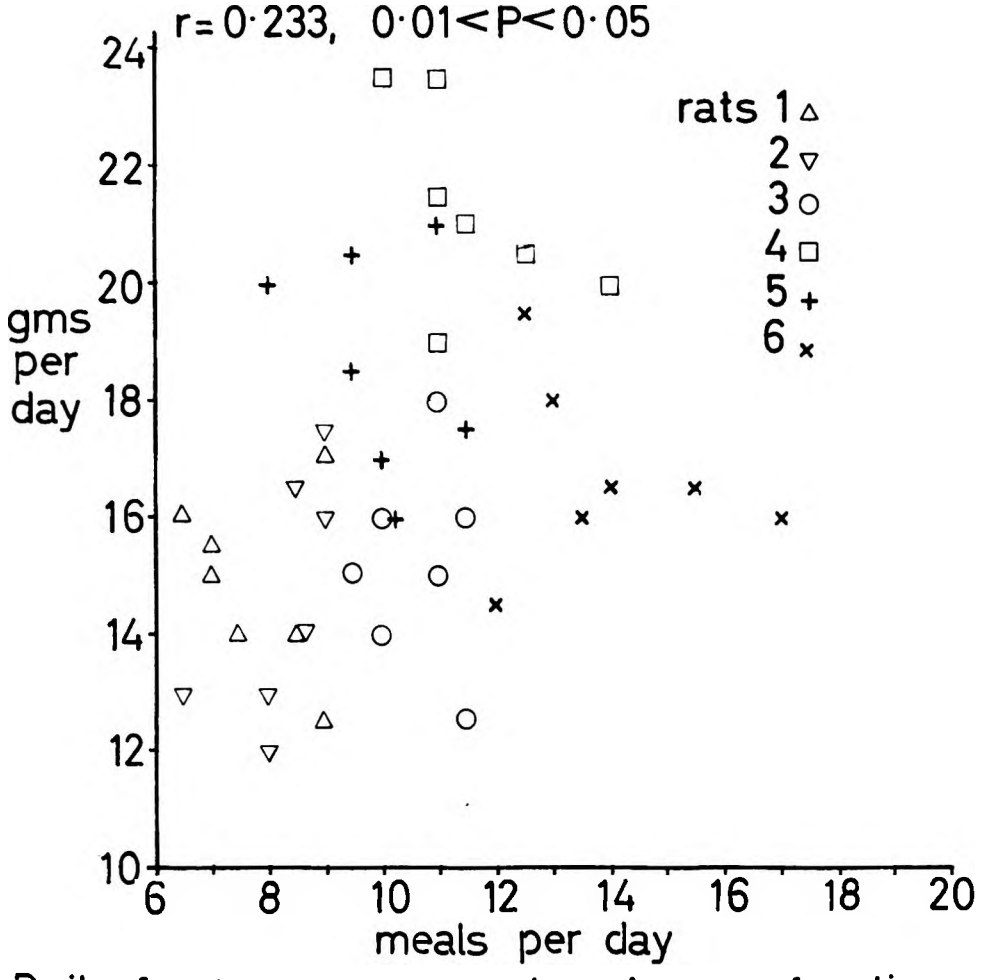
Examining Tables 5 and 6 in search of correlations, one notices in Table 5 that the order of rats under food consumption is the same as that under the number of meals per day, while in Table 6, the order is the same for two rats, but number 6 is out of place. Referring to Figure 46, we see at once that if there is a correlation it is spoilt by the position of rat 6. This rat no doubt contributed to the low correlation coefficient of 0.233. The writer therefore computed the correlation coefficient, omitting rat 6, and obtained 0.400, with 68 degrees of freedom and a probability of a type I error of less than 0.01, although the figure itself is not impressive. In an effort to test the matter further, the writer ignored the division into littermates and non-littermates and arranged the six rats in order of magnitude of their means for the two parameters concerned (Table 7).

Table 7.

The six rats placed in order of magnitude of their means for food consumption and the number of meals per day. Significant differences are marked with asterisks.

Food consumption (gms/day)	Rat order	4	5	6	3	1	2
	Means	21.3	18.6	16.8	15.2	14.9	14.6
	Differences		2.7**	1.8*	1.6*	0.3	0.3
Number of meals/day	Rat order	6	4	3	5	2	1
	Means	14.50	11.57	10.64	9.93	8.21	7.79
	Differences		2.93**	0.92	0.71	1.72**	0.42

Had we chosen a group of rats on the basis of food consumption, we would undoubtedly have chosen 3, 1, and 2. Had we chosen another,



Daily food consumption (gms) as a function of the number of meals per day.

Figure 46.

group based on number 5, the choice would not have been very good, nor, indeed, would a group based on number 4.

The conclusion is the rather depressing one that there appears to be no basis for grouping, even in an inbred colony. The reason for this is that we are measuring the temporal distribution of feeding behaviour and the breeder could not possibly be expected to pair rats on this basis. This raises the interesting question "If we performed experiments using rats of both sexes and mated rats with similar temporal patterns, could we introduce uniformity in this respect into the offspring?" It is reasonable to suppose we could, since the temporal patterns must be based on metabolic patterns which must surely be influenced by genetic factors. By this means, an insight into those factors controlling temporal behaviour patterns might be obtained.

Rat number 6 is interesting in that, it will be remembered, when dealing with the regressions (Table 3) we found a significant negative regression of the number of meals consumed per day by rat 6 in time. It is therefore possible that rat 6 might ultimately have taken up a more reasonable position in Table 7.

DRINKING BEHAVIOUR.

Drinking behaviour is of less importance to the future experiments than feeding behaviour, becoming of significance only when considering the ingestion of dietary constituents provided in aqueous solution. The water requirement of an animal is a function of the sodium chloride

ingestion and uncontrolled water loss. In this experiment the sodium chloride content of the diet remained constant and (Table 3) the amount of food consumed showed no significant changes, with the proviso that the measurement was not fully reliable. Thus the water requirement of the six rats was a function of their uncontrolled loss. The uncontrolled water loss is, in turn, a function of the humid state of the atmosphere and body surface, which we must presume is measured by body weight. Table 3 shows that there was an increase in the body weight of all rats throughout the experiment, but the biggest rate of increase, that of rat 3, was 5.6 grams per week which, with a rat having a mean body weight of 364.7 is less than 2 per cent per week. Thus the rate of increase in surface area was small and the main factor controlling water loss was the humid state. Although the rat room in which the apparatus is housed is temperature controlled, there is no similar control over the humidity which varies with two factors. The experiment started at midsummer, when the incidence of thunderstorms and more general rain is high. The humidity rises ahead of a thunderstorm and remains high for some hours afterwards and there are days, sometimes preceding rain, when it is high all the time. Superimposed on this is the entirely local fluctuation arising from such proceedings as cleaning and sterilisng of cages. These functions are normally performed in a room constructed for that purpose, opening off the rat room and equipped with an extraction fan. Despite the fan, however, it would be unsupportable

for anyone to work in there with tanks of near-boiling water and with the door shut. The result is that the humidity in the rat room rises on occasion to 100 per cent. However, on a Saturday when such activities are not indulged in, a recording hydrometer showed that at midnight the humidity was 30 per cent. It rose steadily until two o'clock in the morning, when it reached 60 per cent, falling to 58 per cent by 4 a.m. and then suddenly rising to 70 per cent by 5 o'clock. Thereafter, it fell to 55 per cent by 8 a.m., rose to 60 per cent by 10 o'clock falling steadily thence throughout the day and night to reach 50 per cent by midnight.

These very factors which have a major effect on water consumption, exert an equivalent effect on the evaporation of water in the troughs. This must be borne in mind when interpreting the figures on water consumption and their relation to drinking time.

The cylinder used for measuring the water was calibrated in 2 millilitre steps, so that the water measured into and out of the water towers was estimated to the nearest millilitre. On each day, water was first measured into each tower, and then, at the end of the 24 hours, the remainder was measured out. Thus, two errors are possible on each day and might either cancel out or summate, giving a possible error of measurement of between zero and 2 millilitres.

Drinking time, like feeding time, was measured to the nearest quarter of a minute.

The total drinking time can be subdivided quite logically into

drinking at mealtimes, and drinking between meals. It is analysed under all three heads, total, at meals, and between meals.

For the sake of uniformity, drinking behaviour was analysed according to the same model as feeding behaviour, and it is not surprising to find, in view of the factors controlling water requirement, that significant regression coefficients are the exception rather than the rule (Table 8).

Table 8.

The regression coefficients of the parameters of drinking behaviour on time and their significance.

Regression on time of	Rats					
	1	2	3	4	5	6
Water consumption (mls/day)	-0.680	-0.755	-0.881*	0.039	-0.709	0.611
Total drinking time (mns/day)	0.274	-0.754	-0.162	0.133	0.266	-0.502
Drinking time at meals (mns/day)	0.459	0.098	-0.193	0.125	0.114	0.022
Drinking time between meals (mns/day)	-0.186	-0.777**	-0.046	-0.009	0.182	0.536*

The significant regression coefficient for Rat 3's water consumption must be accepted with a certain amount of reservation, since the least significant value at the 5 per cent level was 0.843. The writer prefers to leave the regressions of drinking time between meals for

the moment. As will be seen, he has doubts as to whether this is in fact a measure of drinking time at all.

An examination of the differences between the means reveals, in the first place, that the bulk of the variation arises from differences between littermates and non-littermates (Table 9).

Table 9.

The littermate and non-littermate means with the former expressed as a percentage of the latter. The asterisks indicate the significance of the differences. The last column shows the coefficients of variation (C).

	LM	NLM	$\frac{LM}{NLM} \times 100$	C
Water consumption (mls/day)	19.45	19.76	98.4	18.1%
Total drinking time (mns/day)	9.73	13.23	73.5**	24.9%
Drinking time at meals (mns/day)	8.03	10.66	75.5**	23.0%
Drinking time between meals (mns/day)	1.65	2.59	63.7*	93.9%

Looking first at water consumption, we recall the errors in measurement. The calibration of the measuring cylinder itself introduced an error which could be rather more than 10 per cent of the general mean. This is added to evaporation. The effect of these is clear. In measuring food consumption with all its errors, the coefficient of variation (root mean square for error as a percentage of the general mean) was 10.5%, while for water consumption it is

18.1%. This effectively robs the difference between means of significance and makes the means themselves unreliable estimates.

The moral of this is clear; substances consumed in small quantities must not be provided in aqueous solution. If dilution is necessary, it should be by means of mixing with some innocuous substance such as cellulose flour. Some more accurate method of measuring would be desirable. It might be possible to obtain a measuring cylinder of the requisite size calibrated in millilitres. This will reduce the error involved in measuring the water remaining at the end of the day by half. Measuring the water into the tower at the beginning of the day should clearly be done by means of large pipettes. Alternatively, if more accurate scales can be obtained for weighing the food, it might be possible, if not to kill two birds with one stone, at least to seriously frighten them by weighing the water instead of measuring it. There still remains, of course, the error arising from evaporation.

Turning now to drinking time at meals, we have a general mean of 9.36 minutes per day per rat. With a mean number of meals of 10.44 per day, this gives the average drinking time at one meal of less than one minute (53.8 seconds). The maximum possible error in estimation, with the paper speed used in this experiment is 1/8 of a minute or 7½ seconds, giving a percentage of 13.94. It will be remembered that the motor is geared down to the first gearbox by means of a V belt and pulleys, with a ratio of 3 to 1. This can, if necessary,

be changed to one of 1 to 3, which would reduce this error to about 1.5 per cent. In a long term experiment of this sort, such a speed would have practical objections. This error undoubtedly contributes to the coefficient of variation of 23.0%, which contrasts with the coefficient of variation of feeding time of 8.63%. This difference arises from the fact that the average duration of a meal is 8.32 minutes, which is about nine times the average duration of drinking at meals. The coefficient of variation is not reduced in proportion because there are breaks in feeding which increase the error, and not the whole of the experimental error is accounted for by errors of measurement. If at any time it becomes desirable to reduce this error, it can be done by increasing the paper speed.

When we come to drinking time between meals, we find a remarkable coefficient of variation of 93.9%. Here the general mean is 2.12 minutes per rat per day. Thus the errors already discussed under drinking at meals will be proportionately increased. There is, however, another aspect of the matter. It will be seen, when we come to consider random visits to troughs betwe n meals, that the writer was looking for not only an end effect, but a difference between troughs 1 and 8. He failed to find this difference, and his reason for looking for it as well as his failure to find it is germain to drinking time between meals. It arises from the behaviour of the rats.

Each rat was provided with a bowl in which to sleep. Rats 2,

3, 4, and 5 used these bowls regularly. Number 1 used it at the beginning of the experiment but not at the end, and rat 6 never used it. Rat 6 preferred to sleep in a corner at the back of the cage, but rat 1 chose the corner near the drinking trough, and nothing could persuade him that such was not a desirable practice. However, all the rats from time to time slept on the wire floor of the cage. One mustpresume, though measurements were not made, that these were periods of high humidity and the rats were suffering from a thermal crisis. No doubt it was easier to dissipate heat stretched out on the floor than curled up in the bowl. When they indulged in this practice it was usually at the number 1 trough end and rarely at the number 8 trough end. Some consequences of this are to be seen in Figure 26. Where the rat took up such a position that the beam was only partly obscured by its hair, the drift illustrated there occurred. However, the rat might move in such a way as to cut the beam completely when the clean deflection seen to the left of the drift would occur. Who is to say that this rat did not get up and have a drink? Such clean deflections are counted as drinking time. Had they occurred at the number 8 end, and if number 8 trough was empty, as it was most of the time, such deflections would have counted as random visits. Thus one would have expected more random visits at the number 8 end between meals than at the number 1 end. It speaks volumes for the tendency of the rats to sleep at the number 1 end that the difference between the number 1 and number 8 means is not significant. The

writer is of theopinion that the figures for drinking time between meals are thoroughly unreliable and he doubts whether, in fact, the rats did drink at all between meals. Why this curious habit should have developed is beyond the writer. It could be due to some trick of air currents or to the fact that the presence of the water at that end made that corner of the cage cooler than the rest. Whatever the reason, the result will be entirely eliminated by protecting the troughs.

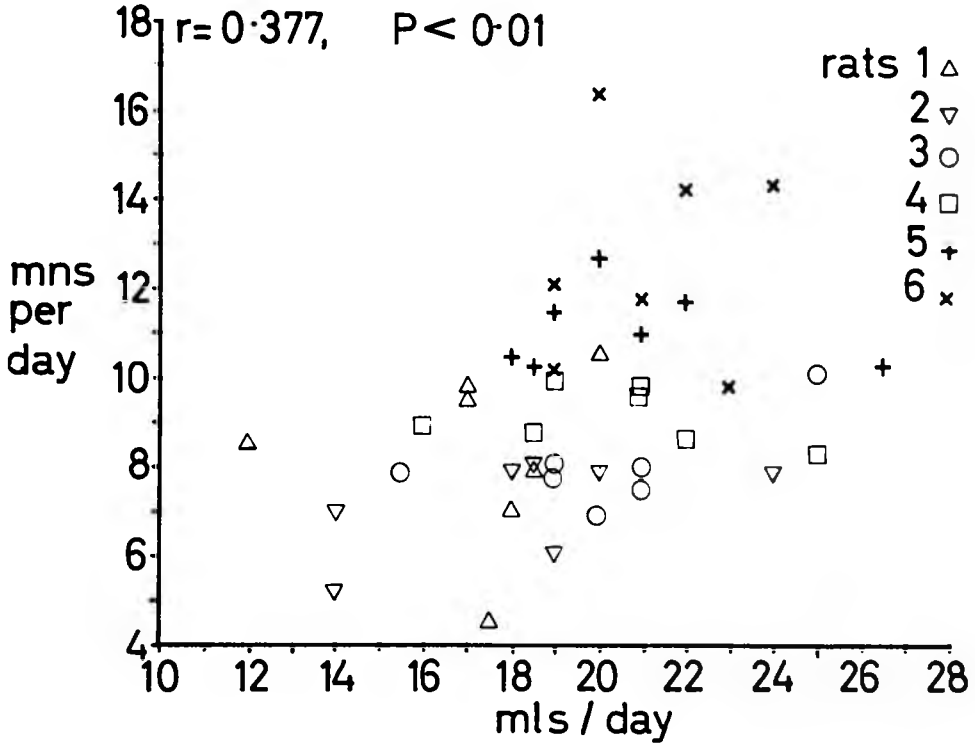
Total drinking time, of course, includes this thoroughly unreliable drinking time between meals. Thus in seeking some correlation between drinking time and water consumption, only drinking time at meals need be considered. The scatter diagram in Figure 47 shows that there is indeed some correlation, as one might expect, but the low coefficient, albeit statistically significant, is an expression of the accumulation of the errors discussed above. Indeed, it is surprising that it is not worse.

Little, then can be learnt from the measurements on water consumption and drinking time, but that little is important.

1. Aqueous solutions must be avoided.

2. When errors in measuring time must be reduced, the paper speed must be increased. Provision was made in the design of the apparatus for this.

3. The need to protect the front of the troughs to avoid accidental cutting of the light beam is emphasised.



Time spent drinking at meals (mns / day) as a function of water consumption (mls / day).

Figure 47.

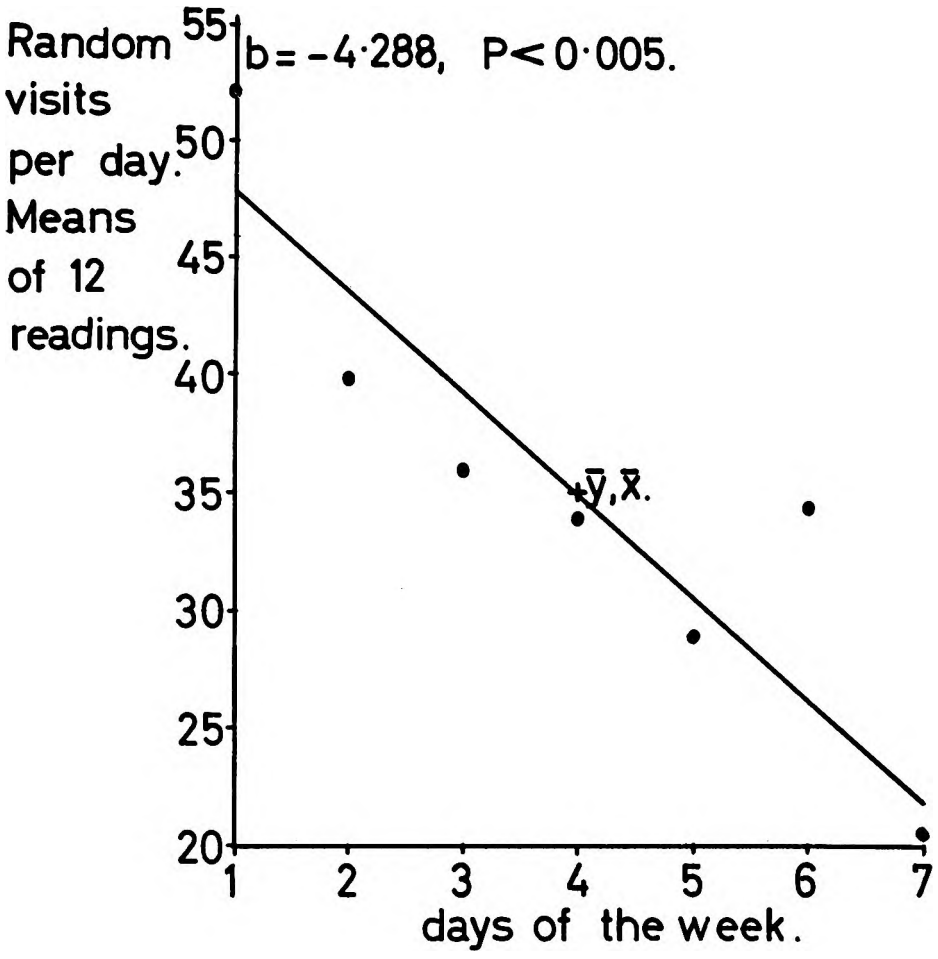
It remains only to comment on the fact that the mean drinking time for littermate rats is less than that for the non-littermates. The only reasonable explanation for this, in view of the hypothesis put forward earlier that the main factor controlling water loss is evaporation and that this is a function of body size, is that there is a familial difference in the rate of drinking. In view of the large errors involved in measurement here, it is unwise to place much reliance on these figures, and fruitless to make far reaching deductions from them. Further, a statistical probability is not a certainty, and this might be the 1 in a 100 chance indicated by the probability at the 1 per cent level.

RANDOM VISITS TO TROUGHS.

The random visits to troughs are immediately and obviously divisible into two groups, those occurring at meal times and those occurring between meals. As these two groups appear to be of different origin and have distinctly different characteristics, they will be treated separately.

Random visits to troughs at meal times.

The question which immediately leaps to the mind is, "Are these random visits "mistakes" on the part of the rats?" If they are, then one would expect the performance to improve with practice. With this idea in mind, the writer drew the graph in Figure 48, calculating the regression coefficient as described in the statistical section.



The decrease in the number of random visits to troughs at meal times as the week progresses.

Figure 48.

Clearly the rats were improving markedly with practice. It is a question, however, how far these random visits would go on decreasing. Looking at the points for days 1, 2, 3, and 4, one gains a strong impression of curvilinearity, whereas the points for days 5 and 7 seem to pull the curve back to the straight. The point for day 6, on the other hand, might well be conforming to a curved line.

This is a matter of some importance, since it is clearly necessary to reduce this random activity to as low a level as possible. One must know how long it takes for such visits to reach a minimum before one can design the details of an experiment. The time period must be longer and more rats employed.

If these visits to the troughs are mistakes, how are they distributed? The various comparisons made are summarised in Table 10.

Table 10.

The mean numbers of random visits at meal times per rat per trough per day, the difference between the means, and the probability of committing a type I error (P). Where no probability is entered in the last column, it is greater than 0.05 and the difference between the means is not significant.

Comparisons	Means	Differences	P less than
Adjacent to food trough	6.28	2.56	0.001
Other troughs	3.72		
Between food and water troughs	4.44	-0.24	
Not between food and water troughs	4.68		
End troughs	4.86	0.56	
Other troughs	4.30		
Food and water troughs	4.09	-0.46	
Empty troughs	4.55		
Food troughs	4.63	0.08	
Empty troughs	4.55		

It is immediately clear that there is a strong tendency for the rats to put their heads into the troughs on either side of the one containing food. This can happen at any time during the period of eating, and it appears as if the rat, having withdrawn its head to chew, becomes slightly disorientated and misses the food trough when inserting its head for the next mouthful. If, as already appears necessary for other reasons, partitions are erected between the troughs so that the rat is in a short passage while eating, this type

of mistake will be markedly reduced if not altogether eliminated.

The fact that there is no difference between the number of visits paid to troughs between the food and water troughs and to those not between, means, simply, that when the rat moves from the water to the food trough, the distance it moves is as likely to fall short of the true distance as it is to exceed it, if, indeed, it makes either type of mistake. On the other hand, on moving from the food trough to the water trough, the rat makes no mistake.

The end troughs do not appear to attract the rat any more or less than the middle ones. In other words, the position of the trough in the row is not, per se, a deciding factor in the number of visits paid to it.

The food and water troughs receive just as many such visits as the rest, suggesting that the distribution has a certain random quality which is assumed in the term "random visits". The writer, however, is not at all satisfied with his own assumption. In so far as the water trough is concerned, there is no serious difficulty since, as we have seen, the period of drinking is, on an average, less than one minute, and during the rest of the meal random visits can be and are paid to the water trough. The actual time spent eating, on the other hand, occupies a much larger proportion of the meal time, sometimes nearly all of it. A visit to the trough, however brief, during this period of eating would by definition, be

counted as eating time and not as a random visit unless separated from the eating time by a minute or more. This suggests very strongly, and is indeed a fact, that the random visits to the feeding trough occur at the beginning or end of the meal. It is as if, before it eats, the rat is uncertain whether it wants to eat or not and sniffs at the food to find out, whereas, after it has eaten, it is uncertain whether it had had enough or not and sniffs the food to find out. The writer has seen dogs do exactly that. That this is not an invariable occurrence is shown by the fact that the average number of such visits is far less than the average number of meals per day (10.44). He can at present offer no suggestion as to why it should happen on some occasions and not on others. Suffice it to say that he is not certain that this is in fact random activity in the strict sense. It might well have a physiological basis.

If the writer is correct in his theorising, one would not expect that the number of visits paid to the food trough would decrease with the passage of time. Indeed, there might be other groupings of troughs which are not affected by the learning process, and presumably these groupings would form the basic level of random visits below which performance would not improve with practice. In order to gain some insight into this, the writer examined the regression coefficients of various groups, virtually the same as those examined for differences between the means. The results of this examination are given in Table 11.

Table 11.

The regression coefficients (b) of the various trough groupings and the probability of committing a type I error in accepting them (P). Where no probability is given, it is greater than 0.05 and the regression coefficients are considered insignificant.

Troughs	b	P less than
Empty	-0.6711	0.001
Middle six	-0.6626	0.005
End two	-0.0672	
Number 8	-0.1331	
Between food and water	-0.8690	0.05
Not between food and water	-0.4732	
Adjacent to food	-0.7630	0.05
Food and water	-0.1798	
Food	-0.2871	
Water	0.0663	

The rats were reducing the number of random visits paid to empty troughs, and presumably this is the learning process. Dividing the regression coefficient into its components, the number of random visits paid to such a trough when it was one of the middle six in the row decreased, but not when it was at the end. Thus, although the means of visits to end troughs and middle troughs did not differ significantly, they might have come to do so had more time been allowed for adaptation.

The term "end two" in the table includes both 1 and 8. Trough number 1 is the water trough and is therefore not empty. The writer examined them separately. There was a slight tendency for the rats to reduce the number of random visits to trough 8 which is statistically insignificant, but no tendency at all to reduce those to the water trough. The number of figures contributing to the daily means of

trough 8 were 12, as opposed to up to 72 for most of the others, and thus the result must be treated with some reserve.

The rats were decreasing the number of visits paid to troughs between the food and water troughs, but the decrease in the visits to those not between was not statistically significant. There was thus a tendency to "run short" when moving from food to water or vice versa which was decreasing with practice and the difference between the static means given in Table 10 might have become significant in time.

The rats were decreasing the tendency to "miss" the food trough and put their heads into the one next to it, clearly a case of improving with practice.

When we come to the food and water troughs, we see a slight tendency to decrease the number of "random" visits, albeit statistically insignificant. When they are treated separately, however, we have a regression coefficient for the water trough which is clearly estimating zero. The result of this is that the regression coefficient for the food trough is somewhat greater than for the combination, but still insignificant. The "t" for 5 degrees of freedom and a probability of 0.1 is 2.015, whereas the "t" obtained by comparing this regression coefficient with its standard error is 2.088. Thus the chance of obtaining such a regression coefficient in a random sample from a population which in fact had none is in the region of 1 in 10. One would be bold to the point of being foolhardy to suggest

that this regression is a reality. Bearing in mind that the food trough appears six times as one of the "middle" group compared with once as an end trough, one must suspect that this regression is, as the statistics show, fictitious. The water trough, on the other hand is always an end trough and is not contaminated with the tendency for the random activity in the middle to decrease. Whether, in the case of these two troughs, we are dealing with truly random activity or whether the phenomenon has a physiological basis cannot be decided on the evidence available. It is something worthy of further investigation.

From the point of view of future experiments, it is desirable that dietary components which are taken in small quantities should not be placed in end troughs, and should be placed between those taken in large quantities and the water trough, provided sufficient time is allowed for the rats to accommodate to the trough positions. Just how long that time should be cannot be deduced from these experiments, but it is clearly longer than a week.

Random visits to troughs between meals.

There are certain very marked differences between the random visits to troughs between meals and at meals. In the first place, the general mean of visits between meals is 0.83 visits per rat per trough per day, contrasted with 4.41 (Appendices XI and XII). In fact, days can pass without any such visits.

In the second place, the number of visits does not decrease with

time, the regression coefficient of -0.655 carrying a probability of committing a type I error in accepting it of very near 20 per cent. Thus we may say that this type of random activity is not subject to a learning process.

The third difference lies in the results of comparing means. Those which seemed relevant to the writer are set out in Table 12.

Table 12.

The mean numbers of random visits between meals per rat per trough per day, the differences between the means, and the probability of committing a type I error (P). Where no probability is entered in the last column, it is greater than 0.05 and the difference between the means is not significant.

Comparisons	Means	Differences	P less than
Food and water	1.317	0.654	0.001
Empty troughs	0.663		
End two troughs	1.341	0.691	0.001
Middle six troughs	0.650		
Number 8	1.195	0.545	0.001
Middle six troughs	0.650		
Number 1	1.500	0.850	0.001
Middle six troughs	0.650		
Number 1	1.500	0.305	
Number 8	1.195		
Adjacent to food	0.592	0.217	
Next but one to food	0.365		
Food troughs	1.134	0.471	0.01
Empty troughs	0.663		
Food trough when not number 8	1.070	0.489	0.01
Empty troughs when not number 8	0.581		

In the first place, the food and water troughs receive more of these visits than do the empty troughs, and the visits are concentrated on the end troughs. One naturally asks whether this is because they are the end troughs or because one of them is the water trough. The water is in number 1, but the number of visits paid to the other end trough, number 8, is significantly greater than those paid to the middle six, as is the number paid to number 1 itself. Further, there is no significant difference between the number of visits paid to numbers 1 and 8.

In view of the discussion of drinking time between meals, this is surprising. Assuming that troughs 1 and 8 are subject to the same hazards and that visits approaching or exceeding ½ of a minute to trough 1 would be counted not as random visits but as drinking time, one would have expected that the number of random visits to trough 8 would be greater than those to trough 1. This is not so. This fact can be interpreted in two ways: either the drinking time between meals is genuine drinking time, or trough number 1 is exposed to greater hazards than trough number 8. In view of his observation of the rats, the writer favours the latter interpretation, though he admits that there is no proof. We can, however, state with confidence that in the random visits to troughs between meals there is a pronounced "end effect".

Continuing with our consideration of position effects, it is interesting to observe that the random visits to the troughs next to

the food trough do not exceed those to the troughs next to them. A similar, though not identical comparison made when examining random visits at meal times, yielded a highly significant result. This difference between the two types of visit tends to confirm that such visits made while the rat is eating are genuine mistakes.

In comparing the number of visits paid to the food and water troughs with those paid to empty troughs, we obtained a highly significant result, but, in view of the end effect, which affects trough number 1, it is necessary to examine the food trough alone. This is done in the last comparison but one in Table 12. Here we see that although the difference between the means and its significance are less, the significance is still sufficient to give confidence that the rat is paying occasional visits to the food trough. However, on one occasion in seven the food trough is number 8 which is subject to the end effect. In an effort to avoid this, the writer made the last comparison in the table, in which he eliminated trough 8 from consideration in arriving at the food trough and empty trough means. The result is not materially changed. The food trough therefore receives more attention than do the empty troughs.

Reverting to the regressions, and remembering his experience with those pertaining to random activity at meal times, the writer wondered whether the lack of significance of the total regression arose from the dilution of one significant result, or from two regressions of opposite sign. He therefore calculated the regression

coefficients for the groupings in Table 12 and estimated their significance. He obtained the rather curious result that, whereas all the coefficients had negative sign, none was significant. This result is curious because one would expect that in a series of groupings which are samples drawn at random from a population having zero coefficient, the "partial" coefficients would vary about zero, that is, some would be negative and some positive. This induces in the writer's mind the notion that had the number of rats been larger, and the period of one week extended, some or all of these coefficients would have become significant. One cannot comment further on this. Additional experimentation is needed.

One is entitled to speculate on the nature of this random activity. It would appear, on the face of it, to be a manifestation of some sort of play, as if the rat, in its nocturnal cavortings, occasionally pokes its head into a trough, for no better reason than that in some obscure way it derives "fun" therefrom, and, granted this much, it would not be surprising if the food trough, which has some meaning for the rat, would attract it more than the others. In addition to this, there is the possibility of accidental cutting of the beam, and this might well be more marked at the ends of the row of troughs than in the middle. With a general mean of less than one visit per rat per trough per day, one might imagine that protecting the troughs would reduce this activity almost to zero, but it is dangerous to be sanguine about this. The protection might well

reduce the "end effect", but rats like entering tunnels, and so might find these visits to troughs more fun under protection than when there is none. Should this prove to be the case, it would seem to be advisable to provide the cages with some tunnels in which the rats can play, and which might even be used as a means of estimating activity.

Consideration of these visits between meals serves to emphasise the importance of not putting dietary constituents taken in small quantities in the end troughs.

SUMMARY AND CONCLUSIONS.

1. The author has described a somewhat elaborate piece of apparatus which was designed to monitor the feeding behaviour of rats which are presented with a choice of several feeding troughs. The apparatus does what it was designed to do; it gives a record of the temporal distribution of feeding behaviour. It can be improved in minor ways, such as affording protection to the troughs and possibly some system of tunnels which might induce the rats to play in some part of the cage where they do not interfere with the troughs. A certain lack of reliability was revealed. Consultation with a member of the staff of the Department of Electrical Engineering suggests that this is due to certain faults in the electronic circuits. A new circuit has now been designed with a much greater difference between the on and off positions and thus with a greater safety margin.

2. By attending to the rats at the same time every day, one tends to induce in them a bout of feeding at that time. This might interfere with their metabolic pattern and feeding behaviour for some time afterwards, and it is proposed to conduct experiments aimed at eliminating this artefact.

3. The mean time spent eating in a day is not related to the mean body weight, but there is a suggestion that feeding time of individuals might increase as their body weight increases. This might be related to the state of obesity and is worthy of further investigation.

4. The time a rat spends eating is not related to the amount of food it eats because it tends to change the rate of eating. In fact, the time spent eating correlates well with the rate of eating. There seems to be a mechanism in operation which is independent of the mechanism controlling the daily food intake.

5. There is a strong suggestion that the number of times a rat eats in 24 hours is fixed and highly characteristic of that rat. When a rat changes the time it spends eating, it does so by changing the duration of its meals and not their number. There might be exceptions to this but there is no evidence to suggest under what circumstances they might occur. It is proposed to attempt confirmation of this rule, and to relate it to the pattern of the animal's carbohydrate metabolism.

6. The means of the different parameters of feeding behaviour are the same for littermate rats and those not related. This could be an accident of sampling and does not necessarily mean that there would be no difference between litters. This warrants further investigation.

7. The possibility of breeding rats according to the temporal distribution of their behaviour has been mooted.

8. The data on drinking behaviour are unreliable but might be improved when the troughs are protected and more accurate means of measurement are introduced, but the problem of errors introduced by evaporation will remain. This means that dietary components must not be in aqueous solution.

9. The number of random visits paid to troughs at meal times tends to decrease with the passage of time, but the week allowed between trough changes was not sufficient for these visits to reach their minimum. This suggests a learning process. The time that must be allowed for this type of activity to reach a minimum must be determined.

10. Not all types of visits to troughs at meal times are subject to this decrease and might either be strictly random or have a physiological basis. Further experiments are necessary to establish which is true.

11. The safest place to put dietary constituents which are taken in small quantities is between those taken in large quantities and the drinking trough. The end troughs should be avoided.

12. The random visits paid to troughs between meals do not decrease and are therefore not subject to a learning process. The total regression coefficient had negative sign and so had all the "partial" coefficients, suggesting that had more rats been used or had the time been longer the finding of this experiment might have been reversed.

13. These random visits tend to be most at the end troughs. Such an effect might be reduced by protecting the troughs but the finding serves to emphasise the danger of using end troughs for dietary constituents taken in small quantities.

14. Where there is food in one trough only, the rat tends to pay visits between meals to that trough more than it does to empty troughs.

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APPENDIX I

Body weight (grams).

	Rats							
Weeks	1	2	3	4	5	6	Totals	Means
1	233	360	338	318	267	300	1816	302.7
2								
3	240	375	360	336	280	322	1913	318.8
4	252	380	370	330	284	322	1938	323.0
5	250	386	368	332	288	328	1952	325.3
6	250	390	372	334	290	330	1966	327.7
7								
8	248	386	380	338	290	340	1982	330.3
Totals	1473	2277	2188	1988	1699	1942	11567	
Means	245.5	379.5	364.7	331.3	279.8	323.7		321.3

APPENDIX II

Daily food consumption (grams).

samples	weeks	Rats						Totals both samples	Means
		1	2	3	4	5	6		
1	1	14	16	18	20	16	18		
	2								
	3	15	19	15	22	22	18		
	4	14	17	18	22	19	15		
	5	15	13	15	20	17	16		
	6	16	18	18	22	20	18		
	7	15	13	14	22	20	14		
	8	13	12	11	22	18	16		
2	1	20	12	18	20	16	14	202	16.83
	2								
	3	17	16	15	21	20	21	221	18.42
	4	16	16	14	25	18	18	212	17.67
	5	16	13	17	18	18	17	195	16.25
	6	12	14	12	20	21	18	209	17.42
	7	13	13	14	19	20	15	192	16.00
	8	12	12	14	25	16	17	188	15.67
Totals		208	204	213	298	261	235	1419	
Means		14.86	14.57	15.21	21.29	18.64	16.79		16.89

Daily food consumption (grams)

	Days						
	1	2	3	4	5	6	7
Totals	198	211	202	192	208	207	201
Means	16.50	17.58	16.83	16.00	17.33	17.25	16.75

APPENDIX III.

Feeding time (minutes per day).

Samples	Weeks	Rats						Totals both samples	Means
		1	2	3	4	5	6		
1	1	65.25	50.25	62.25	82.50	62.25	93.75		
	2								
	3	84.25	68.25	74.25	103.25	76.25	113.00		
	4	89.50	97.25	64.50	88.50	64.75	104.75		
	5	87.25	94.75	82.00	103.75	68.50	105.75		
	6	90.75	95.75	61.00	96.00	67.75	106.25		
	7	94.75	74.75	70.00	107.75	69.50	99.25		
	8	86.00	74.50	62.75	96.00	66.00	100.50		
2	1	65.75	47.00	53.75	76.75	62.25	95.00	818.75	68.23
	2								
	3	69.00	73.00	61.50	87.50	70.25	112.75	993.25	82.77
	4	98.00	84.00	67.75	96.25	62.00	107.25	1023.50	85.29
	5	92.75	94.75	84.75	107.50	77.25	114.50	1113.50	92.79
	6	85.75	107.75	72.25	111.75	76.50	98.75	1068.25	89.02
	7	70.25	70.00	70.00	102.00	69.50	98.50	996.25	83.02
	8	73.00	74.50	74.25	106.25	61.25	85.25	960.25	80.02
Totals		1152.25	1104.50	961.00	1367.75	954.00	1434.25	6973.75	
Means		82.30	74.64	68.64	97.70	68.14	101.45		83.02

Feeding time (minutes per day).

	Days						
	1	2	3	4	5	6	7
Totals	1003.50	973.50	1041.75	982.25	960.25	977.75	1034.75
Means	83.62	81.13	86.12	81.85	80.02	87.48	86.23

APPENDIX IV.

Rate of eating (mgms per minute).

Samples	Weeks	Rats						Totals both Samples	Means
		1	2	3	4	5	6		
1	1	215	318	289	242	257	192		
	2								
	3	178	278	202	213	289	159		
	4	156	175	279	249	293	143		
	5	171	137	183	193	248	151		
	6	176	188	295	229	295	169		
	7	158	174	200	204	288	141		
	8	151	161	175	229	273	159		
2	1	304	255	334	254	257	147	3064	255.3
	2								
	3	246	219	244	240	285	186	2739	228.2
	4	163	190	207	260	290	169	2574	214.5
	5	173	137	200	167	233	148	2141	178.5
	6	140	132	166	179	275	182	2426	202.2
	7	185	186	200	186	288	152	2362	196.8
	8	164	161	189	235	261	199	2357	196.4
Totals		2580	2711	3163	3080	3832	2297	17663	
Means		184.3	193.6	225.9	220.0	273.7	164.1		210.3

Rate of eating (mgms per minute).

	Days						
	1	2	3	4	5	6	7
Totals	2506	2692	2385	2432	2712	2576	2361
Means	208.8	224.3	198.8	202.7	226.0	214.7	196.8

APPENDIX V

The number of meals per day.

Samples	Weeks	Rats						Totals both samples	Means
		1	2	3	4	5	6		
1	1	9	8	12	14	10	18		
	2								
	3	7	10	11	12	12	14		
	4	7	8	9	11	10	14		
	5	7	8	9	11	11	14		
	6	8	10	9	10	9	14		
	7	9	7	10	12	8	12		
	8	9	8	13	9	10	18		
2	1	9	9	10	14	10	20	143	11.92
	2								
	3	6	8	11	10	10	11	122	10.17
	4	7	9	11	11	9	17	123	10.25
	5	7	8	14	11	12	14	126	10.50
	6	7	8	10	13	10	12	120	10.00
	7	8	6	10	13	8	12	115	9.58
	8	9	8	10	11	10	13	128	10.67
Totals		109	115	149	162	139	203	877	
Means		7.79	8.21	10.64	11.57	9.93	14.50		10.44

The number of meals per day

	Days						
	1	2	3	4	5	6	7
Totals	122	124	131	133	121	129	177
Means	10.17	10.33	19.92	11.08	10.08	10.75	9.75

APPENDIX VI

The average duration of meals (minutes)

Samples	Weeks	Rats						Totals both samples	Means
		1	2	3	4	5	6		
1	1	7.25	6.28	5.19	5.89	6.22	5.21		
	2								
	3	12.04	6.82	6.75	8.60	6.35	8.07		
	4	12.79	12.16	7.17	8.05	6.48	7.48		
	5	12.46	11.84	9.11	9.43	6.23	7.55		
	6	11.34	9.58	6.78	9.60	7.53	7.59		
	7	10.53	10.68	7.00	8.98	8.69	8.27		
	8	9.56	9.31	4.83	10.67	6.60	5.58		
2	1	7.31	5.22	5.38	5.62	6.22	4.75	70.54	5.89
	2								
	3	11.50	9.12	5.59	8.75	7.02	10.25	100.86	8.41
	4	14.00	9.33	6.16	8.75	6.89	6.25	105.51	8.79
	5	13.25	11.84	6.05	9.77	6.44	8.18	112.15	9.34
	6	12.25	13.22	7.23	8.60	7.65	8.23	109.60	9.13
	7	8.78	11.67	7.00	7.85	8.69	8.21	106.35	8.86
	8	8.11	9.31	7.42	9.66	6.13	6.56	93.74	7.81
Totals		151.17	136.38	91.06	120.22	97.14	102.18	698.75	
Means		10.80	9.74	6.55	8.59	6.94	7.30		8.32

The average duration of meals (minutes).

	Days						
	1	2	3	4	5	6	7
Totals	101.49	99.08	102.68	94.02	96.31	96.46	108.71
Means	8.46	8.26	8.56	7.84	8.03	8.04	9.06

APPENDIX VII

Water consumption (mls per day).

Samples	Weeks	Rats						Totals both Samples	Means
		1	2	3	4	5	6		
1	1	21	20	20	18	22	18		
	2								
	3	20	24	20	20	28	26		
	4	18	22	18	20	19	20		
	5	18	14	20	20	24	24		
	6	20	20	17	24	16	20		
	7	18	22	21	18	18	20		
	8	14	14	16	16	24	22		
2	1	14	18	30	19	22	30	252	21.00
	2								
	3	19	24	20	30	26	18	275	22.92
	4	22	15	20	12	18	22	226	18.83
	5	16	14	22	22	18	22	234	19.50
	6	16	20	14	20	22	18	227	18.92
	7	16	14	21	24	18	18	228	19.00
	8	10	14	18	22	16	19	205	17.08
Totals		242	255	277	285	291	297	1647	
Means		17.29	18.21	19.79	20.36	20.79	21.21		19.61

Water consumption (mls per day).

	Rats						
	1	2	3	4	5	6	7
Totals	226	258	227	244	237	243	212
Means	18.83	21.50	18.92	20.33	19.75	20.25	11.75

APPENDIX VIII

Total drinking time (minutes per day).

Samples	Weeks	1	2	3	4	5	6	Totals Both Samples	Means
1	1	7.00	9.25	12.00	9.75	11.75	13.75		
	2								
	3	10.50	10.50	9.50	10.00	14.25	20.75		
	4	12.75	11.25	9.25	8.50	13.50	7.50		
	5	13.75	7.75	9.00	10.50	14.50	13.25		
	6	12.00	10.25	8.75	9.50	8.25	14.75		
	7	11.00	9.50	11.50	8.75	14.00	21.25		
	8	10.50	7.00	8.50	8.50	13.32	21.25		
2	1	9.00	13.25	12.00	9.00	11.75	15.00	133.50	11.12
	2								
	3	13.00	17.75	7.25	6.75	12.75	12.25	145.25	12.10
	4	12.00	9.75	11.50	9.25	14.00	15.25	134.50	11.21
	5	10.25	7.75	9.50	10.50	17.00	15.50	138.25	11.52
	6	9.50	8.00	8.75	10.75	14.75	10.25	125.50	10.46
	7	10.50	8.00	11.50	9.00	14.00	19.00	148.00	12.33
	8	12.25	7.00	10.00	11.75	16.00	13.00	139.07	11.59
Totals		154.00	137.00	139.00	132.50	188.82	212.75	964.07	
Means		11.00	9.79	9.93	9.46	13.49	15.20		11.48

Total drinking time (minutes per day).

	Days						
	1	2	3	4	5	6	7
Totals	127.25	142.32	137.50	134.50	134.50	155.00	133.00
Means	10.60	11.86	11.46	11.21	11.21	12.92	11.08

APPENDIX IX

Drinking time at meals (minutes per day).

Samples	Weeks	Rats						Totals Both Samples	Means
		1	2	3	4	5	6		
1	1	5.25	6.00	10.25	9.25	11.75	13.75		
	2								
	3	5.75	9.00	8.25	10.00	10.00	16.25		
	4	12.25	8.50	5.00	8.50	10.75	7.50		
	5	12.25	5.25	8.50	9.50	9.75	9.00		
	6	6.25	9.25	7.75	8.25	8.25	10.50		
	7	11.00	8.00	7.50	8.50	10.50	12.75		
	8	8.50	7.00	8.25	8.50	12.13	21.25		
2	1	3.75	6.25	10.00	8.25	11.75	15.00	111.25	9.27
	2								
	3	10.00	6.75	5.50	6.50	10.50	12.25	110.75	9.23
	4	8.75	7.75	10.50	9.25	9.75	15.25	113.75	9.48
	5	6.75	5.25	7.50	9.75	12.25	10.50	106.25	9.11
	6	7.75	6.50	8.00	9.00	14.75	9.75	107.00	8.83
	7	8.50	7.75	7.50	9.00	10.50	11.50	113.00	9.42
	8	8.50	7.00	8.00	11.25	13.25	11.50	125.13	10.43
Totals		115.25	100.25	112.50	125.50	155.88	176.75	389.75	
Means		8.23	7.16	8.04	8.96	11.13	12.63		9.36

Drinking time at meals (minutes per day).

	Days						
	1	2	3	4	5	6	7
Totals	102.00	115.13	109.50	116.75	98.75	127.50	116.50
Means	8.50	9.59	9.12	9.73	8.23	10.63	9.71

APPENDIX X.

Drinking time between meals (minutes per day).

Samples	Weeks	Rats						Totals both Samples	Means
		1	2	3	4	5	6		
1	1	1.75	3.25	1.75	0.50	0	0		
	2								
	3	4.75	1.50	1.25	0	4.25	4.50		
	4	0.50	2.75	4.25	0	2.75	0		
	5	1.50	2.50	0.50	1.00	3.75	4.25		
	6	5.75	1.00	1.00	1.25	0	4.25		
	7	0	4.00	1.50	0.25	4.00	8.50		
	8	2.00	0	0.25	0	1.19	0		
2	1	5.25	7.00	2.00	0.75	0	0	22.25	1.85
	2								
	3	3.00	11.00	1.75	0.25	2.25	0	18.25	2.87
	4	3.25	2.00	1.00	0	4.25	0	20.75	1.73
	5	3.50	2.50	2.00	0.75	4.75	5.00	32.00	2.67
	6	2.75	1.50	0.75	0.75	0	0.50	18.50	1.54
	7	2.00	0.25	4.00	0	4.00	7.50	36.00	3.00
	8	3.75	0	2.00	0.50	2.75	1.50	13.94	1.16
Totals		38.75	39.25	24.00	6.00	33.94	36.00	177.94	
Means		2.77	2.80	1.73	0.43	2.42	2.57		2.12

Drinking time between meals (minutes per day).

	Days						
	1	2	3	4	5	6	7
Totals	24.25	27.19	29.00	17.75	35.75	27.50	16.50
Means	2.02	2.27	2.42	1.48	2.98	2.29	1.38

APPENDIX XI

The number of random visits to troughs at meal times.

Food troughs are underlined.

		Sample 1								Sample 2								
		Troughs								Troughs								
Weeks	days	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	Totals
1	1	12	7	1	0	3	4	2	6	5	12	7	6	6	1	2	6	80
	2	4	13	11	9	23	7	24	9	4	11	7	5	5	3	6	6	147
	3									2	2	3	5	7	2	6	5	32
	4	3	7	1	1	4	5	1	7	2	7	4	5	9	4	15	11	86
	5	2	6	7	8	5	3	0	0	0	7	2	1	1	2	3	4	51
	6	7	4	1	0	3	6	4	2									27
	7	5	2	0	2	2	2	2	4	5	2	0	2	2	2	2	4	38
3	1	3	7	9	7	7	5	6	10	0	4	3	3	3	2	6	4	79
	2	1	4	3	5	5	8	17	8	2	9	2	2	2	2	3	4	77
	3	1	3	1	0	1	1	4	5									16
	4	17	6	16	17	4	5	0	4	2	3	6	2	4	1	0	3	90
	5									8	5	8	4	2	0	2	11	40
	6	4	7	7	5	3	3	3	8	1	4	5	5	5	3	5	12	80
	7	3	4	0	0	0	1	5	6	9	9	6	4	1	0	0	4	52
4	1	6	35	26	37	5	14	7	3									133
	2	3	9	3	0	6	1	6	3	1	2	3	6	1	2	4	11	76
	3	6	8	5	12	2	3	7	10	4	16	7	21	3	15	2	4	125
	4	1	4	1	1	1	1	2	4	4	4	1	3	1	2	4	5	39
	5	7	4	6	0	2	2	7	6	4	9	5	8	2	2	4	6	74
	6	1	3	3	0	1	2	3	10	2	6	4	2	0	0	1	3	41
	7									2	6	2	0	2	2	2	6	22
5	1	4	8	7	9	4	5	10	6	4	8	7	9	4	5	10	6	106
	2	3	9	3	0	6	1	6	3	6	7	1	4	8	19	7	6	89
	3	2	3	1	4	6	1	1	6	3	2	6	2	5	2	12	11	65
	4	Food in wrong trough																
	5	1	3	3	2	3	7	2	11	1	3	0	1	3	2	4	3	48
	6									5	0	5	3	2	3	4	6	28
	7	9	7	6	3	2	3	5	4	0.	2	1	2	5	2	0	3	54
6	1	4	0	4	2	8	3	2	7	7	5	10	7	11	1	3	5	79
	2	4	3	6	4	7	1	2	2	4	3	0	2	6	3	3	7	57
	3	1	10	4	28	13	6	10	5	1	5	3	2	2	3	14	18	125
	4									0	2	1	4	7	2	1	3	20
	5	2	6	0	2	3	1	1	6									21
	6	8	7	4	0	1	0	0	3	3	10	1	18	14	4	6	3	82
	7	6	9	1	1	0	0	5	16	5	11	2	0	0	1	0	4	61

The number of random visits to troughs at meal times.

		Sample 1								Sample 2								
		Troughs								Troughs								
Weeks	days	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	Totals
7	1	0	1	7	17	4	2	2	2	6	11	8	12	10	8	15	4	109
	2																	
	3	1	0	0	0	0	0	1	3	1	0	0	0	0	0	1	3	10
	4	7	7	3	4	6	1	1	4	2	7	6	4	1	0	2	1	56
	5	1	0	1	0	2	2	6	9	1	0	1	0	2	2	6	9	42
	6	8	7	10	2	2	3	4	9	1	2	1	3	2	0	2	4	60
	7	2	4	0	0	1	0	2	1	2	5	2	1	0	1	7	5	33
8	1									4	6	1	0	4	2	5	7	29
	2									5	3	1	0	4	4	0	9	26
	3	3	5	0	0	4	2	0	10	5	7	1	0	7	1	1	9	55
	4	4	8	8	1	2	4	4	6	4	8	8	1	2	4	4	6	64
	5	1	3	0	1	0	0	1	3	2	6	7	1	21	8	7	11	72
	6	3	7	9	3	24	6	7	13	2	3	3	0	1	1	0	1	83
	7	0	1	2	0	1	0	0	7									11
		missing values																
																		2960

Days	Totals	Number	Means
1	625	12	52.08
2	472	11	42.91
3	430	12	35.83
4	365	11	33.18
5	348	12	29.00
6	401	12	33.42
7	248	12	20.67

Mean number of visits per rat per day 35.30

Mean number of visits per rat per trough per day 4.41

APPENDIX XII

The number of random visits to troughs between meals.

Food troughs are underlined.

Weeks	days	Sample 1 Troughs 1	2	3	4	5	6	7	8	Sample 2 Troughs 1	2	3	4	5	6	7	8	Totals
1	1	5	9	1	0	3	3	3	1	0	1	0	0	0	0	0	1	27
	2	0	0	0	0	0	0	0	0	5	5	3	2	1	0	1	1	18
	3									0	0	0	3	0	0	0	0	3
	4	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
	5	3	7	1	1	1	1	0	0	0	1	0	0	0	0	0	2	17
	6	2	1	0	0	0	1	0	0									4
	7	2	0	0	1	3	0	2	4	2	0	0	1	3	0	2	4	24
3	1	2	1	0	2	0	0	0	1	1	3	0	1	1	0	3	0	15
	2	0	1	3	0	0	0	2	1	1	1	0	0	0	0	1	0	10
	3	0	0	0	0	0	0	1	1									2
	4	5	1	1	0	0	0	1	0	1	1	0	2	0	0	1	4	17
	5									1	3	1	2	1	0	0	5	13
	6	1	1	0	0	0	0	0	0	0	3	2	0	0	3	2	3	15
	7	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
4	1	0	0	0	0	0	0	0	0									0
	2	2	1	0	0	1	0	0	0	0	2	0	2	0	1	0	0	9
	3	3	15	0	0	0	0	0	1	2	0	0	0	0	0	0	0	21
	4	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	3
	5	2	1	1	0	0	0	1	0	3	3	1	0	1	0	4	7	24
	6	0	1	0	0	1	2	1	1	0	0	0	0	0	0	0	0	6
	7									1	0	0	0	0	0	0	0	1
5	1	5	3	1	3	0	0	0	5	5	3	1	3	0	0	0	5	34
	2	2	1	0	0	0	0	0	1	0	1	0	0	0	0	1	2	8
	3	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	3
	4	food in wrong trough																
	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	6									1	2	1	0	0	1	1	4	10
	7	1	1	0	0	0	0	0	1	0	7	0	3	3	0	0	0	16
6	1	2	0	0	0	1	5	3	3	2	0	0	1	1	0	1	0	19
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	1	6	2	0	0	0	2	2	13
	4									0	1	0	3	4	0	0	0	8
	5	0	0	0	0	0	0	1	2									3
	6	0	1	0	0	1	0	0	0	4	1	0	0	0	0	0	0	7
	7	1	1	0	0	0	0	0	0	1	2	0	0	0	0	0	0	5

The number of random visits to troughs between meals.

Weeks	days	Sample 1 Troughs 1	2	3	4	5	6	7	8	Sample 2 Troughs 1	2	3	4	5	6	7	8	Totals
7	1	0	1	3	1	1	1	1	2	4	2	0	0	0	0	0	0	16
	2																	
	3	4	4	1	1	0	0	0	2	4	4	1	1	0	0	0	2	24
	4	2	2	2	3	4	0	0	1	0	0	0	1	0	0	0	0	15
	5	6	0	0	0	0	0	3	4	6	0	0	0	0	0	3	4	26
	6	1	2	0	0	0	0	0	0	2	6	0	3	0	2	0	1	17
	7	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
8	1									5	1	6	2	0	0	5	7	26
	2	missing values								2	2	0	0	0	0	0	1	5
	3	0	0	0	0	1	2	1	1	4	4	0	1	5	0	0	1	20
	4	1	0	0	0	0	0	2	2	1	0	0	0	0	0	2	2	10
	5	0	0	3	1	0	0	1	0	2	1	0	0	0	0	0	0	8
	6	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
	7	0	1	0	4	0	0	1	3									9
																		550

Days	Totals	Numbers	Means
1	137	12	11.42
2	50	11	4.55
3	86	12	7.17
4	66	11	6.00
5	93	12	7.75
6	61	12	5.08
7	57	12	4.75

Mean number of visits per rat per day 6.67

Mean number of vists per rat per trough per day 0.83