ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. Louis-Paul Dugal for his able direction of the experimental work and for many inspiring discussions which accompanied it.

Thanks are also due to Dr. André DesMarais who was consulted at various times and generously gave advice and assistance on various aspects of the project.

I wish to express special appreciation to my husband, Donald A. Smith, for his encouragement and able assistance throughout all the stages of this investigation.

Acknowledgement for technical assistance is made to Mrs. Irena Ben-Tchavtchavadze who was an extremely willing and capable assistant, to Mr. Robert Daoust who spent considerable time adjusting the frictional resistance of the activity cages and to Miss Lise Fournier who assisted in various ways.

Appreciation is also expressed to the Medical Research Council of Canada whose grant MT-446 to Dr. L. P. Dugal financed this research, and to CIBA Limited, Basle, Switzerland who supplied samples of testosterone propionate pellets.

PART I

TABLE OF CONTENTS

## PART II

## TABLE OF CONTENTS

## ABSTRACT PART I

The spontaneous running activity of male white rats was recorded in standard revolving activity cages under constant conditions of light and temperature. Activity was found to vary with age and was at its peak in rats approximately 85 to 100 days old. Activity was affected by exposure to cold at 2°C. but not always in the same direction. There was no correlation between spontaneous activity and food and water consumption or body weight gain under normal conditions. Nor could spontaneous activity be correlated with decreased liver function (except with extremely large doses of carbon tetrachloride), the level of secretion of thyroid hormone or the excretion of adrenaline. There was a tendency for active rats to excrete more noradrenaline than inactive rats at room temperature but activity did not vary with adrenaline or noradrenaline excretion at 2°C. Spontaneous activity was, however, markedly reduced by castration of male rats in early adulthood and could be returned to pre-castration levels by the implantation of testosterone propionate pellets which were slowly absorbed to give a continuous supply of the hormone. Testosterone pellets also inhibited the decline of activity with age in 'normal' rats.

## ABSTRACT PART II

The amounts of adrenaline and noradrenaline excreted in the urine of male white rats were estimated using a slight modification of the extraction and assay procedure of Drujan et al. (1959). There was some tendency for the catechol amine excretion of rats at 23°C. to increase slightly with age and weight. Exposure to 2°C., however, caused a marked and sustained increase in the excretion of the catechol amines as compared to the excretion in controls at 23°C. Adrenaline excretion increased approximately three to four times while noradrenaline excretion increased about eight times. Excretion of both catechol amines increased immediately on exposure to cold, high levels being reached and maintained from day four at 2°C. onwards. Excretion showed no decrease over 20 weeks of cold exposure; indeed, the values after 50 to 60 days in the cold tended to increase further. There were no differences between Wistar and Sprague-Dawley rats in the amounts of adrenaline and noradrenaline excreted at 23°C. or 2°C. 'Old' rats kept in activity cages excreted much more adrenaline and noradrenaline both at 23°C. and 2°C. than other rats and they showed a peak for adrenaline excretion after one week and for noradrenaline excretion after three to four weeks in the cold. On the average 2.8% of injected adrenaline and 2.5% of injected noradrenaline were excreted unchanged in the urine at 23°C. At 2°C. these values increased to 4.2% for adrenaline and 4.4% for noradrenaline.

# I. EFFECTS OF SEVERAL FACTORS ON THE SPONTANEOUS RUNNING ACTIVITY OF WHITE RATS

## INTRODUCTION

Spontaneous activity is that activity indulged in by an animal on a completely voluntary basis, that is, one that results from a stimulus arising within the animal itself. The influences of numerous internal and external factors on the spontaneous activity of a variety of domesticated and wild small mammals have been studied by many investigators during the past 60 or 70 years and much of the literature has been reviewed by Shirley (1929) and Reed (1947). Although much is now known about the influence of some of the factors, however, there are still many conflicting opinions about how the amount of spontaneous activity is regulated. The present investigation was carried out to attempt to elucidate or resolve some of the points at issue and to establish unequivocally the relative importance of the rôles of several factors in influencing the spontaneous activity of the albino Norway rat, the mammal most extensively used in previous studies of this subject.

In previous studies, three general types of apparatus have been used for the direct recording of spontaneous activity. The revolving cage first used by Stewart (1898) has been widely used with various modifications. Unlike some of the other types of apparatus, the revolving cage does not measure total activity, but only the amount of running in a revolving drum. Thus any activity which takes place in the food- or rest-cage is unrecorded. Stabilimeters or bouncing cages with devices sensitive to all types of body movements which take place in the cage determine the times of activity.

Methods of recording movements between compartments (e.g. the time of each passage of an animal between its nest and other parts of the apparatus) have also been assembled and often combined with the other types of cages. Caution is needed in attempts to compare the results of activity experiments in which different sorts of recording apparatus are used.

The revolving activity cage has perhaps been the most extensively used laboratory instrument for investigating activity. Although it only measures running activity, observations by Hoskins (1927a) have shown that so large a part of the total energy expenditure goes to running in the wheels that a record of the revolutions serves as a rather accurate criterion of the animal's tendency to exercise. The question "Why do some rats run so much and others so little in this cage?" has never been adequately answered, although wide individual differences in activity even among littermates were noted by early workers in this field. Rundquist (1933) was able to obtain both active and inactive strains of rats by selective breeding. Only the inactive rats bred true but unfortunately the genetic factors were obscured by environmental influences. With the same rats Brody (1942) concluded that for activity the difference between strains resulted from a single gene pair, a conclusion which was unwarranted from the data presented.

The environmental factors which exert the greatest influence on activity are food, water, sound, illumination, temperature and vapour pressure. The running activity of white rats in revolving cages increases markedly when their food is removed (Reed,1947). Similar effects have been shown to result from the deprivation or restriction of water supply. High frequency sounds tend to increase activity (Richter,1952). The white rat is largely active at night during normal cycles of light and darkness. Once a diel rhythm is set up through normal light-darkness cycles, it tends to persist

despite continuous light or dark. Both the pattern of light and darkness and the amount of illumination can, however, alter the pattern and amount of activity. The effects of both temperature and vapour pressure are probably related to body temperature regulation. Both high and low temperatures have been reported to reduce spontaneous running activity in small mammals; that is, there is a range of ambient temperatures where activity is maximal. In studies of rats in revolving cages, Shirley (1928a) found that the amounts of running were the same at 20°C. and 26°C., Browman (1943) that the amount decreased above 27°C. and was greater at 16° than at 27°C., Fregly (1956) that it increased as the temperature fell from 30° to 10°C. and Fregly (1961) that there was an increased running response to cold observed in normal rats kept for four days at 8°C. In cold temperatures activity increases heat loss and metabolic rate and hence the restriction of activity of small mammals at low temperatures will result in the conservation of heat. An inescapable conclusion is that extensive control of environmental conditions is absolutely necessary for work on the relation of activity to internal changes. Even when as many factors as possible are held constant, however, there are still marked individual variations in the amount of activity of different rats.

## Internal Factors Affecting Spontaneous Activity

### Age and Sex

The running activity of the male rat is generally less on the average than that of the female (Hitchcock,1926; Hoskins,1927a; Wang et al.,1925; Lee and Van Buskirk,1928; Tainter,1943). The activity of the male rat, however, lacks the cyclic characteristics caused by the oestrous cycle of the female, hence males have been chosen for experimentation on spontaneous

activity by most workers. Spontaneous running in the revolving cages appears for any rat to depend on its age as well as its experience in the wheel. Most investigators agree that the preliminary period for the rat to adapt to the cage should be 10 to 20 days (e.g. Shirley,1928b,1929; Slonaker,1924; Heller,1932; Richter,1952; Jones et al.,1953; Eayrs,1954) at which time the activity should have increased to a fairly constant level. Richter and Uhlenhuth (1954) state,however, that a constant level of activity is reached only after 35-60 days for domesticated rats and 20-50 days for wild rats. Tainter (1943) kept rats in the cages for several weeks before they had reached a level which did not change progressively and he replaced the occasional rat which did not run satisfactorily during this preliminary period. Jones et al.(1953) and Fregly (1961) found that within three weeks after the rats were placed in the activity cages running activity reached a maximum and then subsequently slowly declined.

It is rather difficult to assess the earlier studies of the effect of age on spontaneous activity either because observations were on single animals or because the studies were not appropriately designed for that purpose. There is general agreement, however, that the activity of rats increases to a relatively constant level and then declines until death. Slonaker (1912) measured the activity of four male rats from birth to death and concluded that the rats were most active at ten months although he had earlier reported (1907) that four other rats were most active between 87 and 120 days of age. Richter (1933b) reports 100 to 120 days of age as the time of greatest activity. Shirley (1928b) concluded from her studies that male rats are most active at nine months of age although activity was essentially constant at a high level from the fifth to the ninth month. At any age level, however, she found the same wide range of activity, that

is, the least active rat ran 500 and the most active 10,000 revolutions per day. Unfortunately her rats were not left in the cages throughout the experiment and what bearing this had on her results is hard to judge. That activity decreases with increasing senility has been noted by many workers (e.g. Slonaker,1912; Hoskins,1927a; Shirley,1928b; Lee and Van Buskirk,1928) although Shirley found many individual exceptions. Jones et al.(1953) measured the activity of groups of 15 to 25 Sprague-Dawley rats of different ages for seven weeks and showed that activity was greatest in the youngest (51 days) rats and progressively decreased as the age increased (72, 100, 142 and 212 days old). This could perhaps explain the observation by Wang et al.(1925) that the higher running levels are reached by animals that learn to run when they are very young.

Food and Water Consumption

Although it is well known that starvation and lack of water will greatly increase activity, there have been but few studies on the relationship of low and high spontaneous running levels with food and water intake and weight gain. Hitchcock (1927) measured the daily activity and food consumption of 62 rats for five days and found the variations were too great to draw a curve from individual determinations. He concluded that there must be other factors than activity involved in determining total food consumption. Shirley (1928a) found no correlation between activity and weight attained after running in the cages. Rundquist (1933) found no consistent weight differences between his active and inactive strains of rats. Using 12 female Sprague-Dawley rats four to ten months of age and averaging daily activity and weight changes over 5-day periods, Brobeck (1945) showed that if food intake and environmental temperature are held constant, there is a negative correlation between activity and weight gain.

This result is most likely a reflection of the known fact that starved rats will increase activity and simultaneously their body weights will fall. Tribe (1954) submitted three groups of eight hooded Wistar rats to three different levels of 'forced' exercise and found that at no time did the voluntary caloric intake vary with the amount of exercise undergone. The rate of body weight increase was slowed down, however, as the amount of exercise increased.

Liver

The effect of removal of 75% of the liver of five male rats was compared by Dugal and Ross (1943) with that of five sham-operated rats. Immediately after the operation, all activity disappeared in both partially hepatectomized and control rats but the activity was normal after three days in the controls and only after seven days in the hepatectomized rats. Seven days is the time required for regeneration of the remaining liver to take over the normal function of the entire organ.

Pituitary Gland

The search for a neural centre which controls activity has led to contradictory evidence. In various kinds of endocrine imbalance or deficiency, decreases in activity have often been reported. Various kinds of replacement therapy have also been reported to increase activity. Tainter (1943) used control injections of dilute hydrochloric acid and determined that activity was practically unaffected by the pain of injections. He also stated that the stimulation of handling changed the activity of animals very little.

Removal of the pituitary gland is generally reported to cause a marked decrease in running activity. Richter and Wislocki (1930) reported that

10 of 25 operated rats became almost totally inactive. Injections of 1 cc. alkaline extract of anterior lobe of sheep for 30-33 days produced a pronounced increase in body weight and food intake but had no effect on activity. Richter and Eckert (1937) reported that daily injections of emulsions of fresh anterior lobe increased the activity, which had been markedly reduced by hypophysectomy, to the original level in only one of seven female rats. Viable hypophyseal transplants to the anterior chamber of the eye had a small stimulating effect in four of thirteen animals. It is also reported by Richter (1954) that hypophysectomy has a much less depressive effect on some wild rats. The hypophysis is much smaller in wild rats than in domesticated rats. Stone and Obias (1956) found that thirteen young rats hypophysectomized at 35 days of age showed more activity than controls when placed in activity cages every third day; however, the activity of the controls was unusually low. In addition they suppose that the smaller hypophysectomized rats could run more easily in the cages. Müller and Giersberg (1957) also report that removal of the pituitary causes activity to decrease but does not affect the day-night rhythms.

Thyroid Gland

The early studies on spontaneous running activity and thyroid function yielded conflicting results. Hoskins (1927a) and Lee and Van Buskirk (1928) concluded that there was no relation between metabolic rate and amount of activity as activity could be markedly changed with no change in metabolism. On the other hand, Hall and Lindsay (1938) and Rundquist and Bellis (1933) noted a tendency for rats with higher metabolic rates to show greater spontaneous activity, although female rats which normally are more active than males did not have a higher metabolic rate. Surgical thyroidectomy was reported to cause no change in spontaneous activity by Hoskins (1927a),

and Lee and Van Buskirk (1928) and to cause a decrease in activity by Richter (1933a) and Hall and Lindsay (1938). Mann (1945) used thiouracil to block synthesis of thyroid hormone and also reported a decrease in activity. The recent experiments of Fregly (1961) showed that the running activity of male rats at 25°C. was not reduced by hypothyroidism produced by propylthiouracil administration.

Appraisal of the actual data from which these conclusions were drawn has shown the following. In a series of 20 thyroidectomized and 20 control rats Lee (in Hoskins,1927a) found that the average daily activity was almost the same in both groups. Thyroidectomy of 25 rats had no effect on spontaneous activity in the experiments of Hoskins (1927a) who at autopsy found that in nearly all cases the tissue of the thyroid gland had been completely removed. The unpublished data of Wang and Richter (in Richter,1933a) are similar. Lee and Van Buskirk (1928) studied 18 adult thyroidectomized and nine control rats 93-160 days old and found that the average daily activity was almost the same in each group over a three month period and that at autopsy there was no evidence of regeneration of thyroid tissue. Richter's results (1933a) are based on 12 rats thyroidectomized at 60 to 90 days of age. He had no controls and while some of the thyroidectomized rats showed a definite but moderate decrease in activity, others (four out of 12) showed no effect when their activity 10 days before operation was compared with that of 50 days post-operatively. As very small portions of thyroid tissue were found at autopsy in those rats in which no change was noted, he concluded that even a small portion of thyroid was sufficient to sustain activity. He also stated that thyroidectomy caused a small decrease or an arrest of growth. Hall and Lindsay (1938) report a 50% decrease in activity of 35 rats at least 100 days old when comparing the average daily revolutions two weeks before operation with those of two weeks post-operatively. The fall in activity was completed within the first three days after the operation. As they had observed previously no demonstrable effect of a control operation on activity, their 11 controls were not sham-operated and their activity did not decrease. Mann (1945) also obtained a 50% decrease in spontaneous activity of 18 female rats when comparing the average activity two weeks before treatment with 0.1% thiouracil in the diet with the last two weeks of the 30-day treatment. Fregly (1961) administered 0.1% PTU (by weight) mixed with the diet for four weeks to two sets of five male rats (220-250 grams and 350-370 grams) at 25°C. and found no change in spontaneous activity when compared with controls. The treated rats were anaemic and had enlarged thyroid glands thus the drug was effective.

Feeding desiccated thyroid gland to rats is generally reported to cause a decrease in spontaneous activity (Hoskins,1927b; Lee and Van Buskirk, 1928; Hall and Lindsay,1938). On the other hand Richter (1933a) reported that small amounts of thyroid extract caused a return of activity to normal in thyroidectomized rats although large doses caused activity to decrease. Hall and Lindsay (1938) reported that restoration of the metabolic rate of thyroidectomized rats to normal by dinitrophenol (which increases the rate of oxidation of cells by direct action upon them) caused activity to decrease further.

Iodine (and hence $I^{131}$) is taken up by the thyroid gland where it is converted into the thyroid hormone and subsequently released into the circulation. Thus the activity of the gland is assumed to be high if the $I^{131}$ is taken up quickly in large amounts and is released at a rapid rate. Although these measurements of uptake and release by the thyroid gland are widely established as good evidence of thyroid activity, however, they are in the opinion of Livingstone (1962) subject to many misinterpretations. The thyroid gland is not uniform and there is a more rapid turnover of $I^{131}$ in the smaller follicles. From his experiments Livingstone had concluded that there is as yet no good method of measuring the absolute thyroid secretion rate.

The literature on the effects of cold exposure on thyroid function has been well summarized in the monograph by Pitt-Rivers and Tata (1959), in the reviews by Hart (1958), D'Angelo (1960) and Cottle (1960) and in the thesis of Livingstone (1962). Several workers have shown that moderate exposure of rats to cold caused a stimulation of $I^{131}$ uptake by the thyroid and an increased rate of $I^{131}$ release from the gland as well as histological evidence of increased thyroid activity. It has also been shown that short-term exposure to cold is more effective in stimulating the thyroid than prolonged exposure. It has been suggested that moderate exposure of animals to cold causes an increased secretion of the thyrotropic hormone which results in thyroid stimulation but that prolonged exposure to cold may result in a stressful situation which depresses thyroid function. In any case there is a fair amount of evidence that the thyroid gland reaches a maximum of activity early during cold exposure and gradually returns to normal or near normal conditions.

After prolonged exposure to cold in a number of species there is reported to be an increase in the weight of the thyroid gland, a decrease of

colloid within follicles and an increased vascularity (Sellers,1957; Cottle, 1960). Livingstone (1962) did not find any significant increase in thyroid weights of male rats at 2°C. Cottle and Carlson (1956) found that the gland weight was almost normal after 60 days at 5°C. although it had increased on preliminary exposure up to 26 days.

The rate of hormone secretion has been evaluated by following the biological decay of radioactivity over the thyroid gland after the administration of $I^{131}$. Cold-acclimated rats at 5°C. tested in this manner with the intraperitoneal injection of 1-2 μc. by Cottle and Carlson (1956) showed a more rapid release of hormone up to 60 days as compared to warm-acclimated controls at 26°C. The graph presented by Cottle (1960), however, showed no difference between rats at 8°C. for 15 days and controls at 28°C. D'Angelo (1960) found that the increased release of $I^{131}$ from the gland in rats and guinea pigs was maintained after 6-8 weeks exposure to 7°C. Woods and Carlson (1956) also reported that thyroxine secretion remained at a high level for 60 days at 5°C. Livingstone (1962) found that thyroid weights and the release of $I^{131}$ by the thyroid in male rats at 2°C. was not affected by the cold although other methods of measuring the thyroid secretion rate showed a general increase in thyroid activity in all animals after one week, following which the activity returned to normal levels. This contrasts with the statement of Cottle (1960) that there is some evidence that thyroid activity in rats reaches a maximum with about three weeks exposure to cold and returns to near normal conditions after 6 to 10 weeks.

Radioactive iodine is lost more rapidly in the urine of cold-exposed rats than in normals (Cottle,1960). Thus Cottle and Carlson (1956) found less $I^{131}$ in glands of cold-exposed animals than in controls as measured by the 24 and 48-hour uptake counts. On the other hand, Catz et al.(1953) found an increased thyroidal $I^{131}$ uptake at 1, 2 and 4 hours after injection but at 24 hours cold-exposed rats and controls were the same.

Although partly due to muscular activity, the increased metabolic rate produced by exposure to temperatures near the freezing point is caused by an increased heat production by means other than shivering, especially in the cold-acclimated rat. The hormones of the thyroid, adrenal cortex and the sympathetic nervous system are considered important in producing this non-shivering thermogenesis. Hart (1958) reported, however, that thyroidectomized-curarized rats can respond to cold, and thyroidectomized rats can be maintained in the cold with an elevated metabolic rate if they are given minimal amounts of thyroxine. When acclimatized animals were thyroidectomized surgically or by feeding propylthiouracil, most rats survived in the cold for several weeks although they ultimately lost weight and died (Sellers,1957). Since rats kept at 5°C. reduced their food intake and lost weight following thyroidectomy but maintained a high metabolic rate, Hsieh and Carlson (1957) concluded that the metabolic response to cold is not directly dependent upon the amount of circulating thyroxine.

Adrenal Gland

Durrant (1924) found a marked degree of adynamia in 21 rats 40 to 60 days old studied for three days to six weeks after ablation of both adrenal glands as compared to 16 severely traumatized controls, although on super-

ficial observation the rats appeared to be normally alert and active. Bacq (1931) found that abdominal sympathectomy in 10 rats and adrenal inactivation in 13 rats did not affect their spontaneous activity more than in controls. Further, sympathetic denervation of the four limbs did not reduce the muscular activity of the rat to the extent of decreasing its voluntary activity.

Hoskins (1927a) reported that entire removal of the adrenals leads to a marked reduction in running. Richter (1936) also reported that in 10 of 26 adrenalectomized rats which survived there was an immediate and maintained (48 days) decrease of approximately 90% in activity in all the animals. He also reported higher levels of running in 12 of 27 adrenalectomized rats which had implants of adrenal glands in the ovaries. Histologically there was viable cortical tissue but no medulla. An increase of salt in the diet, however, produced a definite but partial increase in activity as did injections or feeding of cortical extract.

Injections of adrenaline are reported to be ineffective in increasing the spontaneous running activity of rats (Richter,1936; Tainter,1943) even when given in doses approaching the maximal tolerated level. On the other hand Lacey (1945) reported a decrease in the average daily diffuse activity of rats after injections of adrenaline chloride.

Ingle and Harris (1936) reported that when adrenals were transplanted to the ovaries in 17 rats, there was, after temporary depression of activity, a return to the pre-operation level and 28 days after the operation the activity was equal to that of the 17 controls. Histological examination showed that only the cortical tissue of the gland survived. Richter (1952) reported that cortisone in the diet (5 mgm.per day) would maintain the activity of gonadectomized domesticated rats. Richter (1950) and Richter

and Uhlenhuth (1954) found that wild rats did not survive adrenalectomy on salt in any amounts or form and not consistently even on treatment with cortisone or DOCA.

According to Hart (1958) when a rat is first exposed to cold, there is an increase in size of the adrenal cortex which regresses to a size just a little greater than normal as the animal adapts. D'Angelo (1960) reported that the hypertrophy of the adrenal gland in rats kept for seven weeks at 7°C. signifies that adrenal function is enhanced. Sellers (1957) also reported an increased size of the adrenals after prolonged exposure. If acclimatized rats are adrenalectomized and re-exposed to cold, they can survive for a number of days (average 12) provided saline is allowed in lieu of water.

A review of the literature on catechol amines and the changes in their excretion on exposure to cold is given in Part II of this thesis. It is assumed that the excretion of these catechol amines in the urine is proportional to the amount produced in the body.

Gonads

Gonadectomy (or castration) in the white rat is reported by many to cause a sharply marked and easily recorded depression in spontaneous activity. Wang (1923) and Wang et al.(1925) reported that the activity of two castrated female rats was much lower than that of two controls while a third female rat exhibited a marked drop in activity after castration and this drop was maintained. Slonaker (1924,1930) stated that ovariectomy not only reduced activity to a much lower level but it also largely abolished its periodicity. Hoskins (1925a) compared the average activity of 16 male rats castrated at about 75 days of age with that of 16 controls. His graph of the following 100 days shows that the activity of the castrated rats remained low while

the activity of the controls increased to about five times that of the castrates after 45 days (average age of rats then 120 days) and then gradually fell approaching that of the castrates. Durrant (1925) compared the activity of eight female castrated rats with that of eight controls and found that although castration decreased the activity, four of the eight rats regained their activity to some extent after the operation, but within 20 days activity was again at a low level. Hoskins (1927a) stated that Wang's observation that extirpation of ovaries markedly reduced the spontaneous activity of the rat was readily confirmed. He quoted the work of Lee which showed that a decrease of 60% or more in activity is found in castrated rats, a decrease which is not correlated with any decrease in metabolic rate. In a later experiment on thyroid administration in senility, however, Hoskins (1927b) used four rats which had previously been castrated because activity was the same as in non-castrates. Wang et al.(1925) reported that castrated male rats maintained a constant low level of activity as compared to their pre-castration level. Castration after the animals had reached a high running level caused a decrease in activity but this was not as great as that observed when castration was performed at an early age or before the animal learned to run a great deal. The body weights of male rats which had been very active showed an increase after castration while males that were almost completely inactive did not show a change from normal.

Fractional castration of male rats by Gans (1927b) indicated that spontaneous activity was lower if more of the testes was removed, the most marked effect being after removal of the last half of the second testis. He further stated that if castration took place after puberty, the castrated animal only began to lag behind the control about the 12th day after castration. Further, the effect of castration was much more marked in rats

with a normally high level of activity but that it usually could be demonstrated even when the activity of early adulthood was low and towards old age when the activity was lessened but still of a fair degree. Gans (1927a) also reported that when 25 rats were castrated within the first two weeks of life, their spontaneous activity was normal as compared to 18 litter controls up to 100 days of age. This result was questioned by Richter (1933) whose 15 rats castrated one to ten days after birth had low activity and he ascribed Gans' results to the relative inactivity of his controls.

Castration of 14 male rats by Heller (1932) was followed by a depression of activity in 12 but a rise in two when the 40 days before and after the operation were compared. Hunt and Schlosberg (1939) reported that five castrated male rats were slightly less active than normals when tested on a spring-mounted cage.

Gans (1927b) noted that castration affects the voluntary activity of rats to varying degrees in different strains and even in different members of a given litter. Thus the effect was quite marked in the case of the Wistar rat, while the Swift animal, a Wistar-Norway hybrid was much less affected. In the latter the fall in activity was rather slow and never attained as low a level as in the castrated Wistar animal. Richter (1952) and Richter and Uhlenhuth (1954) found that castration of wild Norway rats had no effect on their activity; they remained quite as active as before, although castration greatly reduced the running activity of domesticated Norway rats. Castration caused atrophy of the secondary sex organs in both wild and domesticated rats. The adrenals which are normally heavy in wild rats, after castration became even heavier in wild males, less so in wild females; they showed a small increase in domesticated males and a decrease in domesticated female rats. The activity of domesticated

castrated rats (with smaller adrenals and larger gonads than in wild rats) could be maintained on a cortisone diet of 5 mgm. per day in the food. Thus they suggest that running activity of wild rats is maintained largely by adrenal secretion whereas the activity of domesticated rats is largely by gonadal secretion.

Durrant (1925) was unsuccessful in increasing the activity of ovariectomized rats with the daily feeding for three weeks of glycerine extracts of whole pig ovaries. Hoskins (1927a) was also unsuccessful in restoring the activity of castrated female rats by the administration of ovarian products or of increasing the activity of castrated males by feeding desiccated testis extracts or by subcutaneous injections of macerated testis extracts. Furthermore, his experiments with testicular grafts (Hoskins, 1925b) were also negative as in practically all the cases entire resorption of the grafts occurred. The intramuscular or intraperitoneal injections of single doses of 10 to 25 mgm. testosterone propionate into nine male rats by Hoskins et al.(1939) also had negative results. On the other hand Wang (according to Hoskins,1927a) was successful in increasing the activity of castrated female rats with ovarian grafts and Bugbee and Simond (1926) obtained an increase in activity and a restoration of oestrous activity rhythm by the injection of ovarian hormone. Tripling the dosage did not increase the activity level further. Hoskins and Bevin (1940) also observed an augmentation of activity in 11 senile male rats after the injection five times weekly for three to five weeks of 2 cc. estriol glucuronide.

The transplantation of ovaries into the recti muscles of 24 castrated male rats by Wang et al.(1925) produced a marked increase in activity in eight rats, a slight increase in nine and a decrease in seven when the 20 days before and after transplantation were compared. The animals which

ran the most following the transplantation of ovaries also exhibited the greatest flattening of the body weight curve. Removal of the grafts in the seven most active rats caused a large decrease in activity and an increase in body weight. Richter and Wislocki (1928) concluded that the activity of castrated rats could be increased by testicular grafts. They implanted testes into 17♂ and 19♀ castrated rats and found increases in activity in seven (5♂ and 2♀) of the 36 rats in which the grafts appeared to be well preserved. Richter (1934) reported that pregnancy urine of women seven to nine months pregnant given in the drinking water in 1:2 to 1:100 dilution kept up normal activity in castrated (60 to 80 day old) male and female rats. The hormone maintained the female reproductive tract in a normal condition but did not stop the atrophy of the secondary sex organs of the castrated males. Richter and Hartman (1934) further reported that oestrin (5 rat units daily) injected into seven castrated females maintained normal reproductive tracts and normal levels of spontaneous activity. With this same hormone eight castrated male rats showed an increase in activity although this was less marked and took place despite the involution of the male secondary sex organs. Extracts of this hormone injected into five normal rats (3♀ 2♂) caused an immediate drop in activity in all animals for 15 days after which they became as active as before but not more so.

Careful experiments by Heller (1932) showed that low-running male rats were actively secreting testis hormone and hence spontaneous activity was by no means proportional to testis hormone secretion. Normal male rats with "established" activity levels were injected with heavy doses of testis hormone or with gonadatropin for 10 to 25 days. The results were not constant; some animals showed a drop in activity, others a rise. Injections of testis hormone into four castrates did not produce a definite change in

activity although it restored accessary glands to a normal state.

Young and Fish (1945) were able to restore the activity of female rats to levels prevailing before castration by the administration of estrone in pellet form implanted subcutaneously so that a constant source of the hormone was maintained. They found that only a certain small amount of estrone was necessary for running activity and that further estrone did not produce a further increase in activity. It is interesting to note that the amount of activity was generally proportional to the amount shown prior to gonadectomy rather than to the amount of hormone given. Hoskins and Bevin (1940) noted this relationship in their study of the effect of estriol glucuronide on the running activity of senile male rats. Progesterone did not appear to be involved in the maintenance of running activity. Spontaneous activity was maintained at a high level in castrated female rats only when a more or less constant source of estrone was maintained. The great variation in the amount of daily running activity in animals having a constant source of estrogenic stimulation could not be accounted for.

* * * *

This literature survey clearly indicates that a host of investigators have contributed a plethora of isolated items of information about the many factors which affect the spontaneous running activity of rats. It is understandably rather difficult to generalize on previous results for there is much conflicting evidence on the absolute effect of any one factor and on the relative effects of various factors on spontaneous activity. Most of the data are difficult to evaluate and compare because different conditions, methods, apparatus and animals have been used. Obviously much more work needs to be done to elucidate the complex problem of the internal

control of spontaneous running activity in rats, and it would seem most profitable for such studies to be carried out using standardized techniques, conditions and animals. Accordingly, the present investigation was carried out using the same type of animals, standard apparatus, and standard experimental techniques and conditions, to study the effects of several internal factors on the spontaneous running activity of rats.

## MATERIALS

White rats of the Wistar strain obtained from Robidoux Animal Laboratory, St. Constant (La Prairie), Quebec, were used in the present investigation. Only males were used as the amount of spontaneous running activity of female rats shows marked cycles associated with the oestrous cycle. At the beginning of an experiment, the rats were usually about six weeks old and weighed about 110 grams. They were maintained on standard Purina laboratory chow and tap water. Disturbance of the rats was kept to a minimum. Food and water supplies were replenished when necessary, the rats were weighed twice a week and the cages were cleaned weekly.

In order to study the effects of a number of factors upon the spontaneous activity, it is desirable to have a uniform apparatus, measuring the amount of a known type of activity. The apparatus used in the present investigation was the standard revolving activity cage which measures a single type of activity i.e. running activity. The complete revolving activity cage unit (animal cage LC-34 manufactured by Geo. H. Wahmann Mfg. Co., Baltimore) consists of a drum, 14 inches in diameter, that revolves on ball bearings so that friction is reduced to a minimum, a rest cage, a base with a removable tray and a standard mechanical Veeder counter. The drum with a running surface of 4.5 inches in width is made to rotate by the running of a single animal within it. The water bottle holders on the rest cages were changed so that water bottles containing a larger volume (240 cc.) could be used.

To ensure that the frictional torque of each cage was similar and thus to avoid significant errors in measurements of activity between cages, our cages were adjusted and calibrated by the method of Lacey (1944). It was

necessary to check the cages daily throughout the experiments to remedy occasional mechanical breakdowns of several types. Some rats carried food into the drum where it interfered with running and not infrequently became jammed between the support and the drum, thus preventing its rotation. From time to time the revolutions of a drum were not recorded on the counter. Part of each daily inspection involved checking to ensure that water flowed readily from each water bottle. Occasionally rats were unable to obtain water for up to 24 or 48 hours and hence lost weight.

In order to study the effects of certain factors on activity, it was necessary to maintain relatively constant conditions of temperature, light, relative humidity and sound level. All experiments were carried out in one or more of three rooms in which these factors were kept relatively constant. The temperature of one room was maintained at either 29±2°C. or at 23±2°C. The relative humidity measured by a Taylor wet and dry bulb hygrometer (fanned with an electric hair dryer without the heater) was 13-26%. Although a second room was not directly thermostatically controlled, its temperature remained at 25±3°C. and its relative humidity was 9-19%. A cold room maintained at 2±3°C. (mostly 2±2°C.) by a thermostatically controlled refrigeration unit was also used. Its relative humidity, checked at various intervals, was found to be generally 48-52%. The temperature range of each room was read at least weekly on a maximum-minimum thermometer. As well, there were continuous thermograph recordings for the thermostatically controlled rooms.

All rooms were illuminated only by artificial lights which were turned on for 12 hours daily by Paragon time switches. Activity cages were placed side by side on the floor and on a table at the appropriate distances from the light source so that the rest cage and revolving drum of each received approximately five foot candles of illumination.

## METHODS

As some rats in activity cages exhibited marked activity while others were quite inactive, rats of high, medium and low average activity levels were placed in each experimental group so that the averages would be more or less comparable. Furthermore, rats in any experiment were usually divided equally between the room maintained at either 29 ± 2°C. or 23 ± 2°C. and the room at 25 ± 3°C. No noticeable effect on the total daily activity was seen when rats were removed from the cages for 2 hours once a week during cage-cleaning or when the rats were etherized for a short time for measurement of radioactivity in the thyroid experiments. In most experiments, therefore, the total spontaneous running activity in revolutions per week was recorded.

### Food and Water Consumption and Spontaneous Activity

A weighed amount (approximately 50 grams) of pulverized Purina laboratory chow was placed in a porcelain dish fastened to the end of the rest cage to prevent tipping. A metal pan (about five by six inches) beneath the rest cage and immediately under the feeding dish caught any spilled food. The difference between the original weight and the sum of final weight plus the weight of scattered food represented the daily food consumption, and this figure was judged to be accurate to the nearest gram. The weekly water consumption from the 240 cc. bottles (usually filled twice weekly) was measured.

Twenty rats were placed in activity cages at 29±2°C. or 25±3°C. and weekly records of spontaneous activity and body weight were begun. Measurements of food and water consumption were commenced eleven days later and were continued for five weeks.

## Liver Function and Spontaneous Activity

### Carbon Tetrachloride Poisoning

In order to produce extensive liver damage with carbon tetrachloride, it was first necessary to determine what constituted a sufficient but sub-lethal dose. Drill (1952) stated that subcutaneous injection in the rat of 0.016-0.03 cc./kgm. body weight produces minimal lesions while doses of 0.3-1.0 cc./kgm. produce more marked changes which are usually not fatal. Toxic cirrhosis has been produced in rats by the repeated subcutaneous administration of 0.10-0.25 cc. pure carbon tetrachloride once or twice weekly (Radhakrishna-Rao,1936; Cameron and Karunaratne,1936; Lacquet,1932; Reddy et al.,1962) although the higher dosage was lethal to some rats. To produce cirrhosis, Cameron and Karunaratne (1936) have emphasized the necessity for repetition of doses at short intervals over prolonged periods to avoid repair of damage produced by the preceding dose. Indeed, the liver regenerates rapidly and generally appears normal seven days after the last injection.

As a preliminary experiment rats not in activity cages were injected with carbon tetrachloride. Two were injected with 0.15 cc. pure carbon tetrachloride (approximately 0.7 cc./kgm.) twice a week for three weeks (7 injections). The rats continued to grow on this dosage and histological sections of liver stained with Ehrlich's acid haemotoxylin and eosin stain were unexpectedly normal. The dosage was therefore increased to 0.25 cc. and given to three rats every other day. Two of the three rats lost weight on this dose and the third slowly gained. One rat was killed after three weeks, one after four weeks and the third after three weeks on the same dose plus one week on a daily dose of 0.25 cc. The livers of all three rats appeared grossly abnormal. They were extremely pale and greasy with

fatty infiltration and areas of marked necrosis. It was therefore concluded that injections of 0.25 cc. carbon tetrachloride every other day were producing the required degree of liver damage.

Twenty rats were placed in activity cages at 25±3°C. or 23±2°C. Their spontaneous activity was recorded weekly for three weeks and then daily until the end of the experiments. Four weeks after the daily recordings were begun, 14 rats were injected subcutaneously with 0.25 - 0.30 cc. pure carbon tetrachloride every other day for three weeks and daily for two weeks (total five weeks). Some ulceration at the injection sites was observed as reported by Radhakrishna-Rao (1936). The six controls were injected with equivalent amounts of 0.9% sodium chloride on the same schedule. Amounts of spontaneous activity were recorded daily for four weeks after the injections were withdrawn.

Partial Hepatectomy

Reduced liver function was also produced by means of partial hepatectomy using a modification of the method of Selye and Waelsch (1931) as advocated by Dugal and Ross (1943). The method involves removal of approximately 70-75% of the liver (e.g. 9.2 grams were removed from a rat with a total liver weight of 12.8 grams). The cartilaginous xiphoid process is cut off, then the medial bifurcate and right paramedial liver lobes are ligated at the common pedicle and removed. The controls are subjected to a sham operation in which the xiphoid process is cut off but the liver lobes are merely lifted out of the abdominal cavity, handled in the same manner as in the partially hepatectomized rats, then replaced.

Four weeks after the termination of the carbon tetrachloride injections, seven of the 14 rats which had received carbon tetrachloride and three of the six saline controls were partially hepatectomized, and the remaining

ten rats were sham operated. One sham operated and one partially hepatectomized rat tore open its abdominal incision and died shortly after the operation. As the livers of all the rats appeared normal at operation and as there were no observed differences between the rats which had previously received carbon tetrachloride and those which had received saline, it was assumed that all rats had recovered fully from the carbon tetrachloride poisoning. Three weeks after the operations the rats were killed. Daily recordings of the spontaneous activity were made throughout the experimental period.

## Thyroid Function and Spontaneous Activity

### Experiments with Radioactive Iodine

Circulating iodide is actively taken up by the thyroid gland where it is incorporated into the hormone thyroxine which is either stored as thyroglobulin or is released into the circulation. It is assumed that after the administration of radioactive $I^{131}$, the decrease in radioactivity over the thyroid gland reflects secretion of $I^{131}$-labelled thyroxine. Each rat was injected intraperitoneally with a tracer dose of five microcuries ($\mu$c.) of carrier-free $I^{131}$ (obtained as $NaI^{131}$ in a solution of $NaHSO_3$ and diluted with physiological saline to obtain 5 $\mu$c. in 0.1 cc.). At specific times after injection, the amount of radioactivity over the thyroid gland was measured by a probe-type scintillation detector (Nuclear Chicago Model DS-5-1), the counts being recorded on a count rate meter (Nuclear Chicago, Model 181A) with a 2% error level. Light ether anaesthesia was used to immobilize the animal during counting. Two one-minute counts were averaged when the thyroid gland was centered over the opening of the probe while the tissue level was obtained by a one minute count of the radioactivity in the

abdominal region. A standard solution was also prepared and counted as a mock thyroid (Beierwaltes et al.,1957). For this standard 5 $\mu$c. $I^{131}$ were placed in a 10 ml. volumetric flask and water was added until the bulb of the flask was filled. The flask bulb was then submerged in a 100 ml. beaker of water. This was then placed in the same position as the rat on the probe. Under this condition 5 $\mu$c. (equivalent to 111 x $10^5$ disintegrations per minute) gave a fairly constant average reading of 53700 counts per minute. All readings were corrected for isotopic decay and room background. Initially the radioactivity of the thyroid was calculated in counts per minute (c.p.m.) as a percentage of that injected, i.e.

$$\frac{\text{thyroid c.p.m. - tissue c.p.m.}}{\text{standard c.p.m. - room background c.p.m.}} \times 100 \quad .$$

Later the actual counts per minute over the thyroid less room background (corrected for isotopic decay but not tissue background) were used to express the radioactivity of the thyroid as the results were almost identical to those obtained by the use of the above formula.

Twenty-two rats were placed in activity cages at 29±2°C. or 25±3°C. After their activity had been measured for three to six weeks each rat was injected intraperitoneally with 5 $\mu$c. carrier-free $I^{131}$. The average weight at the time of injection was 308 grams. At 24, 48, 72, 96 .... hours after injection, the amounts of radioactivity over the thyroid gland and abdominal region were measured. Two to three weeks after the initial measurement, nine rats were injected with a further 5 $\mu$c. $I^{131}$ and were placed at 2°C. to note if changes in spontaneous activity would follow changes in thyroid activity. A third series of measurements was started after the rats had been at 2°C. for three weeks. The rats were injected with 5 $\mu$c. $I^{131}$ and readings were started one hour after injection. The counts of radioactivity were done in the cold room although the reference standard gave the same

reading both inside and outside the cold room housing the radioactive animals.

After six or seven weeks at 2°C. the seven remaining rats were killed. Their average body weight, thyroid weight and adrenal weight were compared with those of five rats which had been kept in non-revolving cages at room temperature and which were killed at the same time. The thyroids were examined histologically after they were stained with the LaHam-Mallory dichrome stain. Yellow colloid indicates an inactive gland while the active state is shown by blue colloid.

Experiments with L-Thyroxine and Propylthiouracil (PTU)

The effects on spontaneous running activity of changing the level of thyroid function by administration of L-thyroxine and propylthiouracil were tested. Powdered L-thyroxine was dissolved in water by the addition of a small amount of crushed sodium hydroxide. Rats were made hyperthyroid by the daily or twice daily injection of 1 cc. of the solution containing 10 micrograms L-thyroxine. Rats were made hypothyroid by the daily or twice daily injection of PTU which acts by suppressing the synthesis of thyroid hormone by blocking the iodination of tyrosine in the gland. As the blood level of thyroxine falls, there is an augmented output of TSH (thyrotropin) by the anterior pituitary thus causing hypertrophy, hyperplasia and loss of colloid of the thyroid tissue. Such enlarged, hyperplastic goiters produce little, if any, thyroid hormone and the basal metabolic rates of the animals are at or near the thyroidectomy level (Turner, 1960). PTU was made up as a 1% suspension in gum arabic and the rats received 1 cc. of this suspension once or twice daily. Initially the injections were given intraperitoneally but later they were given subcutaneously on the back or side so that the effect of the drug might be longer lasting. The

injection of PTU caused development of sores at the injection sites, but these gradually healed. To test the activity of the chemicals, the metabolic rates of two rats were measured at 28°C. by the method of Depocas and Hart (1957) after daily injections of L-thyroxine into one rat and PTU into the other. The oxygen consumptions in ml./hr. for the two rats were as follows:

| Days of injection | 0 | 1 | 5 | 12 |
|---|---|---|---|---|
| with L-thyroxine | 21.9 | 23.2 | 27.0 | 26.6 |
| with PTU | 19.3 | 27.2 | 20.8 | 16.7 |

As the metabolic rate showed a general increase in the rat receiving L-thyroxine and a general decrease in the rat receiving PTU, the chemicals were judged to be active at these concentrations.

In the first experiment on the effects of L-thyroxine and PTU on activity, eleven rats were placed in activity cages at 29±2°C. and 25±2°C. and left for several weeks before injections were begun. Seven rats were in activity cages for nine weeks, three rats for five weeks and one rat for four weeks. The rats were then injected daily for three weeks (ten days intraperitoneally and 11 days subcutaneously), six with 10 µgm. L-thyroxine and five with 1 cc. 1% PTU. These eleven rats plus five control rats which had been kept in ordinary non-revolving cages were killed the day following the last injection. The thyroids and adrenals were weighed. The thyroids were examined histologically after they were stained with the LaHam-Mallory dichrome stain which colours the colloid yellow when the hormone is in an inactive form and colours the colloid blue when the hormone is in an active state.

The experiment was repeated with slight modifications on a second group of rats in order to confirm the initial results. After five weeks in activity cages twenty rats were injected subcutaneously twice daily for two weeks. Seven rats received L-thyroxine, seven received PTU and six

0.9% sodium chloride. For the first week each received 0.5 cc. twice daily and for the second week 1.0 cc. twice daily. At the end of the two-week injection period, eleven rats were killed (4 thyroxine-injected, 4 PTU-injected and 3 saline-injected) and the remaining nine rats were left in the activity cages for three weeks to see if withdrawal of the drugs changed the activity. The nine rats were then thyroidectomized. Three died shortly after the operation, one died five days later, and the five survivors were kept in activity cages and their activity recorded until they were killed three weeks after thyroidectomy.

## Catechol Amine Excretion and Spontaneous Activity

The methods of collection of urine and measurement of catechol amines are described in Part II of this thesis. The content of both adrenaline and noradrenaline in the urine of twenty rats that had been in activity cages at 29±2°C. or 25±3°C. for six months was measured twice, seven days apart, and the values were averaged for each catechol amine for each rat. When the 20 rats were placed in activity cages their average weight was 130 grams and six months later when the urine samples were collected they averaged 456 grams. It was necessary to remove the rats for one complete day from the activity cages in order to collect a 24 hour urine sample in a metabolism cage. The activity for six days is then computed for seven.

The rats were placed in the cold at 2±3°C. to determine if the spontaneous activity varied in the same direction as the catechol amine excretion. Urine samples were collected and assayed after 1, 2, 3, 4, and 5 weeks in the cold.

## Testicular Function and Spontaneous Activity

Two separate experiments were carried out to determine the effects of removal of the testes and subsequent administration of testosterone on the spontaneous running activity of rats. The first experiment was carried out on 18 rats approximately six weeks old at the beginning of the experiment and with an average weight of 135 grams. After eight weeks in activity cages at either 25±3°C. or 29±2°C., nine rats were castrated by the intra-abdominal method described in Farris and Griffith (1949), and nine were subjected to a sham operation. The rats were then about three and a half months old. Their spontaneous activity was recorded for five weeks after the operation at which time six of the nine castrated rats and six of the nine sham-operated rats were implanted with a 15 milligram CIBA pellet containing pure testosterone propionate. The pellets were in a form to be slowly absorbed over a long period of time. They were placed in the muscular tissue at the back of the neck between the scapulas. The rats were now almost five months old. In order to determine the effects of an increased dosage, the remains of the first pellet were removed when found (average amount of pellet remaining was 4.8 milligrams) after eight weeks and two pellets were implanted. The rats were now almost seven months old. After a further seven to eight weeks the rats were placed at 2°C. in order to observe the effect of a cold stress on the spontaneous activity. The activity was measured for a further five to six week period after which time the 13 living rats were removed from the activity cages and kept in individual metal cages in the cold room where they were included in another experiment. At the end of 149 days in the cold, the remaining seven rats were killed. At autopsy the remnants of the pellets, the testes, secondary sex organs and adrenal glands were weighed for all the rats.

For the second experiment 20 rats of approximately six weeks of age were allowed to run in the activity cages at 24±3°C. for seven weeks. Then ten of these rats were castrated and five of the ten controls were sham-operated. The rats were approximately three months old. Five weeks after the operation when the rats were four and a half months old, six of the castrated rats, three sham-operated and three unoperated controls were implanted with two pellets of testosterone propionate. After a further ten weeks all rats (approximately seven months old) were killed. The remnants of the pellets, the testes, secondary sex organs and adrenal glands were weighed.

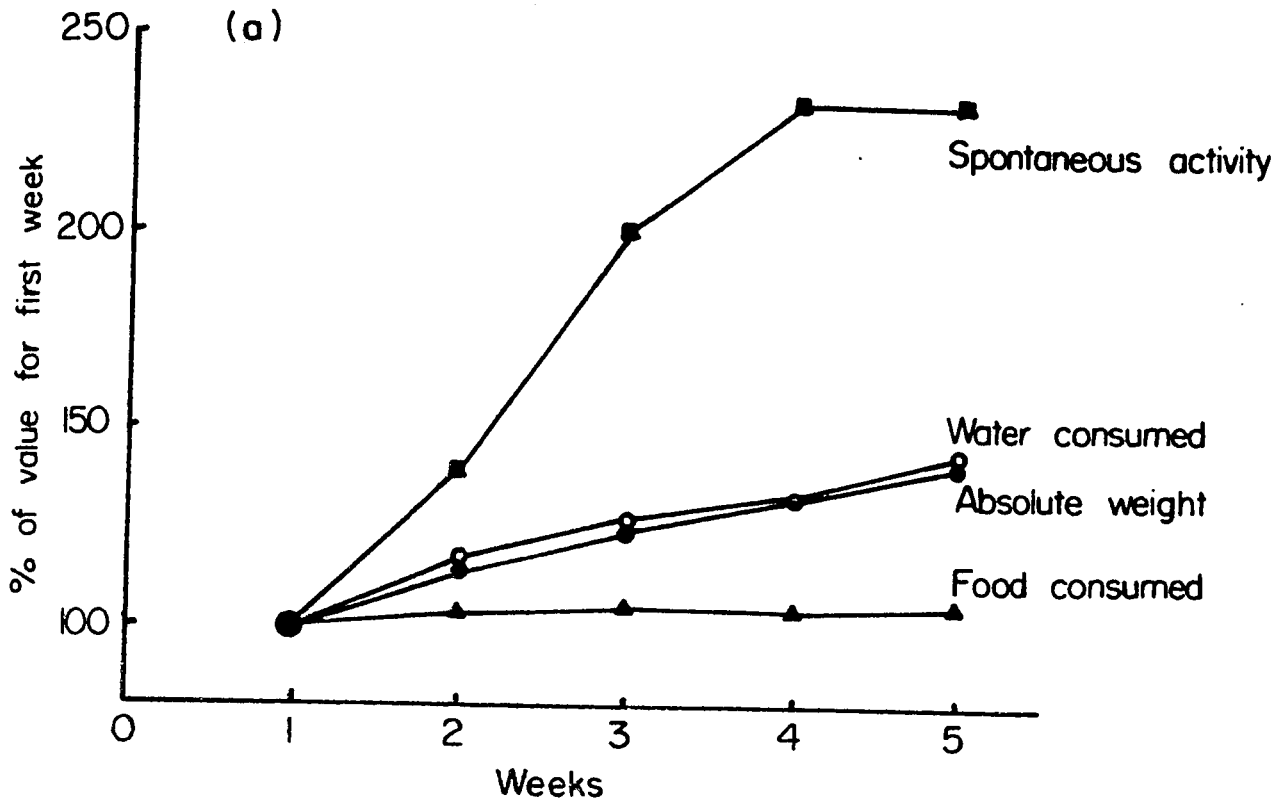
# RESULTS

The amounts of spontaneous activity of rats maintained at 25±3°C. and either 29±2°C. or 23±2°C. were not noticeably different in any of the experiments, hence the results at the two temperatures have been combined.

## Food and Water Consumption and Spontaneous Activity

The weekly averages of food and water consumed, absolute body weight and activity for 20 rats for the five-week experimental period are plotted in Fig. 1(a). Each value is expressed as a percentage of that of the first week which has been taken as 100%. Although the average spontaneous activity increased rapidly from 7418 to 17237 revolutions per week, the average body weight from 179 to 313 grams, and the average water consumption from 235 to 339 cc. per week, the average food consumption changed relatively little, varying from 143 to 152 grams per week.

In Fig. 1(b) the average values of food consumption, water intake and body weight gain for each rat over the five-week experimental period are plotted against each rat's average spontaneous activity. The average food and water consumption and body weight gain for any individual rat can be ascertained from the figure as the average spontaneous activity is the same in each of the three plots. There is no obvious correlation between spontaneous activity and either food consumption, water intake or body weight gain although it is noteworthy that amongst the most active rats there are no low values of any of these parameters.



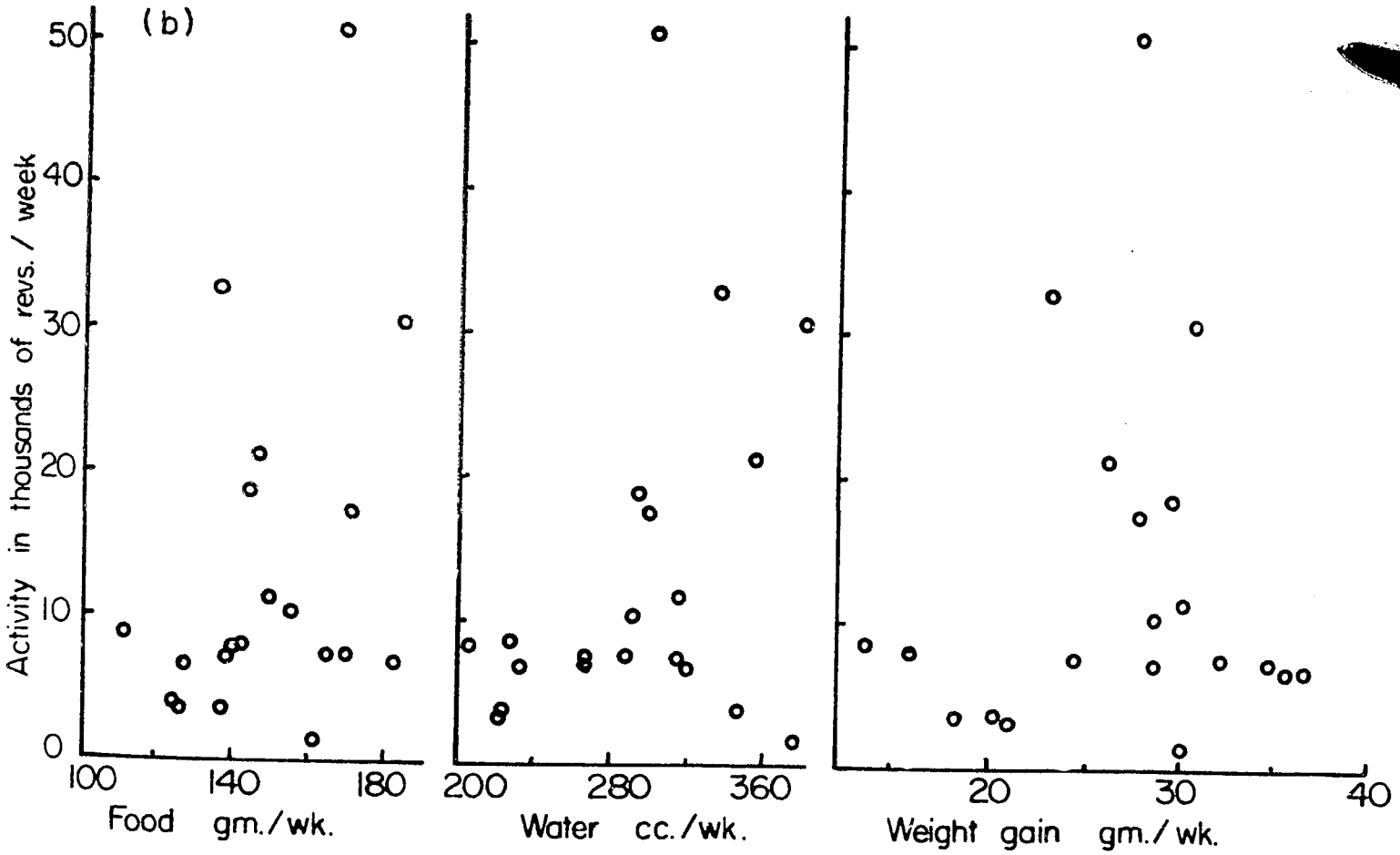




Fig. 1(a) Weekly averages of activity, food and water consumption, and body weight of 20 rats.
(b) Average activity for each rat over five-week experimental period plotted against food and water consumption and body weight gain.

## Liver Function and Spontaneous Activity

The effects of liver poisoning with carbon tetrachloride and of partial hepatectomy on the spontaneous running activity of rats were tested. The average body weight and spontaneous activity are plotted in Fig. 2 for 14 rats which received injections of carbon tetrachloride for five weeks and six saline control rats injected with saline on the same schedule. As the daily activity records did not seem to be critical, only the average numbers of revolutions per week are plotted. Fig.2(a) shows that the repeated injections of carbon tetrachloride caused a loss of body weight (as also reported by Radhakrishna-Rao,1936) and that the body weight rapidly increased once the injections were stopped. The average weight of the rats receiving carbon tetrachloride was 321 grams at the beginning of the injections (i.e. they received approximately 0.85 cc. $CCl_4$/kgm. per injection), 298 grams three weeks later at the beginning of the daily injections (i.e. the dose was now 0.92 cc./kgm.), and 283 grams at the end of the five-week injection period.

Fig. 2(b) shows that the injections of carbon tetrachloride were begun when the spontaneous activity of the rats had probably just passed its peak. There was little difference in activity between the 14 injected with carbon tetrachloride and the six saline injected control rats during the first three weeks of injection but the activity decreased during the last two weeks of injection and was back at the control level approximately two weeks after the injections were stopped.

Fig. 3(a) shows changes in average body weights of the nine sham-operated and nine partially hepatectomized rats, as determined twice weekly. The average body weight decreased in both the sham-operated and partially hepatectomized rats, the decrease being greater for the operated rats from

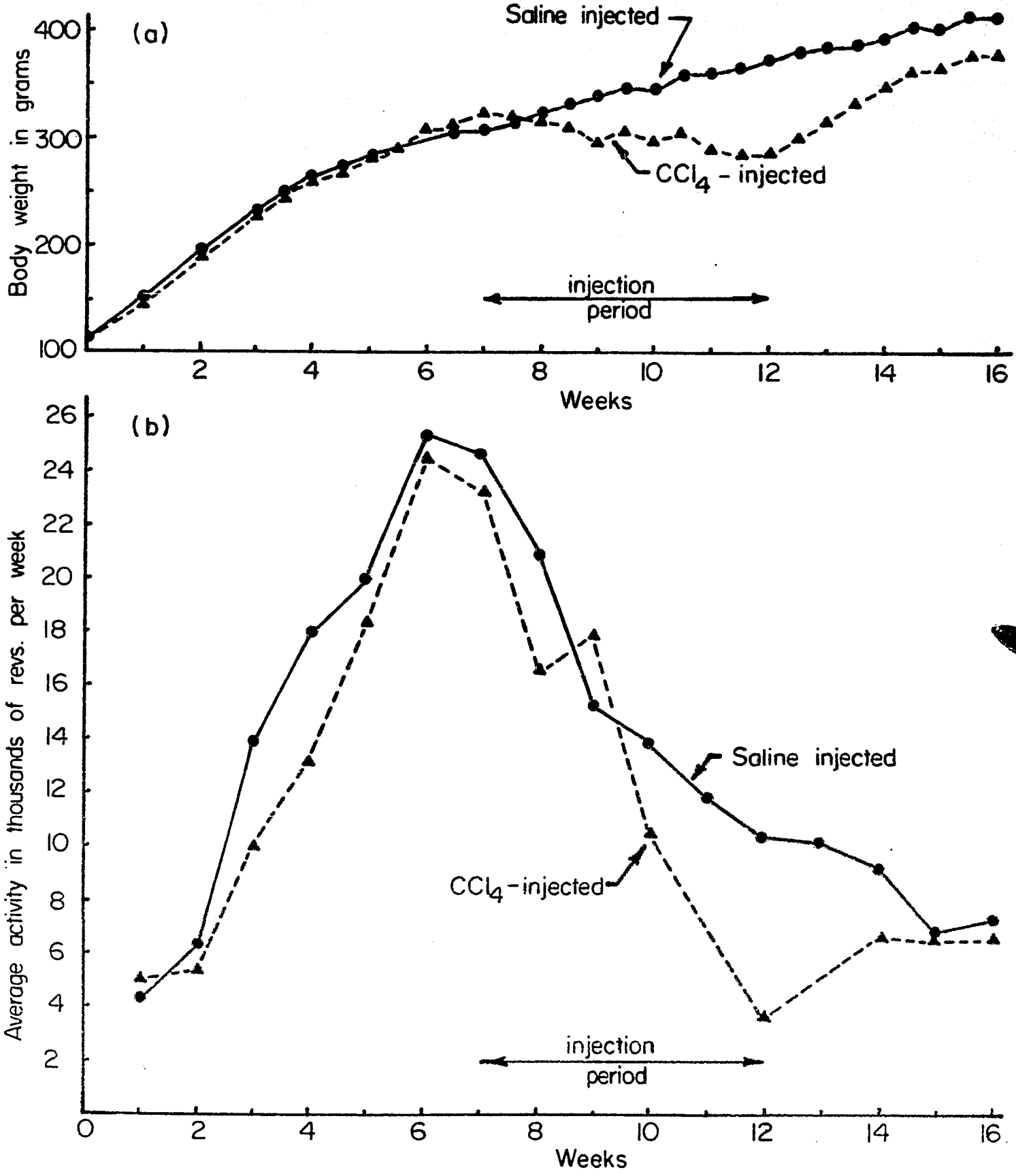



Fig. 2 Changes in (a) body weight and (b) activity before, during and after injection of carbon tetrachloride (averages for 14 rats) or saline (averages for 6 rats).

which eight to twelve grams of liver had been removed. The body weights in both groups began to increase rapidly during the eighth to twelfth day after the operations and then rose more slowly for the rest of the experimental period.

Fig. 3(b) shows that the average spontaneous activity for both the nine sham-operated and nine partially hepatectomized rats decreased immediately almost to zero after the operations. The average post-operative activity of the sham-operated rats returned to the average pre-operative level during the next six to seven days. During this period the average activity of the nine partially hepatectomized rats increased to a level slightly below the pre-operative level. This latter result was due to large decreases in the activity of the two most active rats. The post-operative activity of each of the other seven partially hepatectomized rats returned to its pre-operative level. The post-operative activity of one of the nine sham-operated rats similarly failed to return to the pre-operative level. As the overall level of activity of this rat was fairly low, however, this single low value had no significant depressing effect on the average value for post-operative activity of the group of nine sham-operated rats. Thus partial hepatectomy in these rats appeared to have little more effect on spontaneous activity than did a sham operation.

At autopsy it was observed that the median ventral portions of the liver were adhering strongly to the connective tissue around the site of the missing xiphoid process and to that of the stomach and intestine in many of the partially hepatectomized rats as well as in the sham-operated controls. The liver tissue was normal in appearance in all nine partially hepatectomized rats, but the lobes were irregular in shape and the whole organ weighed 12.8 grams on the average as compared with the mean weight of 16.1 grams in the nine sham-operated rats.

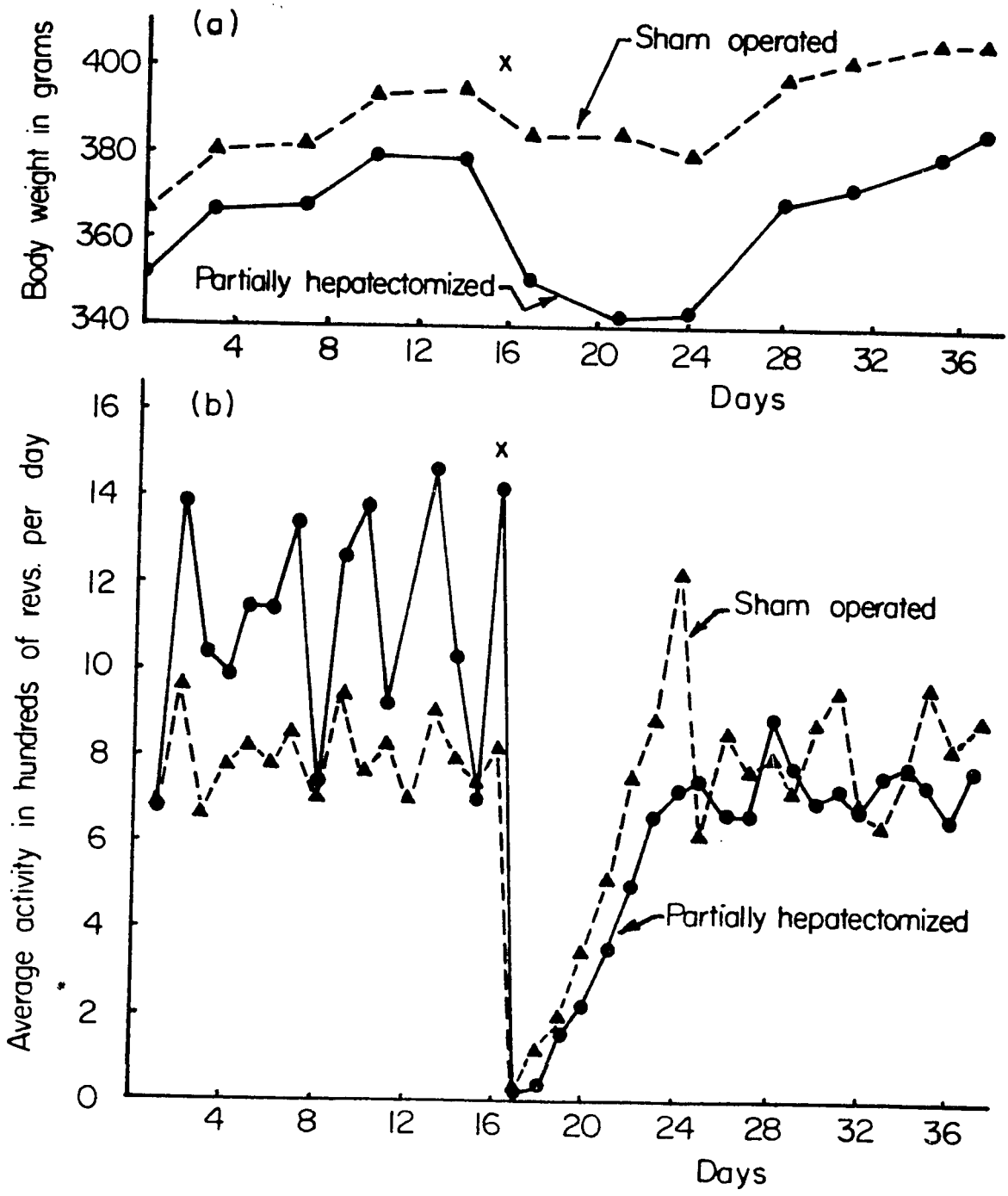



Fig. 3 Changes in (a) body weight and (b) activity before and after partial hepatectomy (averages for 9 rats) or after a sham operation (averages for 9 rats) on day 16 (marked by X).

## Thyroid Function and Spontaneous Activity

### Experiments with Radioactive Iodine

To determine if spontaneous activity was related to thyroid function, experiments were begun using radioactive $I^{131}$. Fig. 4 shows the decrease in radioactivity over the thyroid gland with time after the intraperitoneal injection of 5 $\mu$c. $I^{131}$ for each of 22 rats expressed as (1) a percentage of the injected dose and (2) the actual counts per minute recorded over thyroid corrected for room background and isotopic decay but not for tissue background. The plots for the individual rats are in increasing order of spontaneous activity (i.e. from the least active rat at top left to the most active rat at bottom right) as measured during the week of injection of the radioactive meterial. The shapes of the two curves for each rat are almost identical, thus indicating that both methods of expressing the results are essentially of equal validity. Fig. 4 clearly shows that there is no correlation of thyroid activity and spontaneous activity at room temperature.

The effect of exposure to cold on the release from the thyroid gland of $I^{131}$ after injection of 5 $\mu$c. was tested two to three weeks later on nine of the same rats immediately on exposure to 2°C. and three weeks afterwards for the eight surviving rats. Once again the curves of actual counts per minute were similar to those for radioactivity as a percentage of that injected, so only the former plots have been presented. Thus Fig. 5 illustrates the $I^{131}$ release curves for each of the nine rats in the cold as well as those obtained earlier at room temperature. The initial plotted measurement of radioactivity after the rats had been three weeks in the cold is 12 hours after injection rather than 24 hours and this accounts for the high level recorded in four rats. In each case the curves are similar

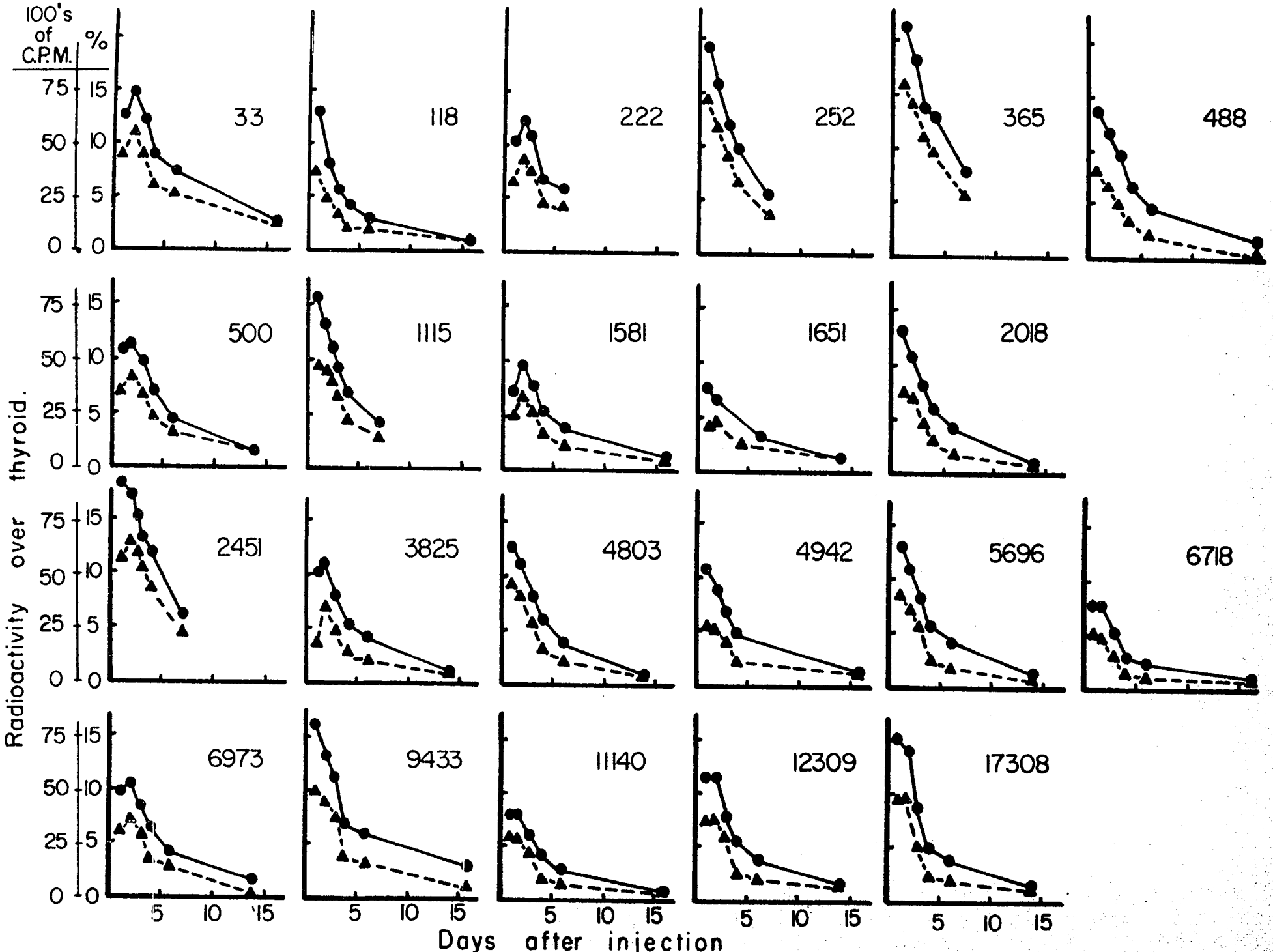



Fig. 4 The decrease in radioactivity over thyroid gland for 22 rats after intraperitoneal injection of 5 μc. $I^{131}$ expressed as: % of injected dose (▲---▲) and absolute counts per minute uncorrected for tissue background (●——●). Numbers indicate activity in number of revolutions for each rat for the first week after injection.

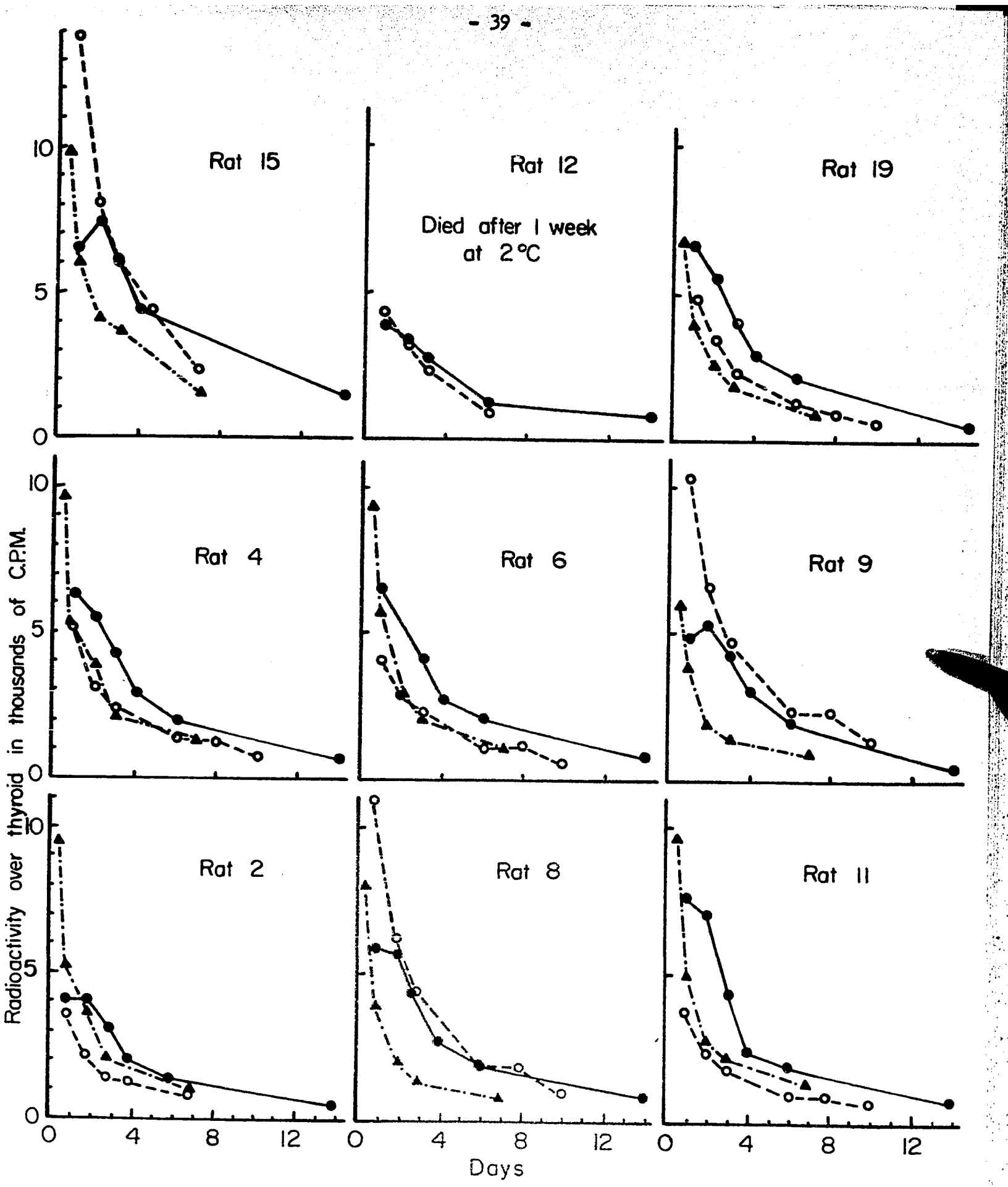



Fig. 5 The decrease of radioactivity over thyroid in nine rats at room temperature(●), immediately on exposure to cold (○), and three weeks after cold exposure (▲).

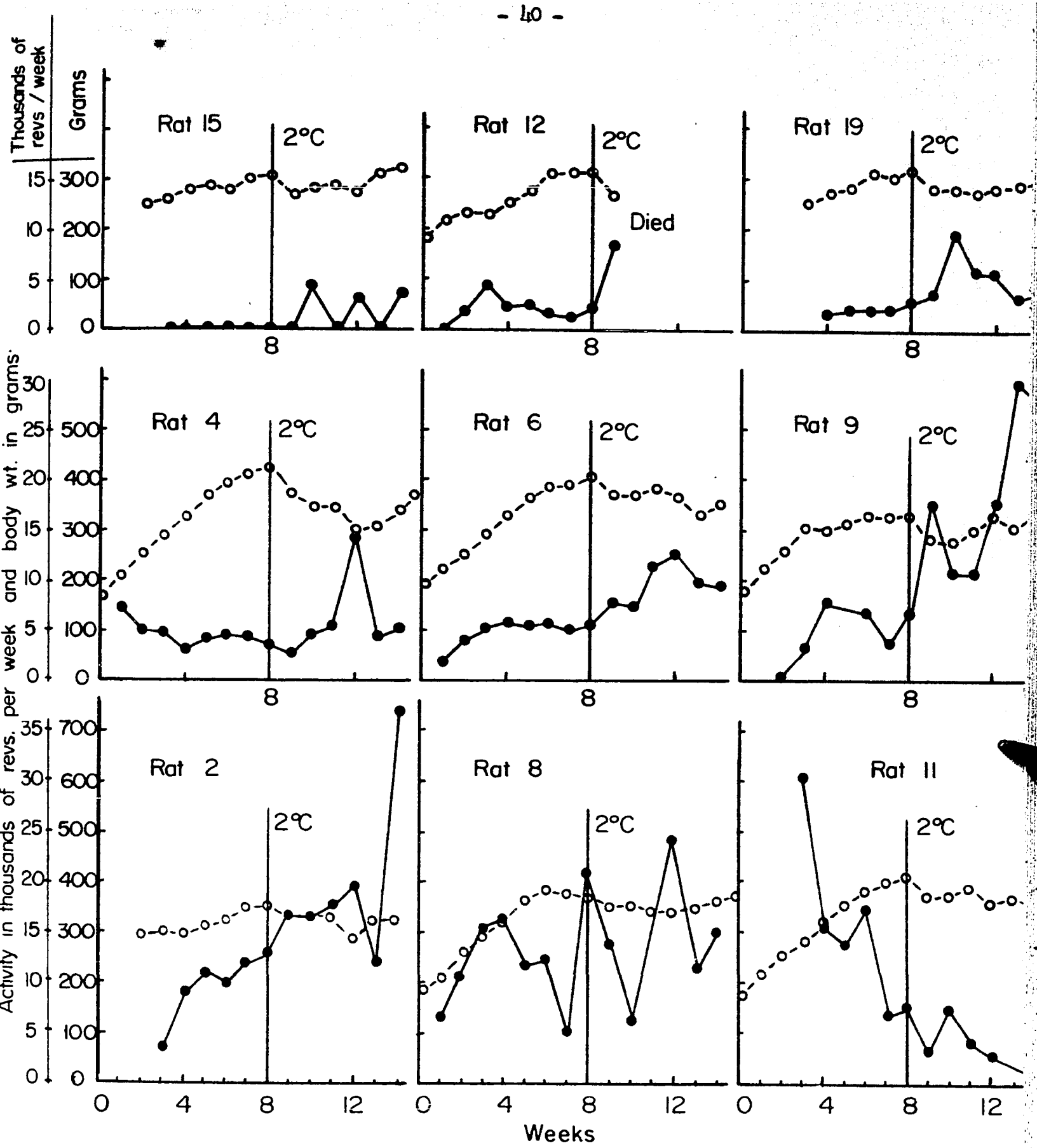



Fig. 6 Spontaneous activity (•——•) in thousands of revolutions per week and body weights in grams (o----o) of nine rats at room temperature (weeks 0 to 8) and at 2°C. (weeks 9 to 14).

although those taken after three weeks in the cold tend to be below the ones taken before cold exposure thus perhaps indicating an initial faster uptake and release of the radioactive iodine by the thyroid. This could be a direct result of an increased speed of absorption into the blood stream from the site of injection.

Fig. 6 shows changes in the weekly spontaneous activity measurements and body weights for the nine rats. There is an initial decrease in body weight on exposure to cold, followed at varying intervals of time by a slow increase. Cold exposure caused spontaneous running activity to increase in seven rats, decrease in one and remain unchanged in one. As the graphs in Fig. 6 have been plotted for the same rats in the same order as in Fig.5 the slopes of the $I^{131}$ release curves from the thyroid (Fig.5) and the level of spontaneous activity (Fig.6) can be easily compared. One would expect that if increases in thyroid function brought about increases in activity, that steep slopes in Fig.5 would be correlated with high levels of activity in Fig.6. This appears not to be so. For example, although the $I^{131}$ release curves for rats 8 and 15 are almost identical, rat 8 was quite active but rat 15 was relatively inactive.

Fig. 7 shows the changes in radioactivity over the thyroid and abdomen during the first 24 hours and at 48, 72 and 168 hours after injection of 5 $\mu$c. $I^{131}$ for the eight rats kept for three weeks at 2°C. The tissue level (as measured over the abdomen) was initially very high but declined rapidly during the first 12 hours and then more slowly. The thyroid contained its greatest amount of radioactivity during the first two hours after injection, after which the activity slowly declined. It is noteworthy that there was relatively little variation from rat to rat, thus further demonstrating the lack of correlation with spontaneous activity.

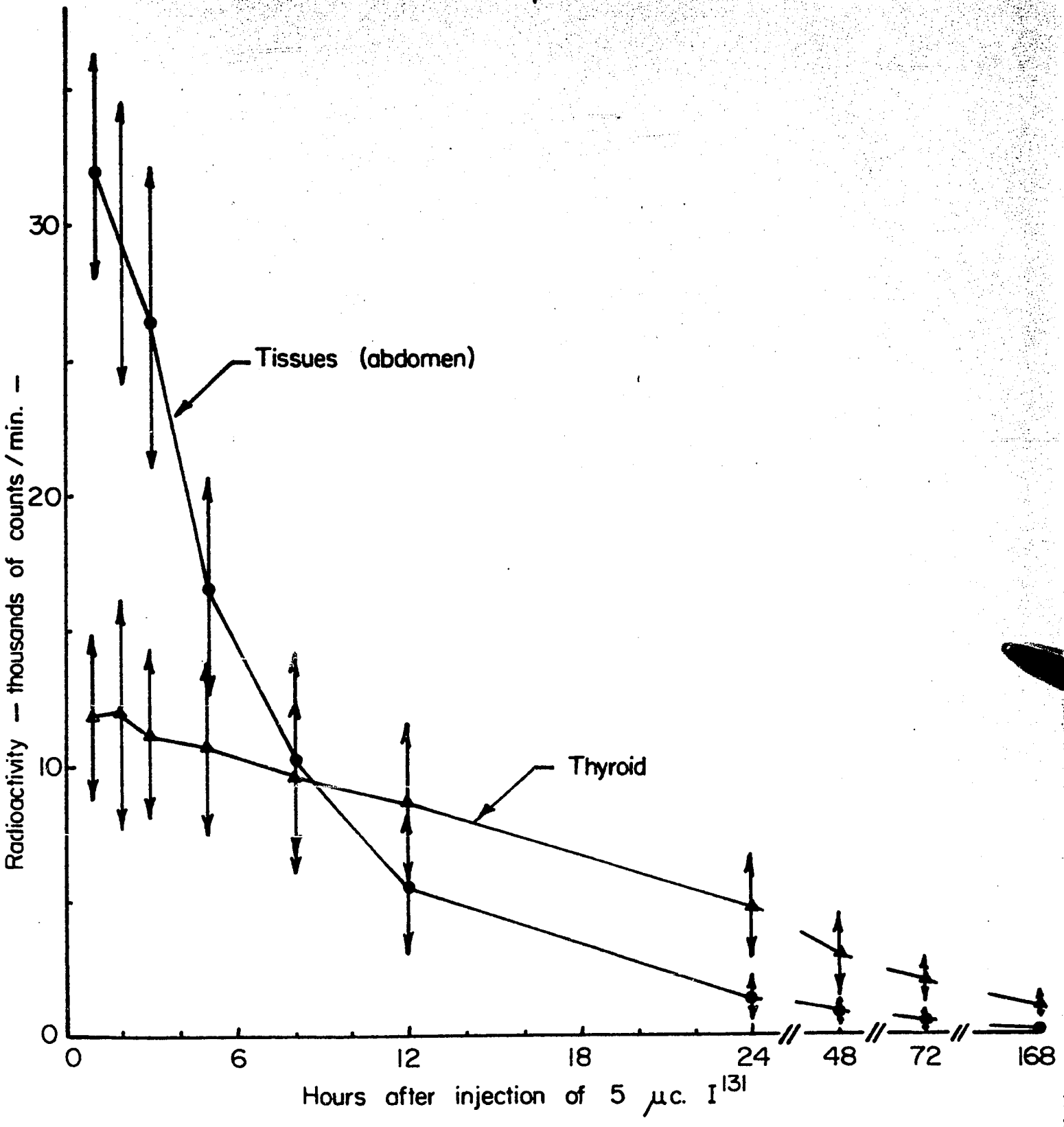



Fig. 7 Average radioactivity ( ± standard deviations) over thyroid and abdomen of 8 rats kept at 2°C. for three weeks.

After three weeks in the cold the rats began to show adverse effects, the tails and ears of many were frostbitten. At the end of the experiment (after six to seven weeks in the cold) when the rats were killed most had only a portion of their tail remaining, the end of which was hard and black as were also the outer edges of the ears. The average body weights, adrenal weights and thyroid weights with their standard errors for the seven rats living at the end of the experiment were compared with those of five rats of the same age which had been kept at room temperature.

| | Cold 2°C. 7 rats | Room Temperature 5 rats |
|---|---|---|
| Body weight - grams | 346 ± 11 | 453 ± 13 |
| Thyroid weight - mgm. | 19.3 ± 1.3 | 26.2 ± 0.8 |
| mgm./100 g. body weight | 5.6 ± 0.1 | 5.8 ± 0.1 |
| Adrenal weight - mgm. | 72.2 ± 4.3 | 43.6 ± 3.1 |

Thus the rats in the cold had smaller body weights and slightly smaller thyroid weights but larger adrenals than those maintained at normal room temperature.

Histologically the thyroids of the rats in the cold stained with the LaHam-Mallory dichrome stain contained only small amounts of colloid which was blue in all cases (i.e. active hormone-containing-thyroglobulin) whereas the control thyroids contained both blue and yellow colloid. In addition, the epithelium in the cold-exposed rats was either high cuboidal or columnar, another indication of an active gland.

Experiments with L-Thyroxine and Propylthiouracil (PTU)

The effect on the spontaneous running activity of rats of increasing the level of circulating thyroid hormone by the injection of L-thyroxine to make the animal hyperthyroid and by decreasing the level by means of injection of PTU to make the animal hypothyroid was tested. Fig. 8 (a & b) shows the average spontaneous running activity before and during the injection of the chemicals. Obviously thyroxine has had no effect while there is only a very slight suggestion that PTU caused a decrease in activity. When the average spontaneous activity of the three weeks before and three weeks during injection are averaged the results are as follows:

| Treatment | Average revolutions per week | |
|---|---|---|
| | Before injections | During injections |
| Thyroxine (6 rats) | 2194 | 2228 |
| PTU (5 rats) | 3734 | 2126 |

The rats were approximately 20 weeks old at the start of the injections and they weighed an average of 397 grams. Fig.9 (a & b) indicates that weight gain stopped when the chemicals were injected.

The rats were killed the day following the last injection as were also five control rats which had been kept in the animal room. At autopsy the thyroids and adrenals were weighed and the results averaged for each group. The averages with their standard errors are as follows:

| Average Weight | Thyroxine 6 rats | PTU 5 rats | Control 5 rats |
|---|---|---|---|
| Body - grams | 393 ± 20 | 390 ± 21 | 453 ± 13 |
| Thyroid - mgm. | 17.5 ± 1.7 | 74.8 ± 7.9 | 26.2 ± 0.8 |
| Adrenal - mgm. | 53.3 ± 1.4 | 44.8 ± 3.9 | 43.6 ± 3.1 |

After staining with the LaHam-Mallory dichrome stain, the thyroids were examined histologically. The follicles of the control rats contained both blue and yellow colloid; the follicles of the rats receiving thyroxine were quite large and filled with yellow colloid and the epithelium was cuboidal;

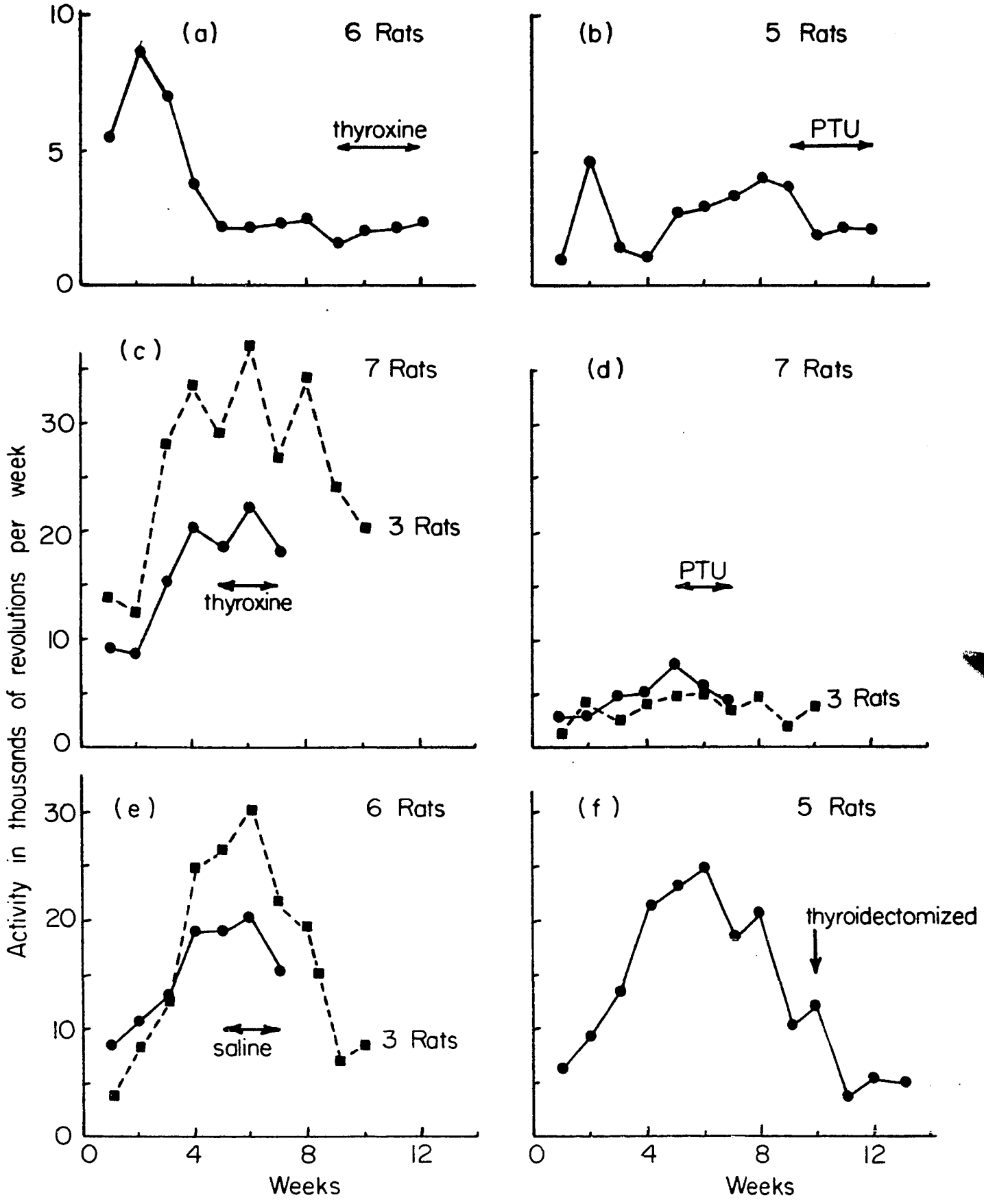



Fig. 8 Activity before, during and after administration of thyroxine, PTU, and saline, and before and after thyroidectomy. Average results for all rats in each group (●——●); average results for 3 rats in each group whose activity was measured for 3 weeks after injections ceased (■---■).

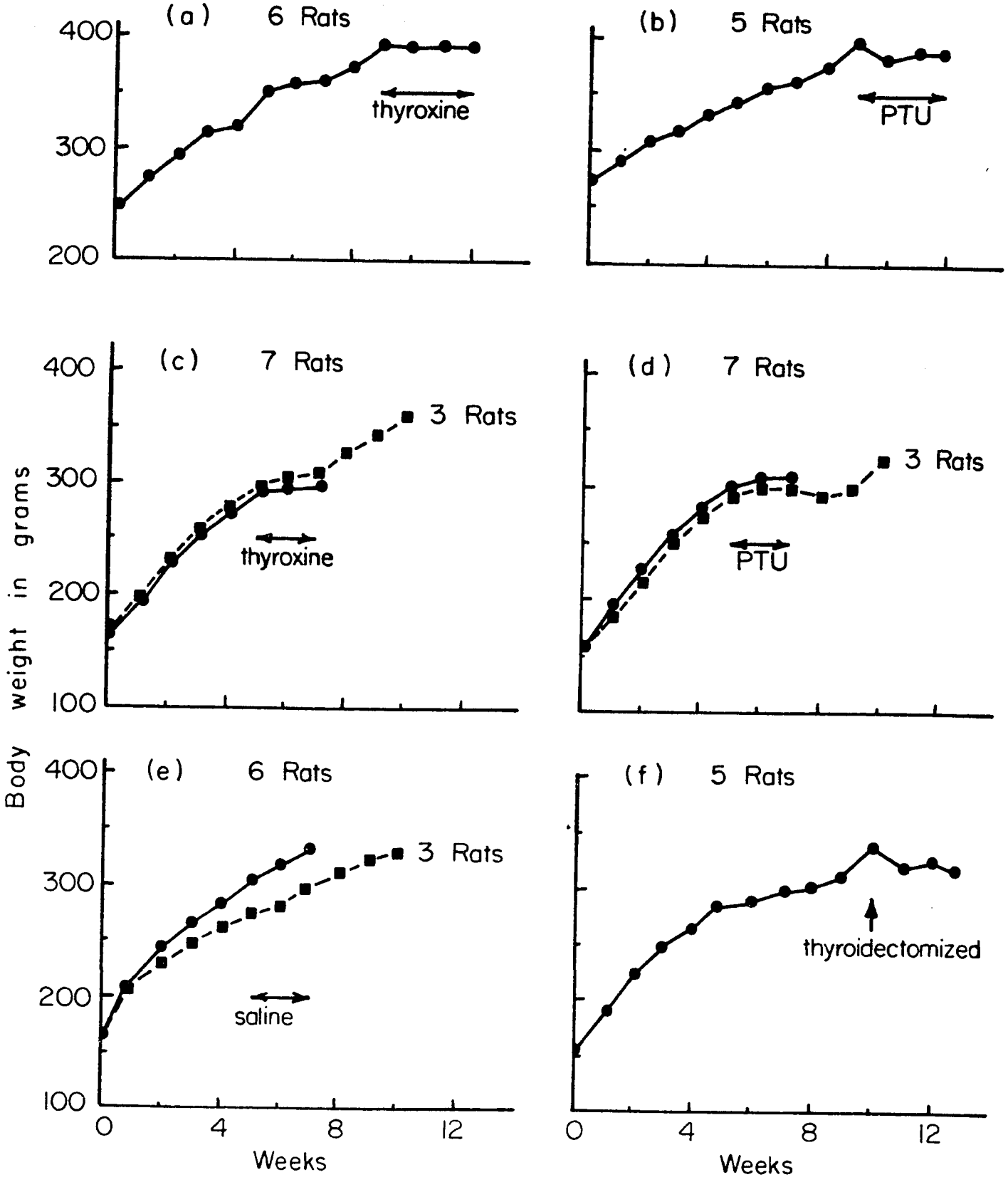



Fig. 9 Body weights before, during and after administration of thyroxine, PTU, and saline, and before and after thyroidectomy. Average results for all rats in each group (●——●); average results for 3 rats in each group which were weighed for 3 weeks after injections ceased (■---■).

and the rats receiving PTU had numerous follicles with columnar epithelium but with no colloid. These observations confirm that the thyroid glands responded to the chemicals in the expected manner.

This experiment was repeated with slight modifications and the activity data are shown in Fig. 8. Changes in the average levels of activity of thyroxine-injected rats in graph (c) and of PTU-injected rats in (d) as compared with those of saline-injected controls in (e) are slight. When the spontaneous activity for the two weeks preceeding and two weeks during injection are averaged the results are as follows:

| Treatment | Average revolutions per week | |
|---|---|---|
| | Before injections | During injections |
| Thyroxine (7 rats) | 19672 | 20179 |
| PTU (7 rats) | 6681 | 5261 |
| Saline (6 rats) | 19345 | 18216 |

When the nine rats with averages for the three weeks following the injection are considered separately the results are as follows:

| Treatment | Average revolutions per week | | |
|---|---|---|---|
| | Before injections | During injections | After injections |
| Thyroxine (3 rats) | 31393 | 32107 | 26654 |
| PTU (3 rats) | 4670 | 4440 | 3756 |
| Saline (3 rats) | 25799 | 26186 | 11726 |

These results are therefore in agreement with those obtained before in that the drugs do not seem to have much effect on spontaneous activity. It appears that the injections were carried out during the most active portion of the rats' lives, consequently the activity values are falling when measured after the injections. The average body weight of the rats at the beginning of the experiment was 161 grams and the average weight at the time of injection when the rats were approximately 13 weeks old was 300 grams, thus these rats were younger than those used in the first experiment. Fig. 9 (c & d) shows again that thyroxine and PTU cause an almost complete

stoppage of body growth in contrast to the saline-injected controls which continued gaining weight as shown in Fig. 9 (e).

As the drugs appeared to have little or no effect on the spontaneous activity, the average spontaneous activity of the five rats which survived the thyroidectomy are plotted in entirety in Fig. 8 (f). The average activity for three weeks before and for three weeks after the operation are 14363 and 4657 revolutions per week respectively. It is felt that as the activity was declining for four weeks prior to thyroidectomy and as it continued to decline at approximately the same rate after thyroidectomy, this latter decrease in activity is due only to the natural decrease in activity of rats of this age and not to the operation. Fig. 9 (f) shows that thyroidectomized rats failed to gain weight.

At autopsy the thyroids and adrenals were weighed and the averages with their standard errors were as follows:

(1) rats killed the day following the last injections

| Average weight | Thyroxine 4 rats | PTU 4 rats | Saline 3 rats |
|---|---|---|---|
| Body - grams | 294 ± 17 | 320 ± 10 | 370 ± 18 |
| Thyroid - mgm. | 12.6 ± 1.5 | 63.4 ± 3.1 | 22.2 ± 2.1 |
| Adrenal - mgm. | 54.1 ± 3.4 | 47.6 ± 1.7 | 59.6 ± 5.3 |

(2) rats killed or thyroidectomized three weeks after cessation of injections

| Average weight | Thyroxine 3 rats | PTU 3 rats | Saline 3 rats |
|---|---|---|---|
| Body - grams | 358 ± 25 | 327 ± 22 | 330 ± 15 |
| Thyroid - mgm. | 19.9 ± 3.2 | 17.6 ± 3.8 | 21.9 ± 1.6 |
| Adrenal - mgm. | 49.7 ± 6.6 | 36.9 ± 3.7 | 49.4 ± 4.6 |

The thyroid glands were again examined histologically using the LaHam-Mallory dichrome stain. All of the saline-injected rats had follicles with cuboidal epithelium and contained both blue and yellow colloid. The thyroxine-injected rats killed immediately after the injections had follicles

with low cuboidal epithelium and contained a large amount of yellow colloid while the thyroids three weeks after injection had numerous small follicles with cuboidal epithelium and filled with blue colloid. The PTU-injected rats killed immediately after cessation of injections had thyroids with numerous extremely small follicles with columnar epithelium and no colloid. The thyroids three weeks after treatment could not be distinguished from those of the saline controls.

## Catechol Amine Excretion and Spontaneous Activity

The content of each of adrenaline and noradrenaline was determined in two 24-hour samples of urine taken one week apart from each of 20 rats removed temporarily from activity cages. Fig. 10 (a) shows the average amount of activity plotted against the average of two determinations of adrenaline for each rat. Fig. 10 (b) gives the comparable picture for noradrenaline. Fig. 10 (a) shows that there is no obvious correlation between excretion of adrenaline and spontaneous activity. On the other hand, Fig. 10 (b) indicates a tendency for rats which excreted more noradrenaline to be distinctly more active than rats which excreted lesser amounts. The coefficient of correlation (r), however, was low being only 0.45. Moreover, the two most active rats excreted only moderate amounts of noradrenaline, and the rat which excreted the second-highest amount was among the least active of all the rats.

While the experiment was being continued for five weeks at 2°C. to determine if the catechol amine excretion and spontaneous activity would change in the same direction, six rats died. Although Fig. 11 gives average values of the various measurements made on 20 rats for 'week 0' (i.e. the control period at room temperature), the averages for succeeding weeks

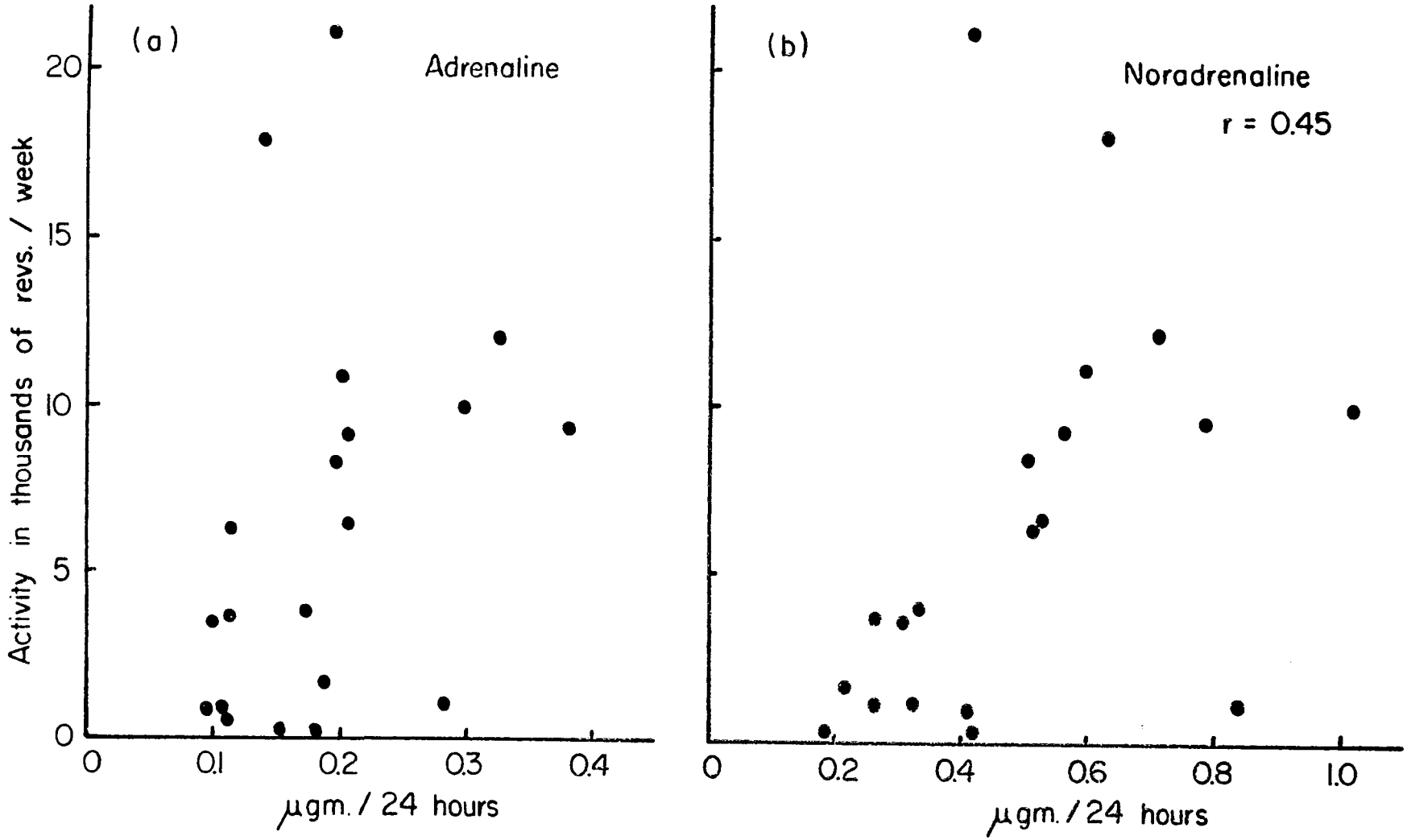



Fig. 10 Average activity of each of 20 rats plotted against (a) adrenaline excretion, and (b) noradrenaline excretion.

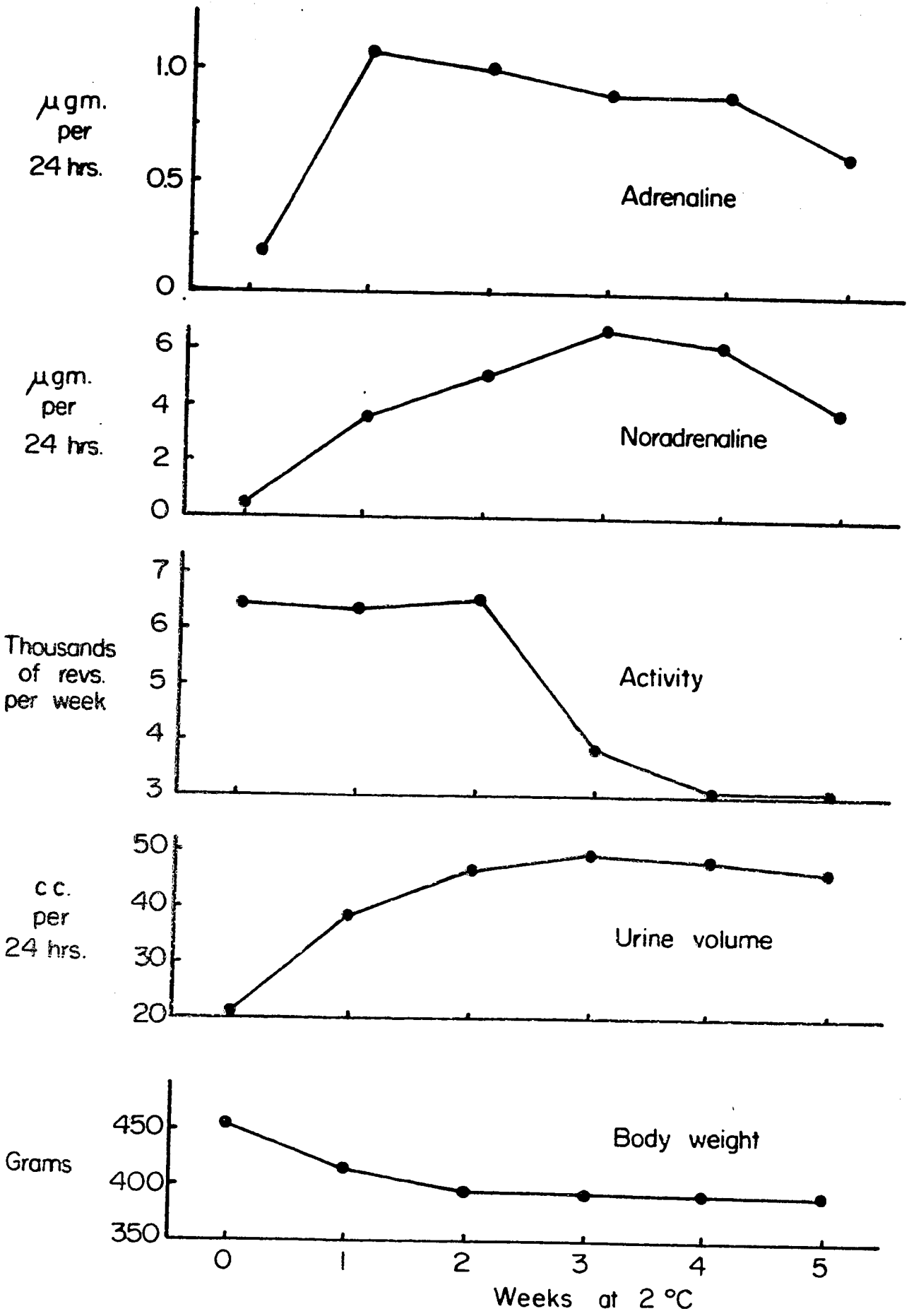



Fig. 11 Changes in adrenaline, noradrenaline, activity, urine volume and body weight after exposure of 20 rats to 2°C.

are based on lesser numbers of rats: 19 for week one, 17 in week two, 16 in week three, 15 in week four and 14 in week five. Fig. 11 shows that both average body weight and average spontaneous activity decreased in the cold. The changes in spontaneous activity on exposure to cold, however, showed marked individual variation; six rats considerably decreased their activity, five rats increased their activity while the activity of the remaining nine rats either remained unchanged or showed both increases and decreases. Fig. 11 also shows that the average urine volume, and excretion levels of adrenaline and noradrenaline increased at 2°C. Marked increases in the adrenaline and noradrenaline levels were observed for each individual rat on exposure to 2°C. Thus none of the parameters appeared to vary with spontaneous activity. The changes in urine volume and noradrenaline levels, however, appeared to take place simultaneously.

## Testicular Function and Spontaneous Activity

Experiments were carried out to test the effect of removal of the testes and subsequent administration of testosterone on the spontaneous activity. The average weekly spontaneous activity and body weights for each group of rats of the first experiment are plotted in Figs. 12 and 13. Fig. 12 (a to d) shows that the operations appear to have been done at a time when the activity of all the rats had probably passed its peak and was slowly declining. Fig. 12(a) shows that the sham operation caused a temporary sharp drop in average activity followed by a rapid return to the slowly declining curve; the same trends are less apparent in (b). Fig. 12 (c & d) show that castration caused an immediate drop in activity to a low level which was maintained. Without the implantation of pellets the activity of the sham-operated controls in Fig. 12(a) slowly declined to approx-

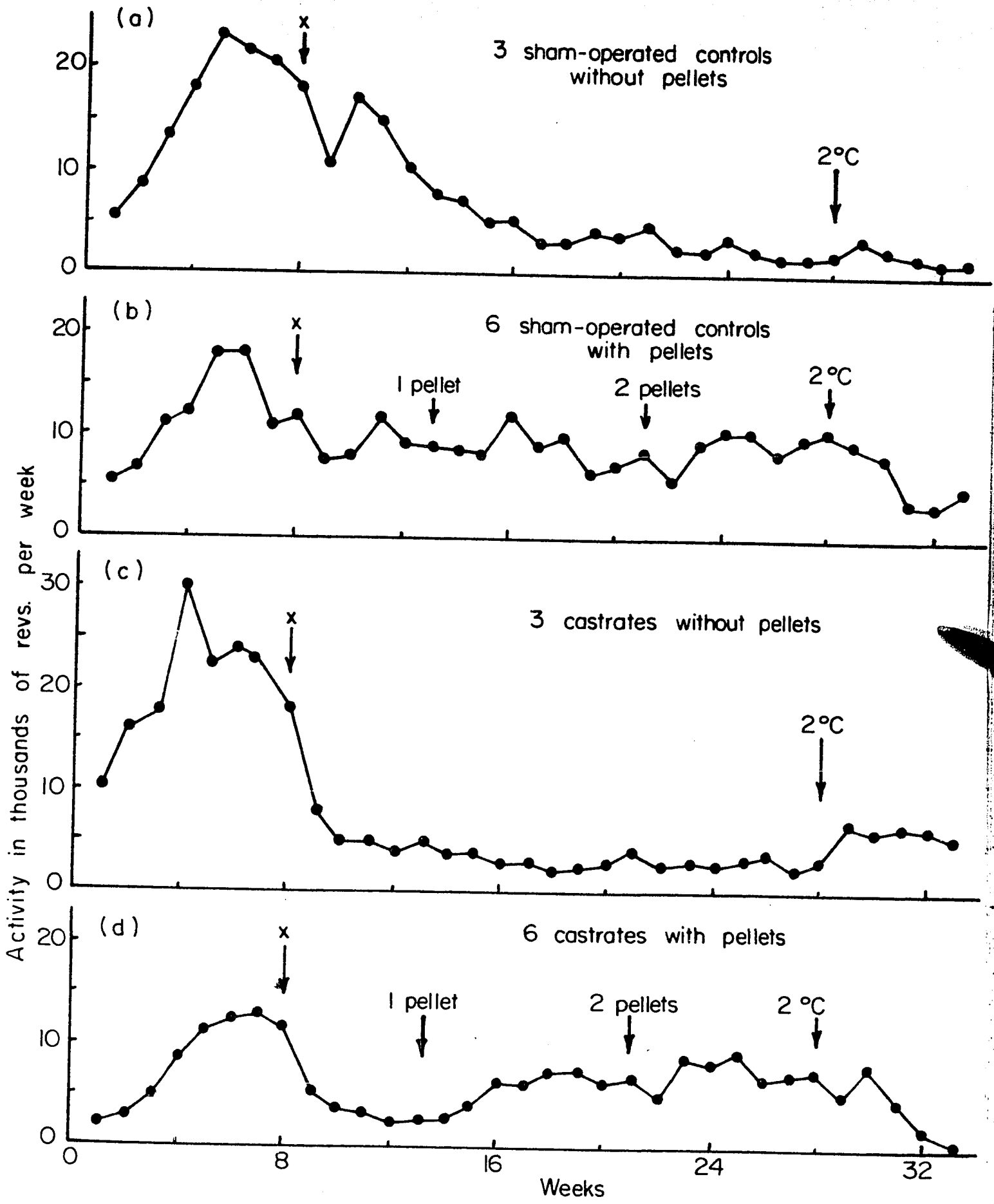



Fig. 12 Average activity of sham-operated and castrated rats with and without testosterone propionate pellets. X indicates when operations were performed.

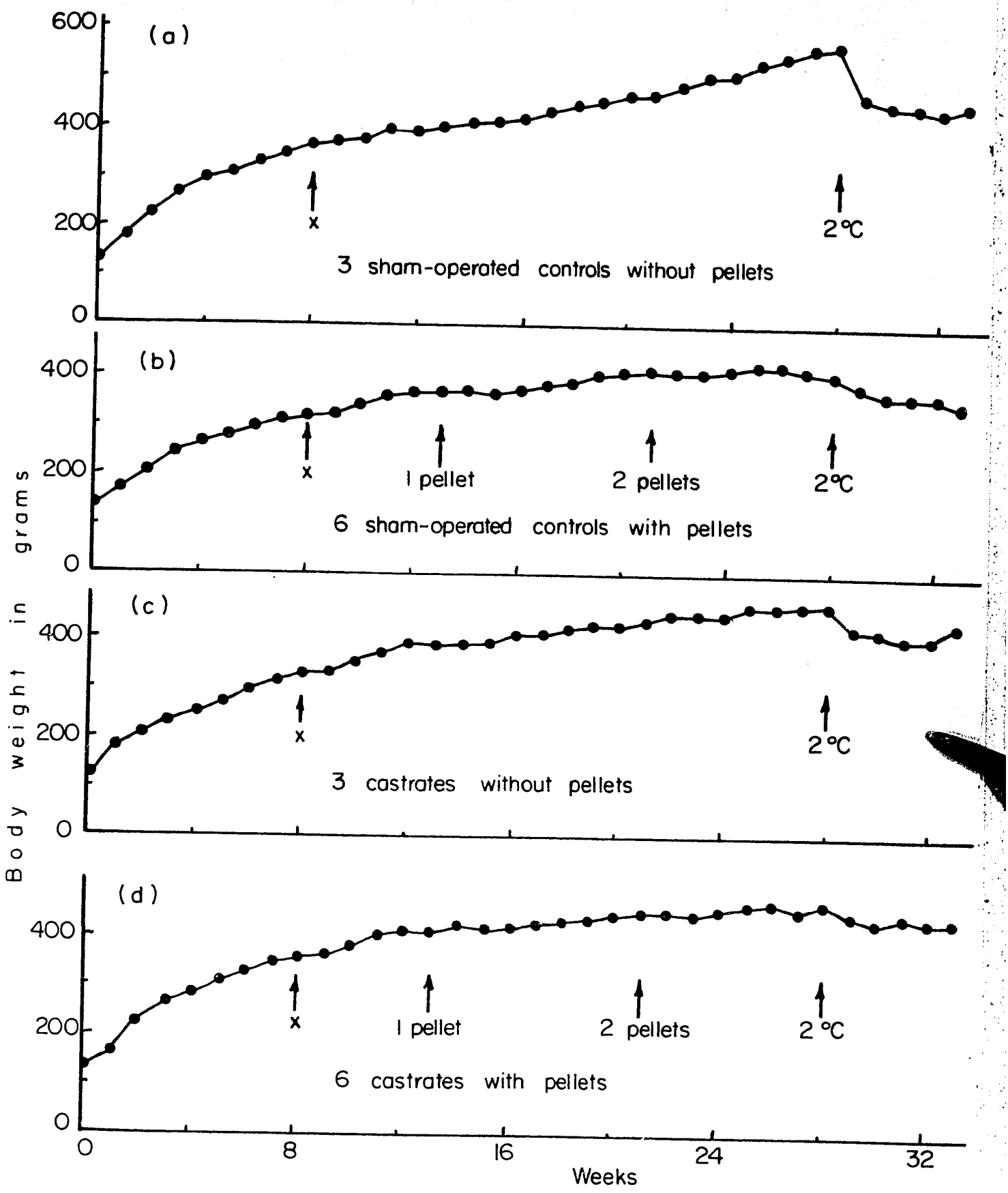



Fig. 13 Average body weight of sham-operated and castrated rats with and without testosterone propionate pellets. X indicates when operations were performed.

imately the same level as that of the castrated rats (c). The implantation of pellets raised the activity of the castrated rats (d) and maintained the activity of the sham-operated controls at a moderately high level (b). For the purpose of giving comparative numerical data, the spontaneous activity of the rats in each group have been averaged over the various time intervals in Table I.

TABLE I.

AVERAGE ACTIVITY OVER FIVE EXPERIMENTAL PERIODS

| Time | Average activity - revolutions per week | | | |
|---|---|---|---|---|
| | Sham operated | | Castrated | |
| | Without pellets (3 rats) | With pellets (6 rats) | Without pellets (3 rats) | With pellets (6 rats) |
| Before operation (5 weeks) | 20577 | 14413 | 23720 | 11457 |
| After operation (5 weeks) | 12441 | 9232 | 5285 | 3540 |
| 1 pellet No pellet (8 weeks) | 4579 | 8641 | 3399 | 6010 |
| 2 pellets No pellet (7-8 weeks) | 2530 | 9020 | 3286 | 7577 |
| Cold 2°C. (5-6 weeks) | 2006 | 6238 | 6420 | 5364 |

Fig. 13 indicates that the body weight increases rapidly at first and then more slowly. Castration did not appear to increase the rate of body weight gain but the implantation of testosterone pellets appears to cause a further slowing of the body weight gain. Exposure to cold causes a fall in body weight in all four groups of rats.

The average weights of various organs at autopsy are recorded in Table II. Pellets were found in the five rats which died during the first 30

days in the cold. On the average, 19 mgm. of the 30 mgm. pellets had been used up after eight weeks at room temperature and 22 days in the cold.

TABLE II

AVERAGES OF BODY WEIGHTS AND VARIOUS ORGAN WEIGHTS (± S.E.)

| Treatment | Days in cold avg. | Body weight g. | Testes g. | Dorsal prostate mgm. | Ventral prostate mgm. | Seminal vesicles mgm. | Adrenals mgm. |
|---|---|---|---|---|---|---|---|
| Controls (3 rats) | 101±48 | 465±26 | 1.46±.13 | 281±37 | 395±135 | 520±30 | 56±1 |
| Controls+ pellets (6 rats) | 94±17 | 397±16 | 1.22±.21 | 282±53 | 606±106 | 684±79 | 54±6 |
| Castrates (3 rats) | 120±25 | 444±35 | - | 23±8 | 78±3 | 61±10 | 81±23 |
| Castrates+ pellets (5 rats)* | 31±11 | 387±20 | - | 758±299 | 1400±123 | 1195±212 | 75±9 |

* One rat was omitted as it lived for 149 days in the cold by which time its pellets had been completely used up and its secondary sex organs were back at castration level.

Table II shows that the secondary sex organs of rats with pellets were extremely enlarged indicating the great potency of the pellets. Because of the cold the testes of the non-castrated rats were intraabdominal and weighed less than they normally would at room temperature.

The results for individual rats are similar to those represented by the averages except in the case of exposure to cold where the spontaneous activity was more varied, and for one rat with very low activity where castration and pellet implantation had no effect. Further, the effects of castration and administration of testosterone on spontaneous activity were most marked in the most active rats.

The average weekly activity and body weights for each group of rats in the replicate experiment are plotted in Figs. 14 and 15. As the values

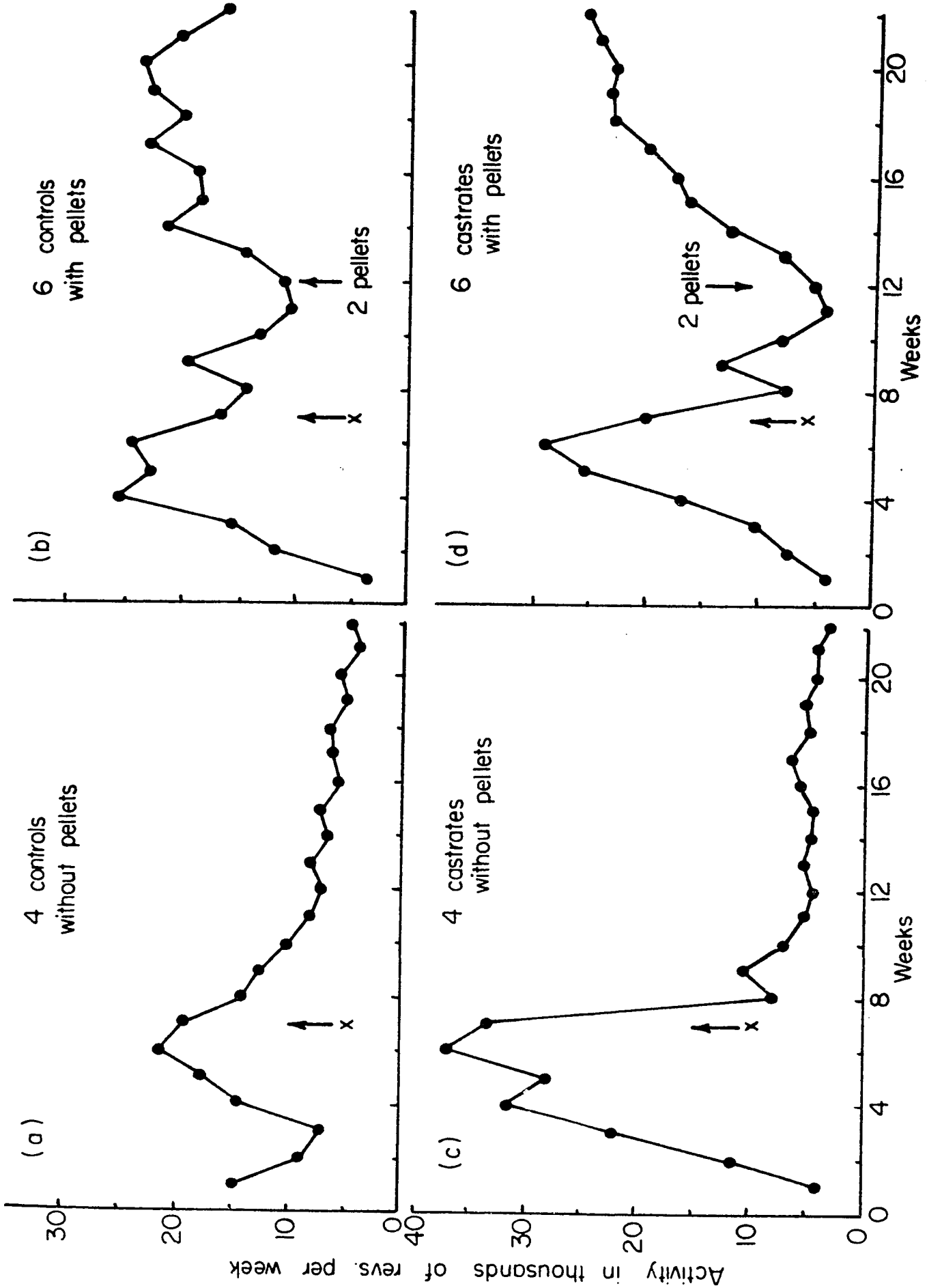



Fig. 14 Average activity of control and castrated rats with and without testosterone propionate pellets. X indicates when the operations were performed.

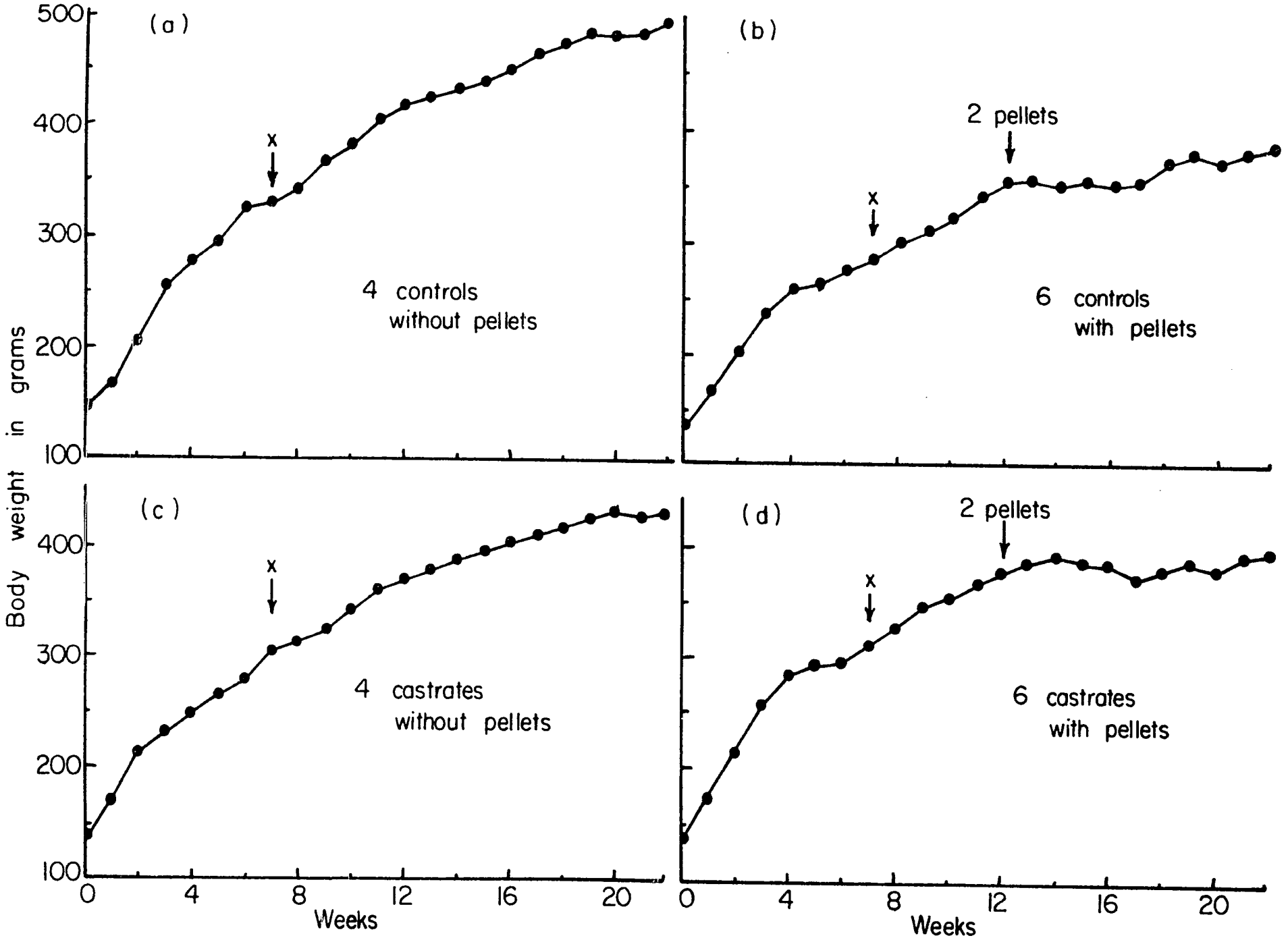



Fig. 15 Average body weight of control and castrated rats with and without pellets of testosterone propionate. X indicates when the operations were performed.

for the sham-operated and unoperated controls were similar, they have been combined. Fig. 14 shows that again the operations were done when the activity of the rats had just passed its peak. Results essentially similar to the previous experiment were obtained. Only one of the ten castrated rats failed to show a significant decrease in activity (it was moderately active). Fig. 14 (b & d) show that the increase of activity after testosterone in the controls and in the castrated rats to levels almost as high as the original peaks was quite dramatic. Individually the results are the same; that is, the pellets caused the activity to increase to the previous high level for any particular rat and in no case was a relatively inactive rat made very active by the pellets, but its activity was increased only to the approximate level that it had been before the operation. As before the numerical data are presented for comparative purposes in Table III.

TABLE III

AVERAGE ACTIVITY OVER FOUR FIVE-WEEK PERIODS

| Time | Average activity - revolutions per week | | | |
|---|---|---|---|---|
| | Controls | | Castrated | |
| | Without pellets (4 rats) | With pellets (6 rats) | Without pellets (4 rats) | With pellets (6 rats) |
| Before operation (5 weeks) | 16004 | 21043 | 30455 | 20315 |
| After operation (5 weeks) | 10476 | 13899 | 7210 | 7849 |
| 2 pellets No pellets (1st 5 weeks) | 6690 | 19190 | 5387 | 15310 |
| 2 pellets No pellets (2nd 5 weeks) | 4932 | 21320 | 4590 | 24456 |

Fig. 15 shows that the presence of the pellets retards increases in body weight. At autopsy at the end of the experiment, the average weight

of the remnants of the pellets removed from the 12 rats concerned was 13.0 mgm. per rat, hence the amount of testosterone propionate absorbed over the ten weeks averaged 17.0 mgm. per rat i.e. 1.7 mgm. per week which compares with the figure of 1.3 mgm. per week at room temperature in the first experiment. The average weights of various organs at autopsy are recorded in Table IV.

TABLE IV

AVERAGES OF BODY WEIGHTS AND VARIOUS ORGAN WEIGHTS (± S.E.)

| Treatment | Body weight g. | Testes g. | Dorsal prostate mgm. | Ventral prostate mgm. | Seminal vesicles mgm. | Adrenals mgm. |
|---|---|---|---|---|---|---|
| Controls (4 rats) | 489±23 | 3.50±.16 | 327±25 | 729±108 | 523±27 | 40±5 |
| Controls+ pellets (6 rats) | 384±24 | 2.27±.09 | 601±19 | 1038±68 | 771±24 | 36±1 |
| Castrates (4 rats) | 427±32 | - | 25±6 | 50±17 | 63±7 | 59±8 |
| Castrates+ pellets (6 rats) | 380±25 | - | 498±63 | 877±101 | 703±41 | 41±3 |

Table IV shows that the secondary sex organs of the castrated rats were extremely small while for the rats with pellets they were again extremely large. The testes of the controls with pellets were smaller than those of the controls probably because so much testosterone was being supplied by the pellets that the rats' own testosterone production was decreased.

To summarize the results of the two experiments on the effects of castration and administration of testosterone on spontaneous activity, it is useful to express all average amounts of activity in relative terms that are readily comparable. To this end, the actual activity data of Tables I

and III have been expressed as percentages of the amount of activity of the five-week pre-operative period for each group of rats in Table V.

TABLE V

SUMMARY OF EXPERIMENTS ON TESTICULAR FUNCTION AND ACTIVITY*

| Activity as % | Controls | | Castrated | |
|---|---|---|---|---|
| | Without pellets | With pellets | Without pellets | With pellets |
| Experiment 1 | | | | |
| Before operation | 100 | 100 | 100 | 100 |
| After operation | 60.5 | 64.1 | 22.3 | 30.9 |
| 1 pellet<br>No pellet | 22.3 | 60.0 | 14.3 | 52.5 |
| 2 pellets<br>No pellet | 12.3 | 62.5 | 13.9 | 66.1 |
| Cold 2°C. | 9.7 | 43.3 | 27.1 | 46.8 |
| Experiment 2 | | | | |
| Before operation | 100 | 100 | 100 | 100 |
| After operation | 65.5 | 66.1 | 23.7 | 38.6 |
| 2 pellets<br>No pellet | 41.8 | 91.2 | 17.7 | 75.4 |
| 2 pellets<br>No pellet | 30.8 | 101.3 | 15.1 | 120.4 |

* Data taken from Table I and III.

Table V shows that castration causes a marked and sustained drop in activity and administration of testosterone brings the activity level up to greater than normal levels for control rats of comparable age and approaches the high levels of activity of early adulthood. Table V also shows that there is a general decrease in activity with time for the control rats so that eventually the level of activity approaches that of the castrated rats. This general decline of activity in control rats can be inhibited by the administration of large doses of testosterone in the form of pellets.

## DISCUSSION

These experiments on spontaneous running activity have confirmed the observations of Hoskins (1925a), Heller (1932), and Young and Fish (1945) that there is no rat in which activity is maintained at a constant level but the activity of any individual rat is often seen to vary considerably from day to day. An extremely wide range of individual performance (less than 50 to over 70,000 revolutions per week) was also observed as has been reported by numerous workers; as yet there is still no adequate explanation of the probably causes.

The rats were generally placed in the activity cages when they were about six weeks of age and weighed over a hundred grams. Their average weekly activity steadily increased until it reached a peak after about six to seven weeks in the cages (85-100 days of age), after which it steadily declined to a low somewhat constant level after 12 to 16 weeks in the cages (125-150 days of age). This compares with the data of Hoskins (1925a) whose control rats reached their peak activity about 120 days of age after nine weeks in the activity cages. As previously stated, it is difficult to assess the earlier studies on the effects of age on activity but the present study confirms the early report by Slonaker (1907) and of Richter (1933b) that rats are most active around 100 days of age, and is in contradiction to that of Jones et al. (1953) that 51- and 72-day old rats reach a higher activity than 100-day old rats. The reports of many workers that activity decreases with increasing age was also confirmed. There were several individual exceptions to the pattern but the general trend was seen in each group of rats. It was endeavored as far as possible to set up each experiment so that there were very active and very inactive rats in both control and experimental groups. It was felt that any investigators

who did not run a group of controls with their experiments were in danger of misinterpreting their results because of this factor of changing activity with age, so that their comparisons of the activity of rats before and after treatment were subjected to this error and would in some cases be completely invalid. Although activity measurements were usually not used in an experiment until the rats had been in the activity cages for at least three weeks, it was felt that this precaution was perhaps unnecessary.

Exposure to cold at 2°C. caused some rats to increase activity, some to decrease while for others the activity was unchanged. There was no relation between survival and change of activity in the cold except that several rats which died became very active in the day or so preceding death. It therefore seems that 2°C. was not a sufficiently low temperature to cause a reduction of activity in all rats.

The fact that there was no correlation between spontaneous activity and either food consumption, water intake or body weight gain is in agreement with reports published by Hitchcock (1927), Rundquist (1933) and Tribe (1954). The negative correlation between activity and body weight gain obtained by Brobeck (1945) is thought to be a result of the restricted food intake of the rats.

There was no marked correlation between greatly impaired liver function and spontaneous running activity. The dose of carbon tetrachloride (approximately 0.80 cc./kilogram of body weight) given to rats every other day for three weeks and then daily for two weeks not only caused a decrease in body weight and fatty infiltration and marked necrosis in all those livers examined but was also at a level reported to cause lethal effects. A decrease

in activity during the last two weeks of large injections of carbon tetrachloride was recorded. In the partial hepatectomy experiments, activity of both partially hepatectomized and sham-operated rats was decreased almost to zero by the operation. Six to seven days were required for the activity of both partially hepatectomized and sham-operated rats to return to a level approximating that of the pre-operative level. Thus the results are in agreement with Dugal and Ross (1943) in that immediately after the operation activity almost completely disappeared in both control and experimental groups and that activity of the partially hepatectomized rats returned to normal in seven days. These results differ from those of Dugal and Ross, however, in that activity of their sham-operated controls was back to normal in three days. Although their controls were subjected to a sham operation to assess the effect of surgical shock, it is possible that their treatment was a less rigorous one. In the present investigation partial hepatectomy per se did not appear to effect spontaneous activity.

The measurement of the uptake and release of $I^{131}$ from the thyroid gland was not correlated with the spontaneous running activity of rats i.e. the curves of the decrease in radioactivity over the thyroid gland showed no obvious differences between the 20 rats which ranged from inactive to active. The release curves of nine rats immediately on exposure to 2°C. and three weeks after cold exposure were similar to those obtained at room temperature except that the curves after exposure to cold were generally below that taken at room temperature (i.e. there was less radioactivity in the thyroid gland 24 hours after injection). This observation agrees with that of Cottle and Carlson (1956) who found less $I^{131}$ in glands of cold exposed animals than in controls as measured by the 24 and 48 hour uptakes.

Perhaps this could be the result of an increased speed of absorption of the $I^{131}$ into the blood stream from the site of injection, thus causing a faster uptake by the thyroid gland. The fact that the release curves (which were taken as valid measurements of thyroid activity) did not change on exposure to cold although the spontaneous activity was altered in eight of the nine rats, is further evidence that there is no correlation between thyroid function and spontaneous activity. Further, the uptake and release of $I^{131}$ by the tissues and the thyroid gland in the eight rats tested after three weeks in the cold during the first 24 hours after injection were remarkably similar. These results, that is, that there is no change in the release of $I^{131}$ from the thyroid gland on cold exposure, are in agreement with those of Livingstone (1962) but are in opposition to those of Cottle and Carlson (1956) and D'Angelo (1960).

After six to seven weeks at 2°C. the thyroid glands of the rats were slightly smaller than those of the controls. This is in contradiction to the general view that thyroid weights increase and then gradually return to normal after prolonged cold exposure. Perhaps the fact that the rats were in activity cages is the cause of this difference. That the adrenal glands were increased in size agrees with the data quoted by Sellers (1957) and D'Angelo (1960).

The conclusion that spontaneous running activity is not correlated with thyroid function was further supported by the experiments with thyroxine and propylthiouracil. The results agree with those of Hoskins (1927a), Lee and van Buskirk (1928) and Fregly (1961) who found that the spontaneous running activity of the rat does not appear to be related to the level of secretion of the thyroid hormone. The results of Richter (1933a) and Mann (1945) who obtained a decreased spontaneous activity with decreased

thyroid function might be explained as the natural decrease of activity with age rather than a result of the experimental treatment. They ran no control animals but used the pre-treatment activity levels as control values. In addition Richter's results are based on relatively few animals. Hall & Lindsay (1938) did run control animals and also found decreased spontaneous activity after thyroidectomy, hence their results are in conflict with those of the present investigation.

In keeping with the results of Richter (1936) and Tainter (1943) who attempted unsuccessfully to change the spontaneous activity of rats by injecting adrenaline chloride, the present investigation provides no evidence for a correlation between spontaneous activity and secretion of adrenaline. This finding agrees also with that of Bacq (1931) who found no change in spontaneous running activity as compared to controls after abdominal sympathectomy or adrenal inactivation. Although adrenalectomy has been reported to decrease spontaneous activity, it appears that this is a result of the imbalance in the mineral metabolism caused by the lack of the adrenal cortical hormones because activity can be returned to normal by giving saline instead of water (Richter, 1936). Moreover, although exposure to cold caused the average activity level to fall (with some individual rats showing increases and others decreases) the excretion of adrenaline was markedly increased. Thus activity seems unrelated to the adrenaline level.

The only suggestion that activity was correlated with the level of a catechol amine was a tendency for the more active rats to excrete more noradrenaline but this was observed at room temperature only. In the cold, noradrenaline excretion levels increased very markedly, as did those of

adrenaline, while the average activity dropped. The high excretion levels of catechol amines at 2°C. were undoubtedly related to the low temperature rather than to the level of activity which was lower than that at room temperature.

Clearly the moderate form of exercise indulged in by the rat is not related to the amount of adrenaline excreted and there is only a tendency for it to be related to excretion of noradrenaline at room temperature. This finding parallels those of many workers on humans. Although under 'stressful' muscular work, humans, rats and mice excrete large quantities of catechol amines (see page 82 in Part II), this condition was not involved in the present experiments.

The effect of castration and implantation of testosterone propionate pellets on the spontaneous running activity of male rats is perhaps the most interesting result obtained. A marked depression of activity after castration, as reported by many workers, was confirmed. This effect of castration was most marked in the very active rats. The fact that the level of the spontaneous activity of the control rats gradually declined and reached approximately the same level of activity as the castrates around 150 days of age has, however, not been generally recognized. It is seen in the graph presented by Hoskins (1925a) when rats were approximately 180 days old and is undoubtedly the reason that Hoskins (1927b) had four senile castrated rats which had activity similar to non-castrates. This also accounts for the statement that the effect of castration is more marked when it is performed at an early age (Wang et al.,1925; Gans,1927a). Castration causes an atrophy of the secondary sex organs and in the present experiments, no obvious change in the rate of body weight gain.

Some of the early workers (Durrant,1925; Hoskins,1927a; Hoskins et al., 1939) were unsuccessful in increasing the activity of castrated rats by some sort of replacement therapy. Perhaps their preparations were not active enough or if active not given at frequent enough intervals. Heller (1932) was able to restore the secondary sex organs of four male castrates to normal with repeated injections of testis hormone but was unable to obtain any consistent change in activity. Wang (in Hoskins 1927a), Hoskins and Bevin (1940) and Bugbee and Simond (1926) were, however, able to increase the activity of female rats by ovarian grafts or hormones. Some success with ovarian grafts in male rats and implantation of testes in both male and female rats was reported by Wang et al.(1925). Richter & Hartman (1934) and Richter (1934) also report success using injections of oestrin or giving human pregnancy urine in the drinking water. Perhaps the most successful experiment, however, was that of Young and Fish (1945) who were able to restore the activity in female rats to levels prevailing before castration by the implantation of estrone pellets. In the present experiment, activity was increased to the pre-castration level by testosterone propionate pellets which allowed the continuous slow absorption of the hormone. The secondary sex organs were enlarged to greater than normal size in the castrated rats with pellets thus indicating the effectiveness of the therapy. It should be noted, however, that in no case was a rat which had been relatively inactive before castration, made more active by the pellets. This observation agrees with that of Bugbee & Simond (1926) and Young & Fish (1945) that activity can only be increased to a certain level by the hormone and with the observation of Heller (1932) that the spontaneous activity of any individual rat is not proportional to the testis hormone secretion in that low running rats can be actively secreting the hormone.

That the activity of castrated rats could be increased by other factors than testosterone was seen on exposure to 2°C. where the activity of the three castrated rats increased. Both increases and decreases in activity were observed in the other groups of rats exposed to cold.

The implantation of testosterone propionate pellets into control rats inhibited the usual decline of activity with age so that the level of activity was maintained at a high level. No adequate explanation is offered for the failure of the animals' own testosterone to maintain its activity but this should provide an interesting field for further investigation.

The adrenal gland weights increased after castration as was also observed by Richter and Uhlenhuth (1954) in domesticated white male rats. The testes of the control rats with pellets were smaller than the controls without pellets undoubtedly due to a decreased production of the animals' own testosterone. The rats exposed to cold had enlarged adrenal glands, smaller testes which were intraabdominal, and smaller ventral prostates although the dorsal prostate and seminal vesicles were approximately the same as those at room temperature.

The rate of weight gain was notably slowed down in all rats in which pellets were implanted. This is in agreement with the results of Wang et al. (1925) who found that ovarian transplants into male rats caused a flattening of the body weight curve and of Hoskins and Bevin (1940) that injections of estriol glucuronide into 11 male rats in all cases caused a diminution of body weight, a decrease, however, that could not be correlated with the dosage of the hormone or the degree of augmented spontaneous activity.

## SUMMARY AND CONCLUSIONS

1. The spontaneous running activity of white male Wistar rats was recorded in standard revolving activity cages under constant conditions of light and temperature. There were very large differences in activity among various rats as well as marked fluctuations in the performance of any one rat. The average activity of a group of rats increased until the rats were approximately 85 to 100 days of age after which it declined to a low level when the rats were approximately 125 to 150 days of age and tended to remain fairly constant at this level. No consistent changes in activity were obtained on exposure to cold at 2°C.

2. No correlation was found between spontaneous running activity and food consumption, water intake or body weight gain.

3. There was practically no correlation between spontaneous running activity and decreased liver function produced either by carbon tetrachloride or by partial hepatectomy although very large doses of carbon tetrachloride did decrease activity to some degree.

4. There was no correlation between spontaneous running activity and secretion of thyroxine. The secretion of thyroxine was measured in normal rats by the release of radioactive iodine from the thyroid and was found to be similar in rats at room temperature and at 2°C. The secretion in the body was increased by subcutaneous injections of thyroxine and decreased (or completely inhibited) by propylthiouracil.

5. There was no proportional correlation between spontaneous running activity and excretion of adrenaline. The more active rats did, however,

show a tendency to excrete more noradrenaline at room temperature. Catechol amine excretion did not vary with spontaneous activity in the cold.

6. Spontaneous running activity is markedly reduced in male rats by castration in early adulthood. Activity can be increased by replacement therapy (implantation of testosterone propionate pellets which allows the continuous slow absorption of the hormone) to the pre-castration level. There is a wide variation in running activity in all animals, however, even with a constant large source of testosterone.

7. As rats grow older there is a steady slow decline in spontaneous activity so that at approximately 150 days of age the activity of control rats and castrated rats are similar. Testosterone propionate pellets in control rats, however, maintain the activity at a relatively high level, inhibiting the decline with age. No adequate explanation has been found for this observation. Castrated rats are also capable of increasing their spontaneous activity in the cold at 2°C.

8. Spontaneous running activity is a complicated phenomenon and is dependent upon the interaction of many internal and external factors.

## REFERENCES

BACQ Z.M.
Endocrinology 15: 34-40, 1931
The effects of abdominal sympathetomy, adrenal inactivation and removal of the stellate ganglia on the spontaneous activity of the albino rat.

BEIERWALTES W.H., JOHNSON P.C. & SOLARI A.J.
Clinical Use of Radioisotopes. W.B.Saunders Co.,Philadelphia, 1957

BROBECK J.R.
Amer. J. Physiol. 143: 1-5, 1945
Effects of variations in activity, food intake and environmental temperature on weight gain in the albino rat.

BRODY E.G.
Comp. Psych. Mono. 17(5): 1-24, 1942
Genetic basis of spontaneous activity in the albino rat.

BROWMAN L.G.
J. Exp. Zool. 94(3): 477-489, 1943
The effect of controlled temperatures upon the spontaneous activity rhythms of the albino rat.

BUGBEE E.P. & SIMOND A.E.
Endocrinology 10: 349-359, 1926
The increase of voluntary activity of ovariectomized albino rats caused by injections of ovarian follicular hormone.

CAMERON G.R. & KARUNARATNE W.A.E.
J. Path. & Bact. 42: 1-21, 1936
Carbon tetrachloride cirrhosis in relation to liver regeneration.

CATZ B., RAWI I., & GEIGER E.
Amer. J. Physiol. 174: 29-32, 1953
Activity of thyroid of cold-exposed rats evaluated by $I^{131}$ uptake and histometric studies.

COTTLE W.H.
Fed. Proc. 19 (Suppl.5): 59-63, 1960
Role of thyroid secretion in cold acclimation.

COTTLE M. & CARLSON L.D.
Endocrinology 59: 1-11, 1956
Turnover of thyroid hormone in cold exposed rats determined by radioactive iodine studies.

D'ANGELO S.A.
Fed. Proc. 19 (Suppl.5): 51-58, 1960
Adenohypophysial function in the guinea pig at low environmental temperature.

DEPOCAS F. & HART J.S.
J. Appl. Physiol. 10: 388-392, 1957
Use of the Pauling Oxygen Analyzer for measurement of oxygen consumption of animals in open-circuit systems and in a short-lag, closed-circuit apparatus.

DRILL V.A.
Pharmac. Reviews 4: 1-42, 1952
Hepatoxic agents: mechanism of action and dietary interrelationship.

DUGAL L.P. & ROSS S.
Rev. Canad. Biol. 2: 435-441, 1943
Effet de l'ablation partielle du foie sur l'activité spontanée du rat blanc.

DURRANT E.P.
Amer. J. Physiol. 70 :344-350, 1924
Studies on vigor. I.Effect of adrenal extirpation on activity of the albino rat.

DURRANT E.P.
Endocrinology 9: 221-228, 1925
Studies on vigor. III.The effect of ovarian extract feeding on the activity of ovariectomized white rats.

EAYRS J.T.
Brit. Jour. Anim. Behaviour 2(1): 20-24, 1954
An apparatus for analyzing the pattern of spontaneous activity in laboratory animals.

FARRIS E.J. & GRIFFITH J.Q.
The Rat in Laboratory Investigation. 2nd edition. J.B.Lippincott Co., Montreal. 1949

FREGLY M.J.
Amer. J. Physiol. 187: 297-301, 1956
Relationship between ambient temperature and the spontaneous running activity of normal and hypertensive rats.

FREGLY M.J.
Can. J. Biochem. Physiol. 39(6): 1085-1096, 1961
Effect of changes of ambient temperature on spontaneous activity of hypothyroid rats.

GANS H.M.
Endocrinology 11: 141-144, 1927 (a)
Studies on vigor. XIII.Effect of early castration on the voluntary activity of male albino rats.

GANS H.M.
Endocrinology 11: 145-148, 1927 (b)
Studies on vigor. Effect of fractional castration on the voluntary activity of male albino rats.

HALL V.E. & LINDSAY M.
Endocrinology 22: 66-79, 1938
The relation of the thyroid gland to the spontaneous activity of the rat.

HART J.S.
Fed. Proc. 17(4): 1045-1054, 1958
Metabolic alterations during chronic exposure to cold.

HELLER R.E.
Endocrinology 16: 626-632, 1932
Spontaneous activity in male rats in relation to testis hormone.

HITCHCOCK F.A.
Amer. J. Physiol. 75: 205-210, 1926
Studies on vigor. V.The comparative activity of male and female albino rats.

HITCHCOCK F.A.
Amer. J. Physiol. 83: 28-36, 1927
The total energy requirement of the albino rat for growth and activity.

HOSKINS R.G.
Amer. J. Physiol. 72: 324-330, 1925 (a)
Studies on vigor. II.The effect of castration on voluntary activity.

HOSKINS R.G.
Endocrinology 9: 277-296, 1925 (b)
Studies on vigor. IV. The effects of testicle grafts on spontaneous activity.

HOSKINS R.G.
Endocrinology 11: 97-105, 1927 (a)
Studies on vigor. XVI.Endocrine factors in vigor.

HOSKINS R.G.
Endocrinology 11: 136-140, 1927 (b)
Studies on vigor. XII.Thyroid administration in senility.

HOSKINS R.G., LEVENE H.M. & BEVIN S.
Endocrinology 25: 143-144, 1939
The relationship of male sex hormone to the level of bodily vigor in senility.

HOSKINS R.G. & BEVIN S.
Endocrinology 26: 829-832, 1940
The effect of estriol glucuronide on the spontaneous activity of senile male rats.

HSIEH A.C.L. & CARLSON L.D.
Amer. J. Physiol. 188: 40-44, 1957
Role of the thyroid in metabolic response to low temperature.

HUNT J.McV. & SCHLOSBERG H.
J. Comp. Psychol. 28: 285-298, 1939
The influence of illumination upon general activity in normal, blinded and castrated white rats.

JONES D.C., KITTELDORF D.J., RUBADEAU D.O. & CASTANERA T.J.
Amer. J. Physiol. 172: 109-114, 1953
Relationship between volitional activity and age in the male rat.

INGLE D.J. & HARRIS R.E.
Amer. J. Physiol. 114: 657-660, 1936
Voluntary activity of the rat after destruction of the adrenal medulla.

LACEY O.L.
Amer. J. Psychol. 57: 412-420, 1944
A revised procedure for the calibration of the activity wheel.

LACEY O.L.
J. Comp. Psychol. 38: 277-284, 1945
The dependence of behavior disorder in the rat upon blood composition.

LACQUET A.A.
Arch. Path. 14: 164-176, 1932
Experimental pathology of the liver. VIII. Effects of carbon tetrachloride on the normal and restored liver after partial hepatectomy.

LEE M.C. & BUSKIRK E.F.v.
Amer. J. Physiol. 84: 321-329, 1928
Studies on vigor. XV. The effect of thyroidectomy on spontaneous activity in the rat, with a consideration of the relation of the basal metabolism to spontaneous activity.

LIVINGSTONE S.D.
Ph.D. Thesis, University of Ottawa, 1962
Effect of vitamin A on the thyroid gland and ascorbic acid biosynthesis and evaluation of various parameters of thyroid function.

MANN C.W.
J. Psychol. 20: 91-99, 1945
The effect of thiouracil upon the heart rate, estrous cycle and spontaneous activity of the white rat.

MÜLLER R. & HIERSBERG H.
Zeitschr. vergl. Physiol. 40(5): 454-472, 1957
Über den Einfluss der inneren Sekretion auf die tagesperiodische Aktivität der weissen Maus.

PITT-RIVERS R. & PAPA J.R.
The Thyroid Hormones. International Series of Monographs on Pure & Applied Biology. Division: Biochemistry. Pergamon Press Inc. New York. 1959

RADHAKRISHNA-RAO M.V.
Indian J. M. Research 23: 1007-1014, 1936
Cirrhosis of the liver following chronic intoxication with carbon tetrachloride.

REDDY D.G., KRISHNAMURTHY K.R. & BHASKAR G.R.
Arch. Path. (Chic.) 74: 73-80, 1962
Carbon tetrachloride cirrhosis in rats.

REED J.D.
Psychol. Bull. 44(5): 393-412, 1947
Spontaneous activity of animals. A review of the literature since 1929.

RICHTER C.P.
Endocrinology 17: 73-87, 1933 (a)
The role played by the thyroid gland in the production of gross body activity.

RICHTER C.P.
Endocrinology 17: 445-450, 1933 (b)
The effect of early gonadectomy on the gross body activity of rats.

RICHTER C.P.
Amer. J. Physiol. 110: 499-512, 1934
Pregnancy urine given by mouth to gonadectomized rats: its effect on spontaneous activity and on the reproductive tract.

RICHTER C.P.
Endocrinology 20: 657-666, 1936
The spontaneous activity of adrenalectomized rats treated with replacement and other therapy.

RICHTER C.P.
CIBA Foundation Colloquia on Endocrinology Vol.III Hormones, Psychology and Behaviour. J.& A.Churchill Ltd., London. 1952
The effects of domestication on the steroids of animals and man.

RICHTER C.P.
J. Nat. Cancer Inst. 15(3): 727-738, 1954
The effects of domestication and selection on the behaviour of the Norway rat.

RICHTER C.P. & ECKERT J.F.
Endocrinology 21: 481-488, 1937
The effect of hypophyseal injection and implants on the activity of hypophysectomized rats.

RICHTER C.P. & HARTMAN C.G.
Amer. J. Physiol. 108: 136-143, 1934
The effect of injection of amiotin on the spontaneous activity of gonadectomized rats.

RICHTER C.P. & UHLENHUTH E.H.
Endocrinology 54: 311-322, 1954
Comparison of the effects of gonadectomy on spontaneous activity of wild and domesticated Norway rats.

RICHTER C.P. & WISLOCKI G.B.
Amer. J. Physiol. 86: 651-660, 1928
Activity studies on castrated male and female rats with testicular grafts, in correlation with histological studies of the grafts.

RICHTER C.P. & WISLOCKI G.B.
Amer. J. Physiol. 95: 481-492, 1930
Anatomical and behaviour changes produced in the rat by complete and partial extirpation of the pituitary gland.

RUNDQUIST E.A.
J. Comp. Psychol. 16: 415-438, 1933.
Inheritance of spontaneous activity in rats.

RUNDQUIST E.A. & BELLIS C.J.
Amer. J. Physiol. 106: 670-675, 1933
Respiratory metabolism of active and inactive rats.

SELLERS E.A.
Revue Canad. Biol. 16(2): 175-188, 1957
Adaptive and related phenomena in rats exposed to cold. A review.

SELYÉ H. & WAELSCH H.
Arch. exper. Path. u. Pharmakol. 161: 115-118, 1931
Beiträge zur Entgiftung im tierischen Organismus. III. Mitteilung: Bedeutung der Leber bei Avertin- und Magnesiumnarkosen.

SHIRLEY M.
J. Comp. Psychol. 8: 23-28, 1928 (a)
Studies of activity. I. Consistency of the revolving drum method of measuring the activity of the rat.

SHIRLEY M.
J. Comp. Psychol. 8: 159-186, 1928 (b)
Studies in activity. II. Activity rhythms; age and activity; activity after rest.

SHIRLEY M.
Psychol. Bull. 26: 341-365, 1929
Spontaneous activity

SLONAKER J.R.
J. Comp Neur. & Psych. 17(4): 342-359, 1907
The normal activity of the white rat at different ages.

SLONAKER J.R.
J. Animal Behavior 2: 20-42, 1912
The normal activity of the albino rat from birth to natural death, its rate of growth, and duration of life.

SLONAKER J.R.
Amer. J. Physiol. 68: 294-315, 1924
The effect of pubescence, oestruation and menopause on the voluntary activity in the albino rat.

SLONAKER J.R.
Amer. J. Physiol. 73: 485-503, 1925
Analysis of daily activity of the albino rat.

SLONAKER J.R.
Amer. J. Physiol. 93: 307-317, 1930
The effect of excision of different sexual organs on the development, growth and longetivity of the albino rat.

STEWART C.C.
Amer. J. Physiol. 1: 40-56, 1898
Variations in daily activity produced by alcohol and by changes in barometric pressure and diet, with a description of recording methods.

STONE C.P. & OBIAS M.D.
J. Comp. & Physiol. Psychol. 49(4): 407-410, 1956
Effects of hypophysectomy on behaviour in rats. V. Wheel turning activity and performance on the Maier reasoning apparatus.

TAINTER M.L.
J. Comp. Psychol. 36(2): 143-155, 1943
Effects of certain analeptic drugs on spontaneous running activity of the white rat.

TRIBE D.E.
Brit. Jour. Animal Behaviour 2(4): 140-143, 1954
The influence of exercise on the selection of food by rats.

TURNER C.D.
General Endocrinology. 3rd. edition. W.B.Saunders Co.,Philadelphia,1960.

WANG G.H.
Comp. Psychol. Monogr. (Balt.) 2,S.6: 1-27, 1923
The relation between "spontaneous" activity and oestrous cycle in the white rat.

WANG G.H., RICHTER C.P. & GUTTMACHER
Amer. J. Physiol. 73: 581-599, 1925
Activity studies on male castrated rats with ovarian transplants, and correlation of the activity with the histology of the grafts.

WOODS R. & CARLSON L.D.
Endocrinology 59: 323-330, 1956
Thyroxine secretion in rats exposed to cold.

YOUNG W.C. & FISH W.R.
Endocrinology 36: 181-189, 1945
The ovarian hormones and spontaneous running activity in the female rat.

# II. EXCRETION OF CATECHOL AMINES BY WHITE RATS AT ROOM TEMPERATURE AND IN THE COLD

## INTRODUCTION

The physiological rôles of the catechol amines have received much attention in recent years, particularly in reference to the part they play in the acclimation of an animal to a cold environment. The catechol amines which appear to be most important are the hormones adrenaline and noradrenaline. Adrenaline (epinephrine) is a secondary amine which has a calorigenic action in animals, increasing their oxygen consumption and the levels of blood glucose. Noradrenaline (norepinephrine or arterenol) is a primary amine which as the physiological mediator of the adrenergic vasomotor nerves is a powerful vasoconstrictor. The catechol amines are synthesized, stored and secreted by the adrenal glands, adrenergic nerves and the chromaffin tissue present in various sites throughout the body.

The steps in the biosynthesis of adrenaline and noradrenaline have been outlined by Goldstein and Contrera (1961). Noradrenaline is the precursor of adrenaline, which is formed by methylation. Cier and Peyrin (1960) showed that the small amounts of noradrenaline in the secretion from the adrenal glands did not result from a demethylation of adrenaline. Newly formed noradrenaline is either stored or is methylated to adrenaline. The proportions of the amines in adrenal extracts are fairly constant and characteristic of a species (Turner, 1960). The presence of two different types of secretory cells in the adrenal medulla has been demonstrated by the iodate reaction of Hillarp and Hökfelt for noradrenaline and the fluorescent test of Eränkö for adrenaline (Mirkin, 1961). The adrenal medulla is, however, generally conceded to be the main source of adrenaline as

adrenalectomy usually causes a marked reduction in adrenaline excretion without generally affecting the noradrenaline excretion. Noradrenaline content is highest in nerves, such as the sympathetic trunk, that are rich in adrenergic fibres; in fact, the noradrenaline content of sympathetic effectors is closely correlated with the number of adrenergic fibres that supply them. Noradrenaline is synthesized by the nerve cell and is stored in the axonic terminals. As it can be released continuously on repeated stimulation over long periods, it must be synthesized rapidly. Bertler et al.(1960) stated that a rapid rate of noradrenaline synthesis seems to be made possible by the fact that stimulation accelerates the production of dopa, a precursor.

The metabolism of adrenaline and noradrenaline has been much elucidated and the metabolic steps are outlined by Axelrod et al. (1961). The major pathway for metabolism involves O-methylation to yield metanephrine and normetanephrine, which are inactive metabolites which are then conjugated or deaminated.

It is generally considered that certain stimuli elicit specifically one or other type of secretion. The ratio of noradrenaline to adrenaline in venous blood taken from the adrenal gland after splanchnic nerve stimulation seemed to be dependent upon the nature of the evoking stimulus (Mirkin, 1961). Effluents obtained from several different organs after electrical stimulation of their autonomic nerve supply contained mostly noradrenaline. Adrenaline and noradrenaline are known to be rapidly removed from the blood and to be inactivated very rapidly in the tissues. The large amounts of catechol amines retained in the heart and other tissues suggests, however, that the hormones are bound to some constituent and protected from enzymatic attack. It has been observed that sympathetic nerve

endings selectively take up and retain circulating catechol amines. Hertting and Axelrod (1961) have shown that stimulation of the sympathetic nerve endings causes release of noradrenaline which was previously taken up from the circulation and bound.

A reflection of the secretion rate of adrenaline and noradrenaline (which are continuously secreted in small quantities from the body tissues) can be obtained from the amounts of the hormones appearing in the urine. Although a large proportion of the secreted hormones are metabolized to various breakdown products before being excreted, an indication of the relationship between the concentration of hormones in the blood and urinary concentrations has been obtained by experiments in which adrenaline and noradrenaline were infused into the blood stream with concurrent estimations of the amounts of these amines appearing in the urine. Although the results are variable, approximately two to four per cent of the noradrenaline and two to five per cent of the adrenaline administered are reported to appear unchanged in the urine (Bickel et al.,1961; Botting,1960; Frankenhaeusser et al.,1961; LeDuc,1961b). Although a proportion of the catechol amines excreted in the urine exist as esterified products which cannot be estimated without very strong acid (pH 1) hydrolysis, it is now generally believed that conjugation plays a minor role in the inactivation of physiological amounts of catechol amines (Axelrod et al.,1961).

The amounts of the hormones and their metabolites which appear in the urine of animals maintained at room temperature seem to depend on several factors. The state of hydration of the animal may be important. Perman (1961) showed that adrenaline excretion was increased at moderate hydration levels in rats and that there was also some increase of noradrenaline excretion with rising diuresis. On the other hand LeDuc (1961) reported that

diuresis by itself had little effect on the excretion of catechol amines in rats at room temperature. Restriction of water, however, significantly increased the output of adrenaline. He also reported that fasting caused a three-fold increase in the adrenaline excretion. Injections of catechol amines into animals may increase or decrease the volume of urine. Diuresis is produced by an elevated arterial pressure and an interference with the tubular reabsorption of water in the kidney. Botting and Lockett (1961) and Botting et al. (1961) reported that the subcutaneous injection of 50-200 μgm. adrenaline/100grams of rat or 3-5 μgm. noradrenaline/100 grams of rat had a diurectic action in water-loaded rats. On the other hand an antidiuresis with retention of sodium and a decreased excretion of potassium without change in either filtration rate or renal plasma flow was obtained with injections of 5-30 μgm. adrenaline/100 grams of rat.

According to LeDuc (1961b) the output of adrenaline and noradrenaline in rats was lower during the summer months than during the winter even in rats kept at the same room temperature. Excretion of catechol amines was found by him to vary also with age and weight. Relative to body weight, there was a decrease in noradrenaline excretion with increasing weight whereas adrenaline excretion increased to a maximum in rats weighing about 200 grams and then slowly declined.

From their experiments with human subjects, Euler and Hellner (1952) concluded that a correlation exists between the degree of 'stress' involved in muscular work and the output of catechol amines, moderate work causing an insignificant rise in the catechol amine secretion and maximal work inducing a high output. This conclusion has been supported by Botting (1960), Hasselman (1960) and Jacobs et al. (1961) who reported that fairly severe exercise was necessary to cause a marked change in urinary catechol amines

of humans. An hour of continuous running or walking was insufficient to show an increase in catechol amine excretion. Although Oesterling et al. (1962) found only a slight increase in free catechol amines during exercise, they reported a significant increase in the excretion rate of conjugated catechol amines. Kärki (1956) has reported that exhausting muscular work of long duration greatly increases the adrenaline and noradrenaline excretion, while less strenuous work appears to cause an increase in noradrenaline only. Goldfien et al. (1961) reported that normal subjects have higher noradrenaline levels during mild activity than at bed rest and that an increase in both adrenaline and noradrenaline levels is observed after strenuous exercise.

Beauvallet et al. (1961) reported that severe muscular work causes a pronounced lowering of the adrenaline and noradrenaline levels in the adrenals of rats. In mice allowed to swim to exhaustion daily for 30 days, the volume of the noradrenaline-containing medullary parenchyma was increased while the volume of the adrenaline-storing parenchyma was not affected in the experiments of Eränkö and Harkonen (1961b). In a parallel study on rats, Eränkö et al. (1962) found that the volume of both the adrenaline-storing and noradrenaline-storing parts of the medulla increased. Forced running of rats in rotating wire cylinders by Eränkö and Harkonen (1961a) caused an almost complete loss of catechol amines (chemically) from the adrenal medulla and the histochemical reactions turned almost negative but returned to normal in six days. The observed depletion may not, however, have been due to muscular work alone but other factors such as general exhaustion, lack of sleep and diminished food and water intake may have been important contributing factors. In any case prolonged muscular work does not seriously interfere with the synthesis and/or storage of adrenaline and noradrenaline in the adrenal medulla.

It has been established that there is an increased production and secretion of catechol amines in animals exposed to cold. An increased excretion in rats chronically exposed to cold is reported by Cottle (1960), LeBlanc and Nadeau (1961) and LeDuc (1961a). The increased levels of catechol amines in cold not only bring about decreased heat loss owing to their vasoconstrictor action, but also increase heat production owing to their stimulating effect on metabolism. Although adrenalectomized rats can increase their adrenaline secretion in the cold, most of the adrenaline secreted on exposure to cold comes from the adrenal medulla (LeBlanc & Nadeau, 1961; LeDuc, 1961). Although acute cooling causes a definite increase in the release of adrenaline and its depletion in the adrenal glands, the catechol amine content in the adrenal gland of cold-adapted rats may be normal or increased (Euler, 1960).

The development of non-shivering thermogenesis in rats acclimated to cold greatly extends their range of activity as the total heat production is sufficient to offset the fall in insulation and body temperature is maintained (Hart, 1961). The suggestion by Hsieh and Carlson (1957) that l-noradrenaline is the mediator of the non-shivering heat production in the cold-acclimated white rat is supported by the results of Depocas (1960) and LeDuc (1961b). While noradrenaline injected intramuscularly had little calorigenic effect in warm-adapted rats, it markedly elevated the oxygen consumption of rats acclimated to 5°C. (Hsieh and Carlson, 1957). Although the calorigenic effects of adrenaline are also intensified in the cold-acclimated rat, noradrenaline is accepted as the more likely mediator of non-shivering thermogenesis because neither cold exposure nor noradrenaline induces marked hyperglycemia (Depocas, 1961; LeDuc, 1961b). The striking sensitivity of cold-acclimated rats to continuous intravenous infusion of

noradrenaline was dependent on the dose and increased with time of exposure to 6°C. being maximal in four weeks (Depocas, 1960). Depocas (1960) has proposed that striated muscle is the site of the major alteration in sensitivity to noradrenaline induced by acclimation to cold. Hart and Jansky (1962) have also concluded that elevated heat production in the absence of shivering occurs in skeletal muscle.

LeDuc (1961b) found that the excretion of noradrenaline in rats exposed to 3°C. was large, being nearly maximal during the first 24 hours, and was sustained for up to six months at a high level about three times as high as that of a control group kept at 22°C. although there was a slow and steady decline which was most pronounced during the second month of cold exposure. The excretion of adrenaline was maximal after one week in the cold and decreased afterwards more rapidly than that of noradrenaline although it always remained at a level one and a half to two times greater than that of a control group at 22°C. LeDuc hypothesized that adrenaline acts as a second line of defense which is only needed when the noradrenaline mechanism becomes saturated. Further, he supposed that the increased sensitivity of cold-acclimated rats to noradrenaline at one month (as shown by Depocas) could be related to the decline he found in the excretion of noradrenaline at this time.

* * * *

Measurements of the amounts of adrenaline and noradrenaline excreted in the urine of cold-exposed rats in activity cages reported in Part I of this thesis were markedly different from those obtained by LeDuc (1961). The results differed not only in the absolute amounts of the catechol amines excreted but also in the changes in levels with the time of cold exposure. In the hope of determining the cause of these discrepancies, the excretion

of adrenaline and noradrenaline was measured at various intervals over 20 weeks in both Wistar and Sprague-Dawley rats exposed to cold at different ages. Sprague-Dawley rats were the strain used by LeDuc. It was also felt necessary to determine if the excess amounts of catechol amines excreted after injections of either adrenaline or noradrenaline were the same at room temperature and in the cold.

# MATERIALS

## Animals

Male white rats of the Wistar strain obtained from Robidoux Animal Laboratory, St. Constant (La Prairie), Quebec were used in the experiments. To investigate the possibility of strain differences, Sprague-Dawley rats obtained from Quebec Breeding Farms, Ste. Eustache, Quebec were used with the Wistar rats during the second experiment. The animals were about six weeks old and weighed about 100 grams when they were received. They were kept in individual wire mesh cages except for the rats at the beginning of experiment 2a where the rats were kept in activity cages. The rats were maintained on Purina Laboratory chow and tap water and were usually weighed twice a week.

All experiments were carried out in one of three rooms in which temperature, light, relative humidity and sound level were kept relatively constant. One room was maintained at 29 ± 2°C. or 23 ± 2°C. (the latter temperature was used for most experiments) with a relative humidity of about 20%. A second room at 25 ± 3°C. and relative humidity 14% was used only for a short time. The cold room was maintained at 2 ± 3°C. (mostly 2 ± 2°C.) and had a relative humidity just over 50%. The refrigeration unit would on occasions stop functioning and the unit began to defrost. The rats were then moved for short periods of time to another cold room at the same temperature. The temperature range of each room was read at least weekly on a maximum-minimum thermometer. All rooms were illuminated by artificial lights which were turned on for 12 hours daily by Paragon time switches.

## Adrenaline and Noradrenaline

Adrenaline and noradrenaline were obtained in both a liquid and a powdered form as follows:

adrenaline
(1) Epinephrine hydrochloride solution
Parke Davis & Co. Ltd., Brockville
-each cc. contains 1 mgm. adrenaline as the hydrochloride dissolved in physiological saline containing not more than 0.1% sodium bisulphite as preservative

(2) l-epinephrine bitartrate powder M.W. 333.3
California Corporation for Biochemical Research
3625 Medford Street, Los Angeles 63, California

noradrenaline
(1) Levophed bitartrate, brand of levarterenol bitartrate
Winthrop Laboratories of Canada Ltd., Aurora
-0.2% (0.1% base) , each cc. contains 2 mgm. of levophed bitartrate monohydrate, equivalent to 1 mgm. levophed base in isotonic saline solution with not more than 2 mgm. sodium bisulfite as preservative

(2) l-arterenol bitartrate hydrate powder M.W. 337.3
California Corporation for Biochemical Research
3625 Medford Street, Los Angeles 63, California

Standard stock solutions containing 100 µgm./ml. were kept in brown bottles in the refrigerator and appeared to retain their activity for several months. The solutions were prepared by diluting with 0.2N acetic acid as follows:

adrenaline
(1) 1 cc. Parke Davis adrenaline made up to 10 cc.
(2) 18.2 mgm. l-epinephrine bitartrate powder made up to 100 cc.

noradrenaline
(1) 4 cc. ampoule Winthrop levophed bitartrate made up to 40 cc.
(2) 19.9 mgm. l-arterenol bitartrate hydrate powder made up to 100 cc.

Working solutions containing 1 µgm./ml. were prepared by diluting 1 cc. of the stock solution to 100 cc. with 0.02N acetic acid and these were made up fresh for each day an assay was done although the working solution appeared to keep without loss of activity for at least one month for adrenaline and one week for noradrenaline.

Throughout the experiments the amounts of catechol amines in urine samples were determined by a fluorescent technique. Standard curves of fluorescence plotted against known concentrations of either adrenaline or noradrenaline were linear. Standard curves of noradrenaline made up from either the powder or from the liquid were identical. At pH 6.0 the standard curves for adrenaline made up from either the powder or the liquid were also identical. At pH 3.0 ,however, the fluorescent values were slightly higher for the powder. The liquid solution in the ampoules was probably contaminated with a slight amount of noradrenaline.

For injection experiments the catechol amines were made up from the solutions in the ampoules in concentrations of 100 µgm./cc. , the dilutions being carried out with a solution of 5% dextrose.

## Fluorometer

Measurements of fluorescence were made on a Turner Model 110 fluorometer (G.K.Turner Associates, 2425 Pulgas Avenue, Palo Alto, California, U.S.A.) using primary filter #47B which isolates the 436 mµ mercury line (also passes the 405 mµ mercury line) and secondary filter #2A-12 which has a sharp cut at 510 mµ. This fluorometer was found to be superior to the Coleman 12C instrument in that it was much more stable and sensitive (high sensitivity was necessary as the catechol amine content of rat urine was small) and used smaller volumes of solution (5cc.). Although the Aminco-Bowman spectrophotofluorometer is undoubtedly a more versatile instrument, it was unnecessary to have a diffraction grating instrument for the present procedure as suitable filters are available. A wide variety of wavelengths is recommended by various investigators in this field. Drujan et al. (1950) activated at 420 mµ and measured the fluorescence at 510 mµ which is essentially the same as the present filters used in the Turner flucrometer.

# METHODS

## Collection of Urine

Rats were placed in metal metabolism cages for collection of urine over 24-hour periods. The cages were thoroughly washed after each 24-hour collection. As the urinary output of some rats at room temperature was extremely small, especially when the rats were young, a second 24-hour specimen was often collected and pooled with the first before extraction and assay. In this way it was possible to determine the catechol amine output of individual rats and there was no need to pool 24-hour samples from several rats in order to obtain sufficient amounts as did LeBlanc and Nadeau (1961).

Most authors agree that at pH 2 to 3 urinary catechol amines are stable for several days at room temperature and for a long time in the cold. At pH 2 or higher no appreciable splitting of conjugated catechol amines takes place (Euler and Orwén, 1955). Euler and Floding (1956) observed no loss of catechol amines when acidified urine with a pH less than 6 was kept for a week at room temperature. Samples with a pH of 6 and above often showed on standing growths of bacteria which caused the solution to become alkaline and thus inactivated it. LeBlanc and Nadeau (1961) collected their rat urine samples in 0.5 cc. 0.1N HCl while LeDuc (1961) added 1N HCl to the rat urine samples so that the pH was maintained around 3. In the present investigation urine was collected for 24 hours in 50 ml. glass beakers containing 1 cc. toluene as preservative (to reduce evaporation) and 0.5 cc. HCl. At room temperature where the urine volumes were small, 0.5 cc. 1N HCl was used; and in the cold room where the urine volumes were considerably larger, 0.5 cc. 6N HCl was added. The pH of the urine samples was then usually about 4. Subsequent studies on the recovery of catechol amines

added to urine revealed no differences between urine acidified with 0.5 cc. of 0.1N, 1N or 6N HCl. It would thus appear that very low pH's are not necessary for the stability of the catechol amines.

All urine samples were measured to the nearest half cc. and were filtered through Whatman #1 filter paper. After the initial experiments when it was observed that the filter paper absorbed about 2 cc. of fluid, the filter papers were dampened with distilled water to reduce loss of volume. If the volume of urine was small (less than 10 cc.) the collection beakers were thoroughly rinsed with distilled water and the washings also filtered. In such cases the urine volume was first recorded and the final volume (urine + distilled water washings) was extracted in entirety.

Evaporation from the collection flasks introduced a slight error in volumes especially at room temperature. Beakers containing 0.5 cc. acid and 1.0 cc. toluene and left for 24 hours were completely dry at room temperature but still had almost 0.5 cc. remaining in the cold. When beakers containing 2, 5, or 10 cc. acidified rat urine + 1 cc. toluene were left for 24 hours, the volume remaining in the cold was 2, 5, and 10 cc. but at room temperature only 0.7, 3.6 and 8.5 cc. Thus there was practically no loss of volume in the cold and only a relatively large loss at room temperature when the urine volume was very small. As the complete volume if less than 10 cc. was taken for extraction and assay, there should have been no loss of catechol amines. There was, however, a small loss of urine in the metabolism cages because all of it did not reach the collection flask.

On occasion water bottles leaked and not only increased the volume and diluted the urine in the collection beakers but sometimes caused them to overflow into the metal tray beneath the beaker. Also at times water dripping from the overhead refrigeration plates in the cold room caused the same

difficulty. Assays were performed on 10 cc. of the diluted samples and results were generally included if they were similar to those of the other rats in the group.

## Extraction and Assay of the Catechol Amines

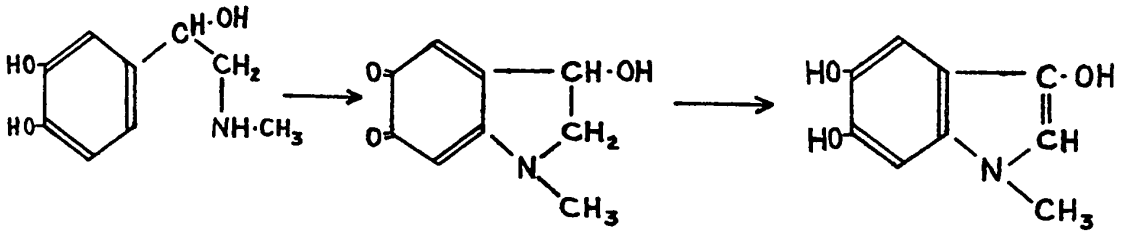
Adrenaline and noradrenaline were determined by a slight modification of the method of Drujan et al. (1959). The catechol amines were extracted from the urine samples usually either the day of collection or on the day following. The samples were then stored in the refrigerator until they were assayed, usually one to three days after the initial collection.

Basically the extraction procedure involves adsorption of the catechol amines on neutral alumina at pH 8 and elution with dilute acetic acid. The filtered urine is adsorbed by the alumina in the presence of the sodium salt of ethylene diamine tetra-acetic acid (versene). This substance is added so that it may chelate with calcium ions present in the sample (Botting, 1960). These ions (Ca) might possibly interfere with the fluorescence later in the procedure. Moreover Euler and Floding (1956) state that versene prevents oxidation of noradrenaline at pH 3.0. Woelm (Germany) alumina is claimed by many workers to be the best although many laboratories quite satisfactorily employ a reagent grade. Recoveries in the present investigation using Burrell Corporation activated alumina (obtained from Canadian Laboratory Supplies) and Woelm neutral aluminum oxide (obtained from Research Specialties Co., 200 South Garrard Blvd., Richmond, California) were almost identical. The adsorption on alumina in alkaline medium probably depends on the formation of a coating of aluminium hydroxide on the surface of the alumina grains (Euler and Orwén, 1955). During elution this layer together with the catechol compounds adsorbed is dissolved in acid leaving the "carrier" grains behind.

Accroding to Euler and Orwén (1955) adsorption on alumina is specific for dihydroxyphenyl compounds, and at pH 8.2 according to Euler and Lishajko (1959) the catechol amines in urine are stable for at least one hour. Euler and Lishajko (1959) also state that titration of urine to pH 8.0 gave slightly less yield and recovery than titration to pH 8.5. At pH 9.0 the recovery was markedly diminished. Euler and Floding (1956) found that the adsorption on a column gave a somewhat better recovery of added catechol amines than stirring with alumina. As rat urine is relatively concentrated and deep in colour, it was hard to judge exactly the phenolphthalein end point. Consequently, the pH of the extraction mixture was closer to pH 9.0-9.5 than to pH 8.0. This may be the reason that recovery experiments gave somewhat lower (although consistent) values than expected. The acetic acid eluates kept in the refrigerator did not appear to lose their activity at least for one to two weeks as assays carried out after that time were similar to those done earlier. This thus agrees with the statement of Euler and Orwén (1955) that in the refrigerator extracts at pH 4 maintained their full activity for a week. When stored at room temperature up to 48 hours, they also noted no inactivation in the eluate.

Although colorimetric methods for the estimation of catechol amines are available, they cannot be applied to urine extracts as the amounts present are too small. The most sensitive chemical method of estimating urinary catechol amines is by the formation of derivatives which fluoresce. This can be carried out in two ways. Firstly, by direct oxidation of the catechol amines to their corresponding chromes and the rearrangement of these to highly fluorescent trihydroxyindole derivatives. Secondly by a condensation reaction of the amines with ethylene diamine which also forms a fluorescent derivative. This latter method is not specific for adrenaline

and noradrenaline and Euler et al. (1955) state that the presence in urine of large amounts of biologically inactive catechol compounds makes the fluorescence reaction with ethylene diamine unsuitable for estimation of catechol amines. Consequently, the catechol amines are assayed in the present investigation by oxidation to the corresponding chromes, adrenachrome and noradrenachrome, rearrangement to the corresponding fluorescent lutines by the addition of strong alkali, and stabilization with ascorbic acid; then the amount of fluorescence is read in the fluorometer.




adrenaline adrenochrome fluorescent adrenolutine
1-methyl-3,5,6 trihydroxyindole

The method of Drujan et al. (1959) was adopted as it required a urine volume of only 10 cc. whereas the majority of other methods required 100 cc. According to Euler and Floding (1955) only those catechol amines which possess a hydroxy group at the $\beta$-carbon (next to the ring) and hydrogen at the $\alpha$-carbon in the side chain are able to form fluorescent compounds from the corresponding chromes. Thus dopamine gives only a weak fluorescence - in normal urine about 3% of that of noradrenaline when oxidized at pH 6.0. In biological material there is little reason to expect any appreciable amounts of catechol amines with a hydroxy group in the side chain other than adrenaline and noradrenaline. The degree of fluorescence produced is compared with the fluorescence resulting from standard solutions of adrenaline and noradrenaline treated similarly. The oxidation is carried out at two different pH's. At pH 6.0 both adrenaline and noradrenaline are oxidized but at pH 3.0 only adrenaline.

It is generally agreed that the oxidation time should be accurately measured although it has been reported that a three-minute oxidation instead of two minutes does not seem to significantly alter fluorescence. Euler and Lishajko (1961) reported that although two minutes was an adequate oxidation time for standards, urine eluates required three minutes oxidation time in order to give optimal fluorescence values. Yet they had earlier reported (1959) that increasing the oxidation time from two to three minutes resulted in a reduced yield. Further increasing the oxidation time did not increase the yield but it was found essential to have thorough mixing of the eluate with the sodium hydroxide-ascorbic acid mixture (Euler and Lishajko, 1961). Although three minutes oxidation time was timed exactly in the present investigation, little difference was observed when the oxidation times were two, three or four minutes. It was absolutely essential to stabilize the fluorescent compounds once they were formed by the addition of the antioxidant vitamin C (ascorbic acid). Although this was always added immediately after stopping of the iodine oxidation by thiosulphate, only small differences were found when it was added one, two or three minutes later. Euler and Floding (1955) stated that maximal fluorescence was obtained in a fairly narrow concentration region and that with large amounts a quenching effect was noted. As observed by Euler and Lishajko (1961), the mixture of ascorbic acid and strong alkali tended to age rapidly and it was thus used within two hours after its preparation in the present investigation.

Drujan et al. (1959) advised allowing the samples to stand 15 minutes before reading in the fluorometer as readings taken earlier would give erratic results perhaps because of the existence of other fluorescent compounds. Euler and Floding (1956) stated that the fluorescent product was

stable for a period up to an hour and Euler and Lishajko (1961) that the net fluorescence values of standard solutions of adrenaline and noradrenaline were well maintained for one hour and thereafter declined slowly. Botting (1960) found only a small diminution in the fluorescence obtained with adrenaline up to 80 minutes after the formation of the fluorescent derivative. In the present investigation the fluorescence value of noradrenaline was found to increase rapidly during the first 15 to 40 minutes but then remained stable from 45 to 105 minutes after which the fluorescence declined slowly as measured up to 180 minutes. The level of adrenaline fluorescence was much more stable with time. At pH 3.0 the reading was relatively constant from 15 to 180 minutes, while at pH 6.0 fluorescence increased up to 30 minutes, was constant from 30 to 150 minutes and decreased very slightly from 150 to 180 minutes. Blanks containing all solutions except the catechol amines gave constant fluorescent readings from 30 to 180 minutes. Consequently, in the present method the solutions were read in the Turner fluorometer exactly one hour after the last reagent was added. The range selector was set for the highest range where the reading was on scale.

The fluorescence of the blanks depended in small degree on the age of the solutions. As read against a bakelite rod (adjusted to zero fluorescence) readings on the x30 scale varied from 8-11% at pH 3.0 and from 12-15% at pH 6.0 but were constant throughout any one assay. The fluorescent readings (read against the blank which was adjusted to zero fluorescence) are expressed by comparison with the fluorescence of standard solutions which are treated in the same way to produce the fluorescent trihydroxyindole derivatives. Using serial dilutions of the stock solutions, calibration curves for both adrenaline and noradrenaline were constructed using

the two most sensitive scales of the instrument (x10 and x30) and found to be linear. On the x30 scale, 0.1 μgm. adrenaline gave a fluorescence of 81% at pH 3.0 and 24% at pH 6.0 while 0.1 μgm. noradrenaline at pH 6.0 gave a fluorescence of 22% during the daytime and 15.5% at night. According to Euler and Floding (1956) 4% of the noradrenaline present is oxidized along with adrenaline at pH 3.0. In the present experiment, it was almost negligible; 1.0 μgm. noradrenaline (more than normally present in a sample to be assayed) at pH 3.0 on x30 scale gave 2 to 4% fluorescence.

Midway in the investigation there was a marked change noted in the calibration curve for noradrenaline - the fluorescence readings being somewhat lower. The reason for this was not immediately apparent and several factors were tested. A complete new set of solutions was prepared and a fresh supply of noradrenaline was obtained. Assays using both noradrenaline made up from either the powder or from the solutions in the ampoules gave almost identical results. The only reason for the difference in assay that could be found was that the fluorescent values for noradrenaline but not adrenaline were higher when the assay was done in the daytime as compared to night. The fluorescent laboratory lights were nearly always on - both day and night. During the second half of the investigation most of the assays were done at night. To test the above hypothesis, however, working solutions of noradrenaline were made up from the stock solution and were assayed both in daylight and after dark on two consecutive days. The results confirmed the above hypothesis. Subsequent assays have also been consistent with this view.

For any assay up to 12 samples each at two concentrations (0.4 and 0.8 cc.) and at two pH's (3.0 and 6.0) could be run along with the blanks and standards. Four samples were read in the fluorometer every five minutes.

The amounts of adrenaline and noradrenaline were calculated as follows:

$$\text{Amount of adrenaline} = \frac{\text{observed reading for sample at pH 3.0}}{\text{calibration factor for adrenaline at pH 3.0}}$$

$$\text{Amount of noradrenaline} = \frac{\text{observed reading for sample at pH 6.0}}{\text{calibration factor for noradrenaline at pH 6.0}}$$

$$- \frac{\text{calibration factor for adrenaline at pH 6.0} \times \text{amount of adrenaline}}{\text{calibration factor for noradrenaline at pH 6.0}}$$

where the calibration factor = fluorescent scale deflection per microgram of catechol amine. After calculating the total amount of catechol amines present in the eluate (3.5 cc.) which was obtained from 10.0 cc. of urine (or less if 10.0 cc. was not collected), the amount of catechol amines in the total 24 hour volume of urine can then be determined.

Recovery of Catechol Amines

Known amounts of adrenaline and noradrenaline were added to urine or to water and put through the extraction and assay procedures. Recovery from the urine samples was fairly constantly about 50% for both adrenaline and noradrenaline. Values in the results section of the thesis have not been corrected for this loss as all the results are on a comparative basis.

There are many factors involved in the procedure which can alter the recovery of catechol amines added to urine and although most workers obtain a fairly consistent range of recovery, they find on some occasions that the recovery for unknown reasons is markedly lower. Botting (1960) found that the percentage recoveries of the amines after extraction and assay varied from 60-76% even after much practice with the method. In the original method of Sourkes et al. (1957), they stated that recovery is variable between runs but is usually above 35%. In their revised method (Drujan et al.,1959) the mean recovery was 70%. Euler and Floding (1956) stated that recovery was usually about 70% but with a newer method Euler and Lishajko

(1961) obtained a recovery of 70 to 100% with only one exception. LeDuc (1961) used the methods of Euler et al. and obtained an average recovery of 75%.

For the recovery experiments in the present investigation five different concentrations of the catechol amines were used (0, 0.525, 1.05, 1.75 and 3.50 µgm.). These were added to 10 cc. of a pooled sample of urine and were acidified with 0.5 cc. 0.1N, 1N or 6N HCl. Six samples of each concentration were prepared, three (one at each acidity level) were extracted immediately while the duplicates were left for 24 hours at room temperature before they were also extracted. The amount recovered (after subtracting the amount present in the urine sample alone) showed that there was no loss of activity due to standing for 24 hours at room temperature nor was there any loss of activity in the less acid samples. Adrenaline recoveries were carried out at pH 3.0 and 6.0 and noradrenaline at pH 6.0. The average recovery in all cases was 50%. When the experiment was repeated using concentrations of 0, 0.15, 0.30, 0.50 and 1.00 µgm. added to either urine or water and left for 24 hours at room temperature approximately 50% was recovered for the noradrenaline from both the urine and water but although 50% of the adrenaline was recovered from the urine samples only 10% was recovered from the water both at pH 3.0 and 6.0. Therefore, there must be something in the urine which stabilizes the adrenaline. Mann (1953) states that both adrenaline and noradrenaline are considerably more stable in urine than in water, even at body temperature and alkaline pH and that this stability is due to the ascorbic acid and phosphates present in the urine.

It is felt that the recoveries although quite consistent might have been higher than 50% if the pH of the extraction mixture had been kept closer to pH 8.5 than to 9.0 or 9.5.

## Experiment 1

### Amounts of Catechol Amines Excreted after Injection

The first experiment was set up to determine if the amounts of catechol amines excreted after the injection of either adrenaline or noradrenaline were similar at room temperature and in the cold, and if the amounts varied with time of exposure to cold. Sixteen rats (average weight 133 grams) were placed in the cold at 2°C. three days after they were received while ten rats (average weight 138 grams) were kept at 23°C. Urine was collected for 24 hours before and 24 hours after the subcutaneous injection of either adrenaline or noradrenaline, and both 'before' and 'after' samples for any one rat were assayed on the same day. The differences in the catechol amines in the two samples were considered to be due to the excretion of the injected catechol amines. Solutions for injection were made up in a concentration of 100 μgm./cc. immediately before the injection although assays indicated that the solutions kept their initial activity on refrigeration. These solutions were assayed with standards diluted from stock on the same day that the urine samples were assayed.

To determine if the amount of injected catechol amine excreted varied with time, injections of either adrenaline or noradrenaline were given subcutaneously to rats at room temperature and then four to five days later to the rats in the cold on the following schedule:

| Noradrenaline | | Adrenaline | |
|---|---|---|---|
| 23°C. | 2°C. | 23°C. | 2°C. |
| Day 1 | Day 5 | Day 8 | Day 12 |
| 14 | 18 | 20 | 25 |
| 42,55 | 46 | 48 | 53 |

where Day 1 = first day for the rats in the cold. For the adrenaline injections half of the rats received 25 μgm. and the other half 50 μgm. while

for the noradrenaline injections half of the rats received 50 µgm. and the other half either 25 µgm. or 100 µgm. Five of the ten rats at 23°C. were injected with 25 µgm. on Day 46 and were subsequently injected with 100 µgm. on Day 55. At the end of the experiment the 11 surviving rats in the cold and the ten control rats were killed.

## Experiment 2

### Excretion of Catechol Amines at Room Temperature and in the Cold

The second experiment to determine the changes in excretion of naturally produced catechol amines on exposure to cold was initially carried out on 20 'old' rats; the first urine samples were taken when the rats were approximately seven and a half months old. This experiment was than repeated using rats approximately six weeks old at the beginning.

(a) Two 24-hour urine samples were collected one week apart from 20 rats (average body weight 456 grams) maintained at 25° or 29°C. in activity cages and the results were averaged. The rats were exposed to 2°C. one week later when they were about 32 weeks old. After six weeks in the cold, the rats were removed from the activity cages and placed in individual metal cages. Urine samples were collected at various intervals (Day 7,14, 21,28,35,49,56,86,116, and 146) over a five-month period in the cold. At the end of this time the seven surviving rats were killed.

(b) Twenty-one Wistar and 21 Sprague-Dawley rats approximately six weeks old were divided into four groups. Groups I, II, and IV each had six Wistar and six Sprague-Dawley rats while Group III had three of each. Group IV served as controls and was maintained at 23°C. throughout the experiment. Although all the rats were of the same age, they were exposed

to cold at different times. Thus Group I was exposed to 2°C. when they were about six weeks old and weighed 123 grams on the average. Group II was exposed to 2°C. 30 days later when they were 10 weeks old and weighed 246 grams on the average. Group III was exposed to 2°C. 70 days after those of Group I when they were 16 weeks old and weighed 368 grams on the average. After 20 weeks at 2°C. the survivors of each group were returned for 10 days to 23°C. after which they were killed.

Urine samples were collected and assayed at various time intervals. For the rats exposed to cold these were at Day 0,1,2,4,8,11,15,18,22,29,36, 50,64,85,113, and 141. Following the collection of urine on Day 141, the rats were returned to 23°C. and urine samples were collected for Day 1, 4, and 8 of their return to room temperature. Urine samples from the control rats were collected and assayed at the following times as measured by the time scale for Group I : Day 0,10,17,24,39,46,53,61,73,87,109,137,159,196, and 215. Thus Day 1 in the cold for Group II corresponds to Day 31 for the controls and Day 1 in the cold for Group III corresponds to Day 71 for the controls.

Several rats including four of the six Sprague-Dawley controls died during the experiments. When the rats of Group II were killed, half of the control rats were also killed (equivalent to Day 189 for the controls). Thus three of the six Wistar and one of the three surviving Sprague-Dawley rats were killed at this time. One of the two remaining Sprague-Dawley rats subsequently died before the end of the experiment.

## RESULTS

Approximately 50% of the adrenaline and noradrenaline (as determined by studies of recovery of catechol amines added to urine) are lost during the extraction procedure. In general, as only comparative values are important in the present investigation, no correction for this loss has been applied to the values obtained. It is assumed, however, that the correct absolute amounts would be approximately double those reported.

The Purina Laboratory chow was tested for fluorescent material. LeDuc (1961b) reported that one unspecified brand of commercial food contained a fluorescent material which interfered with the estimation of catechol amines, while 10 grams of another gave after oxidation a fluorescence which would influence the excretion values of catechol amines in rats 170-180 grams by 10% at most. Ten and 20 grams of Purina Laboratory chow were dissolved in acidified distilled water and left overnight. After centrifugation the supernatant yielded the equivalent of .03 $\mu$gm. adrenaline per gram of food and .10 $\mu$gm. noradrenaline per gram of food. This fluorescent material could cause the apparent amount of catechol amines excreted by rats to be too high by contaminating the urine sample directly or after being eaten and excreted unchanged. The catechol amine excretion of the rats which ate more food did not appear to be any higher than that of those rats which ate less. Although food dishes were supplied to each rat in the metabolism cages, some rats invariably scattered this food, most of it being caught on the wire mesh, but some very small pieces reaching the urine collection flask. Higher excretion values of catechol amines were generally not recorded for those rats which scattered their food. Thus although the food did contain fluorescent material, this was not of sufficient magnitude to interfere with the assay.

## Experiment 1

### Amounts of Catechol Amines Excreted after Injection

Analyses of variance using R x 2 tables as outlined in Chapter 13 of Steel and Torrie (1960) were carried out on some of the data of the present investigation. If the F values were significant, the multiple range test set up by Duncan (1955) and modified for group means with unequal numbers of replications by Kramer (1956) was used if more than two factors were involved. Generally only a "t" test was necessary.

The solutions of adrenaline and noradrenaline used for injection were assayed against prepared standard solutions at the same time as the urine samples and gave the expected concentration. As the extra catechol amines excreted 24 hours after injection expressed as a percentage of the amount injected did not appear to differ with the absolute amount injected (25, 50 or 100 µgm.) the results have been combined. The average excess amount of adrenaline excreted in 24 hours after the subcutaneous injection to 10 rats maintained at 23°C. and for 16 or fewer (five rats died during the experimental period) at 2°C. expressed as a percentage of the injected dose at various time intervals is given below.

| 23°C. | | | 2°C. | | |
|---|---|---|---|---|---|
| Day | No. of rats | % excreted in 24 hours | Day | No. of rats | % excreted in 24 hours |
| 8 | 10 | 1.38 | 12 | 16 | 2.00 |
| 20 | 10 | 1.43 | 25 | 14 | 2.08 |
| 48 | 10 | 1.44 | 53 | 11 | 2.24 |
| Mean ± S.E. | | 1.42 ± .10 | Mean ± S.E. | | 2.09 ± .12 |

The analysis of variance gave a significant difference for temperature but not for time. The excretion of 1.5 times as much injected adrenaline at 2°C. as at 23°C. was significant at the 1% level (t=3.10, d.f.69).

The average excess amount of noradrenaline excreted in 24 hours after injection to 10 rats at 23°C. and 16 or fewer rats at 2°C. expressed as a percentage of the injected dose was measured at various time intervals as follows:

| 23°C. | | | 2°C. | | |
|---|---|---|---|---|---|
| Day | No. of rats | % excreted in 24 hours | Day | No. of rats | % excreted in 24 hours |
| 1 | 10 | 0.46 | 5 | 16 | 2.37 |
| 14 | 10 | 1.25 | 18 | 16 | 1.65 |
| 42,55 | 15 | 1.81 | 46 | 11 | 2.74 |
| Mean ± S.E. | | 1.27 ± .11 | Mean ± S.E. | | 2.20 ± .25 |

Again the analysis of variance gave a significant difference for temperature but not for time. The excretion of 1.7 times as much injected noradrenaline at 2°C. as compared to 23°C. was statistically significant at the 1% level ($t = 3.15$, d.f.76). Considering the data at 23°C. alone, the amount of noradrenaline excreted at Day 42,55 is significantly more than at Day 1 (at 1% level).

Most of the rats exposed to 2°C. lost a portion of the tail, the remaining end becoming black and hard. Often the tips of the ears were frostbitten, became black and were also eventually lost. In a few rats the penis became swollen and inflamed.

The average body weights of the rats at 23°C. and 2°C. are plotted in Fig. 16a. Although the rate of growth was initially slowed down by the cold, the rats grew fairly well. The normal excretion of adrenaline and noradrenaline as measured for the 24-hour periods prior to the injections is plotted in Fig. 16 c and d. The numerical data are given in Table VII in the Appendix. The values of both catechol amines are much higher in the cold. When analyzed statistically the excretion of adrenaline in μgm./24 hrs.

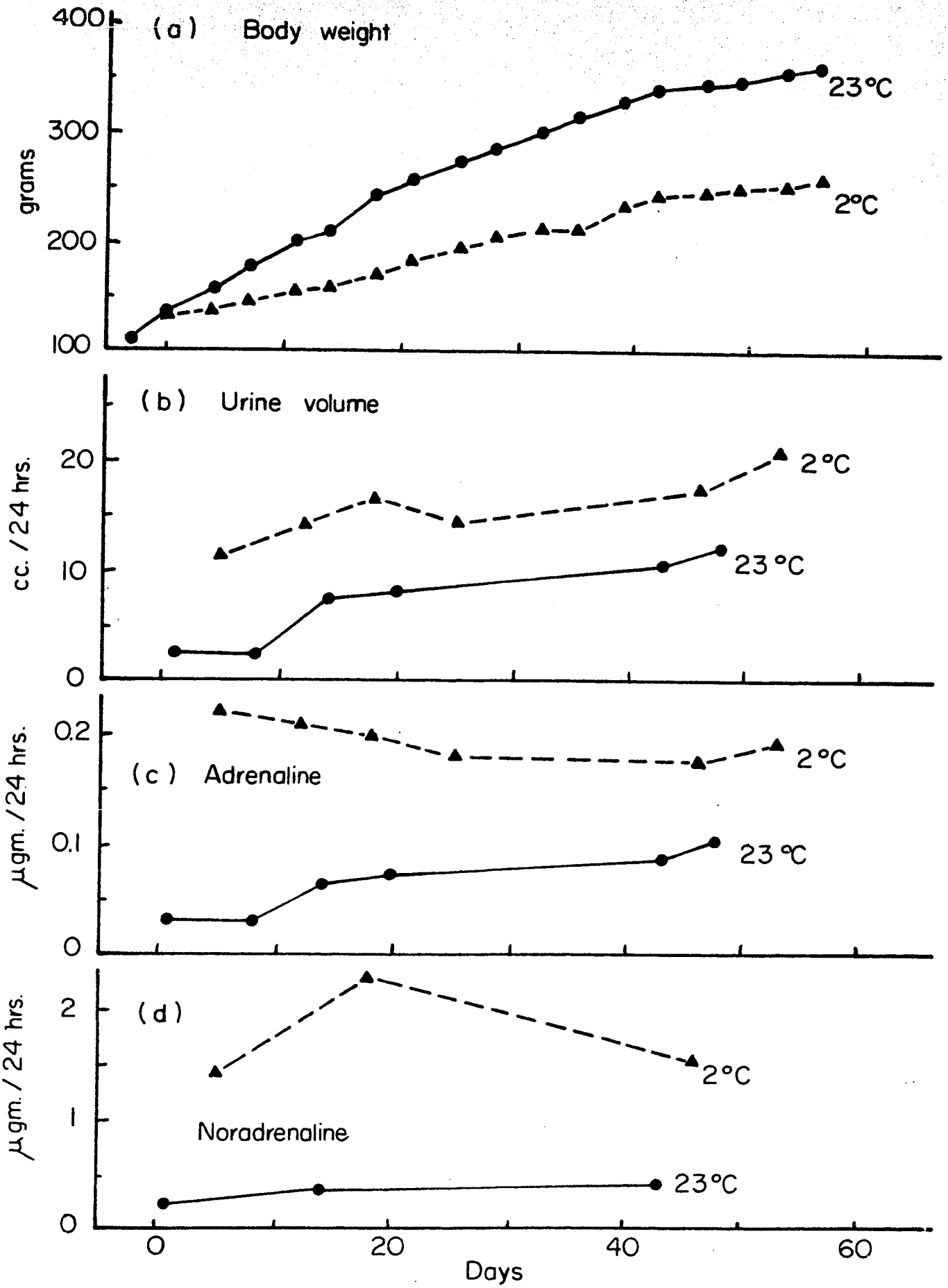



Fig. 16 Average body weight (a), urine volume (b) and excretion of adrenaline (c) and noradrenaline (d) in rats at 23°C. and 2°C. as measured at various times.

varied with temperature but not with time being significantly greater at 2°C. (at 1% level) than at 23°C. ($t = 12.34$, d.f.147). The same was true for noradrenaline ($t = 12.10$, d.f.76).

The average excretion of adrenaline at 2°C. was about three times the average value of .068 μgm./24 hrs. recorded for 23°C. while the average excretion of noradrenaline at 2°C. was approximately six times the average value of 0.344 μgm./24 hrs. recorded at 23°C. When the values at 23°C. are analyzed separately by Duncan's test, the excretion of adrenaline at Day 1 and Day 8 are significantly lower (at 1% level) than subsequent measurements.

Urine volumes of samples collected in the 24 hours prior to the injection are plotted in Fig. 16b. The urine volumes in the cold are about double those at 23°C. The injection of the catechol amines to rats at 23°C. appeared to cause a diuresis which was most marked for the first two injections. No consistent changes in urine volumes were observed after injection of the catechol amines to rats in the cold. The injection of noradrenaline appeared to cause increases in the normal output of adrenaline, which was increased on the average 0.03 μgm./24 hrs. at 23°C. and 0.05 μgm./24 hrs. at 2°C. In one case, however, at the time of the second injection of noradrenaline to a rat at 2°C. which died shortly afterwards, most of the noradrenaline appeared to be excreted as adrenaline.

## Experiment 2

### Excretion of Catechol Amines at Room Temperature and in the Cold

The average body weight, urine volume, and excretion values for adrenaline and noradrenaline of rats at room temperature and in the cold are plotted in Figs. 17, 18, 19 and 20; the numerical data are recorded in Tables VIII to XII in the Appendix. Thirteen of the 20 'old' rats of experiment 2a died in the cold, two deaths were accidental and one rat appeared to die from a urinary infection. Twelve of the 36 rats exposed to 2°C. in experiment 2b died. For seven rats there was evidence of infection of the urinary tract or the presence of enlarged and diseased kidneys. In addition four of the six Sprague-Dawley control rats at 23°C. died after 130 days of the experimental period. Before death they lost weight and exhibited marked difficulty in breathing. At autopsy infection of the lungs was evident in the four that died and also in one of the two survivors which was having difficulty breathing at the time it was killed. In most cases very high excretion values of adrenaline and noradrenaline were recorded just before death. For this reason the values for the control rats towards the end of the experimental period in experiment 2b are unreliable, and some have been omitted. As the same controls served for each group of rats exposed to cold, it is mostly for Group III that the control values are unreliable toward the end of the experiment. As no marked differences were seen between the Wistar and Sprague-Dawley rats used in the experiment, the values have been combined for plotting the figures.

Fig. 17a shows that the average body weight of the 'old' rats in activity cages decreased considerably on exposure to 2°C. Only after 50 days in the cold (that is, shortly after the rats were removed from the activity

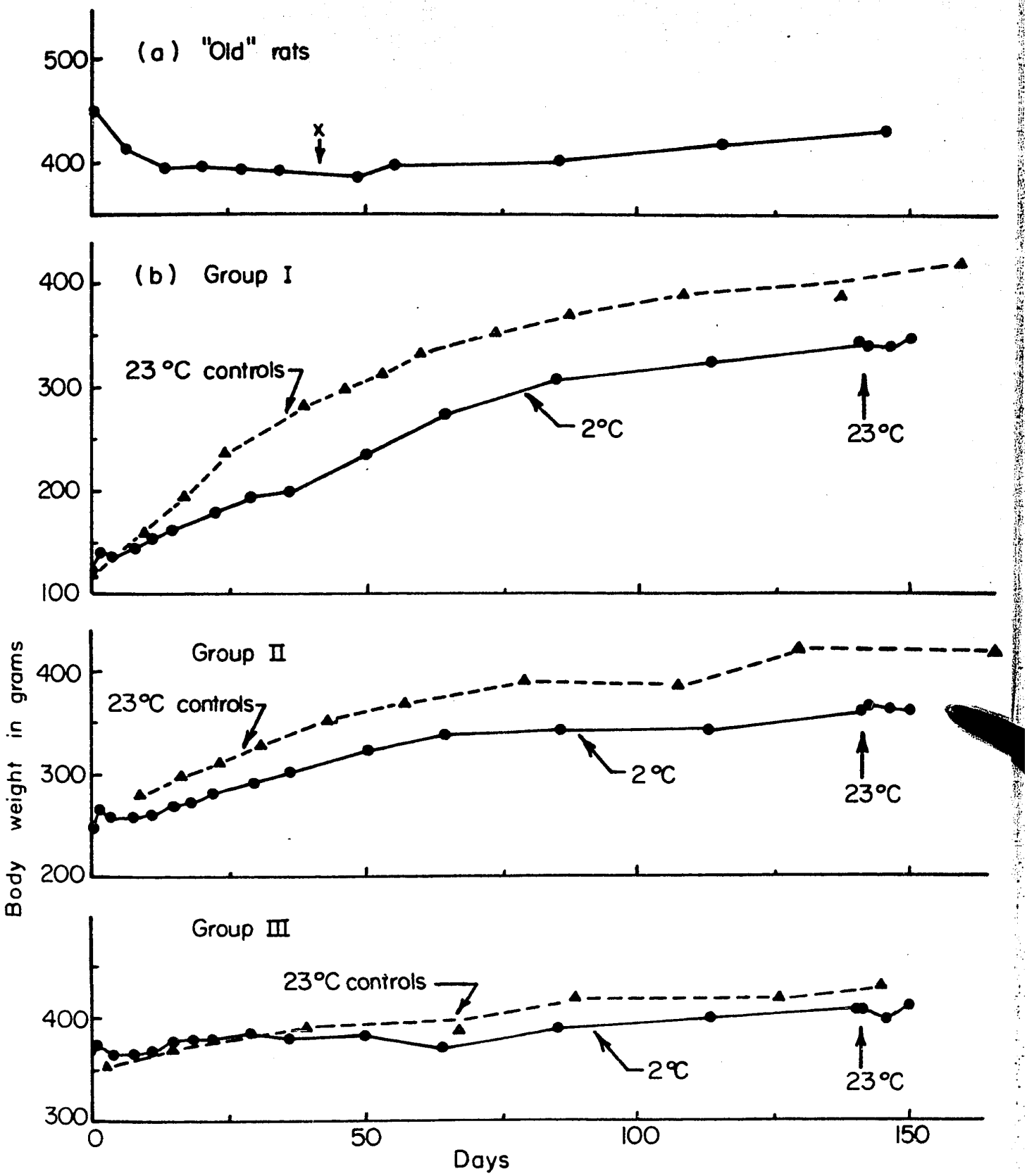



Fig. 17(a) Experiment 2a - Average body weight of 'old' rats at 2°C.
X indicates when rats were removed from activity cages.
(b) Experiment 2b - Average body weights of Groups I, II and III (●) at 2°C. compared with controls (▲) at 23°C.

cages) did the body weight begin to increase. Fig. 17b shows that the weight gain of the rats in Group I of experiment 2b was initially slowed down after exposure to 2°C. but after about four days the rats began to gain weight fairly rapidly. Weight gain of the rats in Group II exposed to cold 30 days after those of Group I was rather similar although the rapid gain of weight was completed about mid-way in the experiment. As the rats of Group III had passed the rapid growth stage before they were exposed to cold 70 days after those of Group I, their weight gain over the experimental period was relatively small but comparable to that of the controls at 23°C. Return of the cold-exposed rats to 23°C. caused a slight decrease in weight in all groups.

Fig. 18a shows that the average urine volumes of the 'old' rats in activity cages, although quite high before exposure to cold, increased to very high levels at 2°C. being highest three weeks after exposure to 2°C. and then decreasing to levels almost double that observed before exposure. Fig. 18b shows that the average urine volume of the control rats of experiment 2b was relatively constant around 5 cc. throughout most of the experiment. Values for the 24-hour collection, however, varied from 0.5 up to 14 cc. although they tended to be relatively constant for any one rat. Fig. 16b showed that the urine volumes of the rats in experiment 1 were initially lower and increased over the experimental period. The average urine volume of the rats exposed to cold (Fig. 18b) increased during the first four days of exposure to 2°C., remaining somewhat constant up to 40 days in the cold after which it tended to increase still further. The urine volumes were two to six times greater at 2°C. than in controls kept at 23°C. both in experiment 1 and 2. Although the urine volumes decreased when the cold-exposed rats were returned to 23°C., they were still above

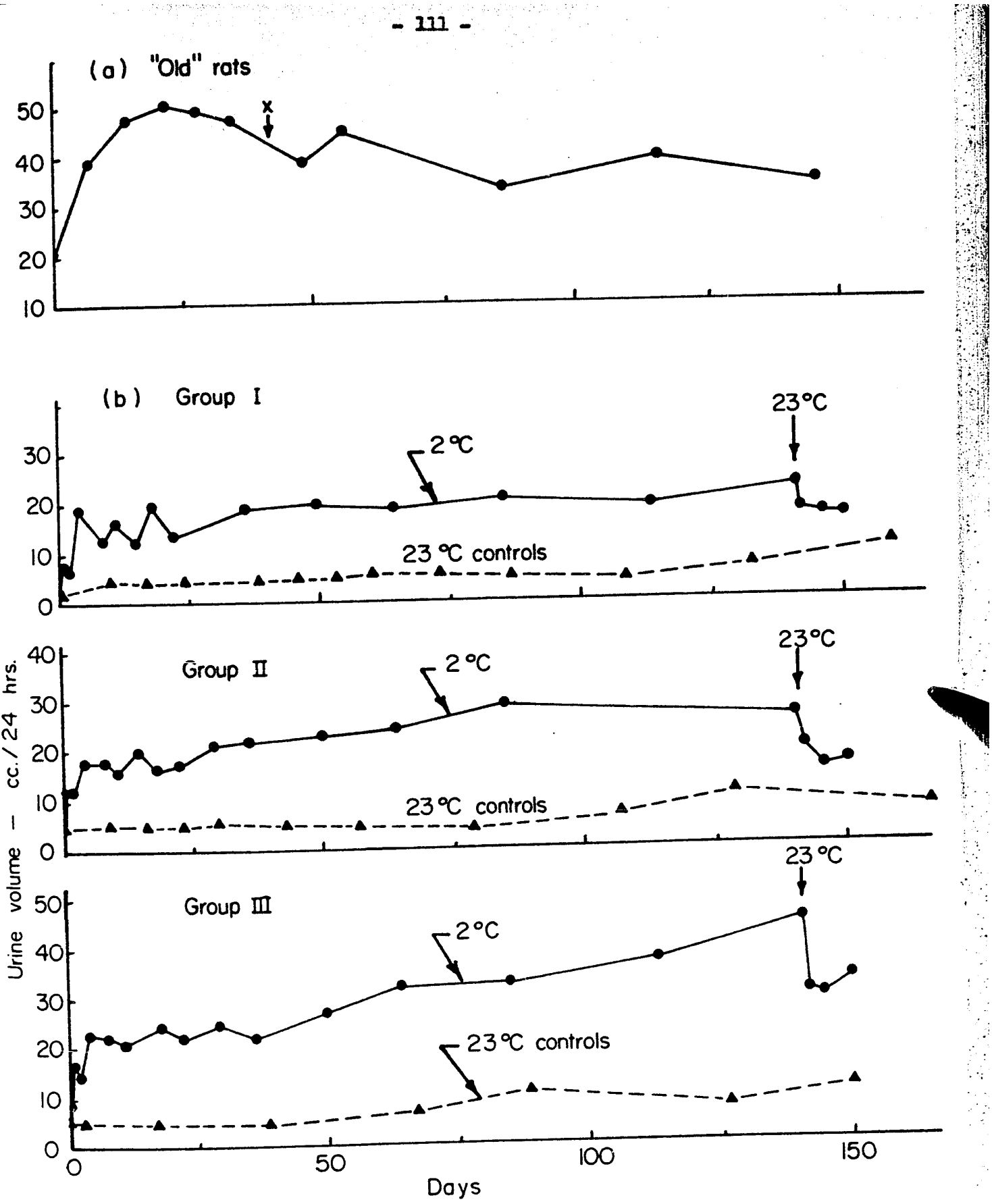



Fig. 18(a) Experiment 2a - Average urine volume of 'old' rats at 2°C.
X indicates when rats were removed from activity cages.
(b) Experiment 2b - Average urine volumes of Groups I, II and III (●) at 2°C. compared with controls at 23°C. (▲).

the control level after 8 days.

Fig. 19 shows that the average 24-hour excretion values for adrenaline were much higher for the 'old' rats (a) than for the other groups (b). The peak value for the 'old' rats was recorded at one week in the cold. The excretion then continually decreased until after 86 days the values were approximately the same as those recorded for Group III. The average 24-hour excretion of adrenaline by the control rats of experiment 2b in Fig. 19b remained relatively constant around 0.05 $\mu$gm. up until the time that some of the rats became sick and died. This contrasts to the values obtained in experiment 1 (Fig. 16c) where the adrenaline concentration tended to increase with age. In contrast to the urine volumes which showed rather wide individual variation, the adrenaline excretion was much more constant from rat to rat. Fig. 19b shows that the adrenaline excretion for the rats of experiment 2b exposed to cold markedly increased, reaching a high value after four days and remaining somewhat constant up to 50 days, after which further increases were noted especially in Groups II and III. The values for the rats in experiment 1 corresponded well with those Group I, both sets of rats being approximately the same age and weight when exposed to cold. The high value at Day 50 recorded for Group III is undoubtedly a result of the fact that the cold room temperature was below freezing for about three days before the measurement was taken. The highest levels of excretion of adrenaline were recorded in the last group of rats (Group III) exposed to cold. Adrenaline excretion in the cold was increased about three to four times over that at room temperature and tended to average between 0.2 to 0.4 $\mu$gm./24 hours. On the return of the cold exposed rats to 23°C. the adrenaline excretion immediately decreased but tended to be slightly above the control level values even after eight days. A review of the

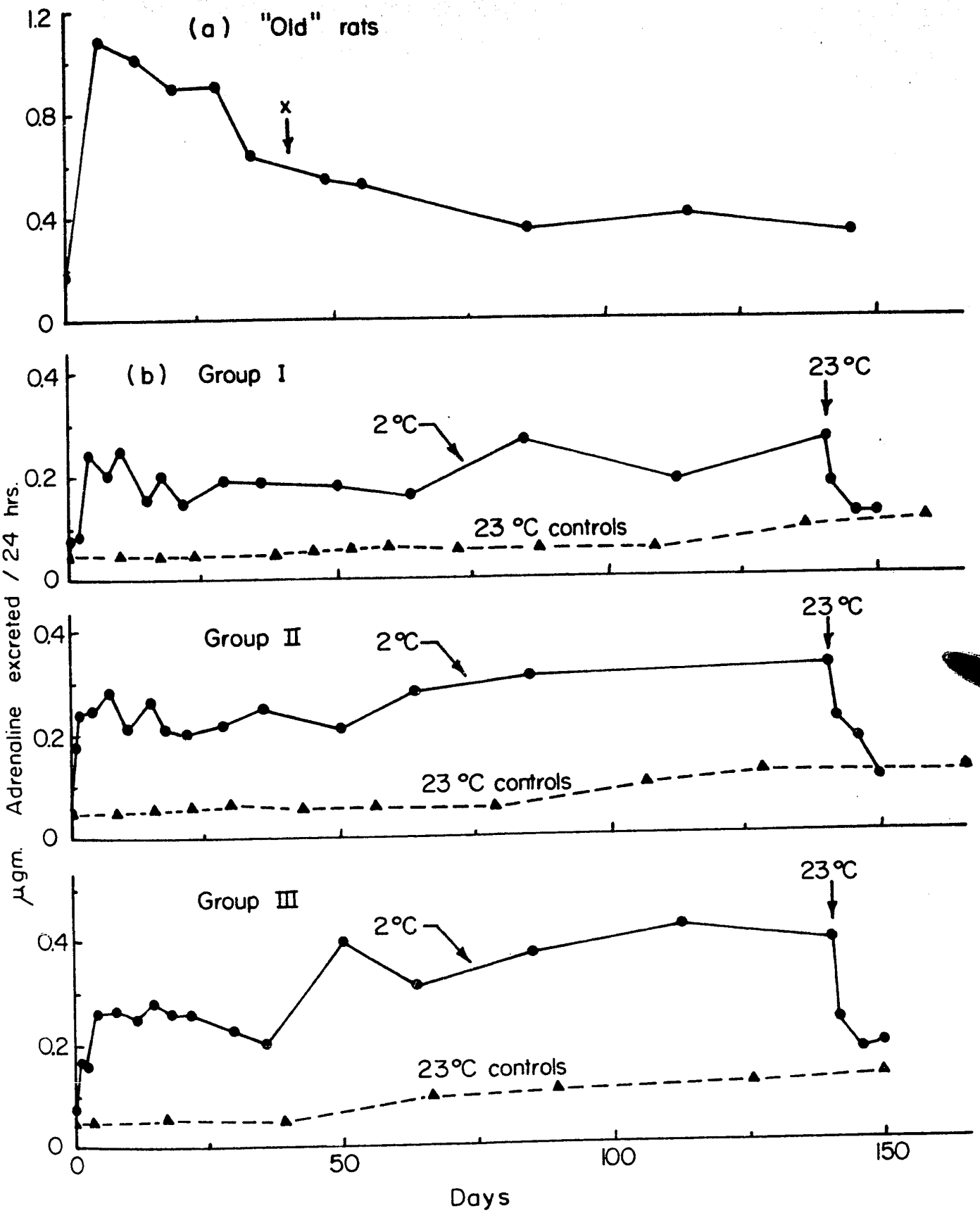



Fig. 19(a) Experiment 2a - Average adrenaline excretion of 'old' rats at 2°C., X indicates when rats were removed from activity cages.
(b) Experiment 2b - Average adrenaline excretion of Groups I, II and III (●) at 2°C. compared with controls (▲) at 23°C.

individual results shows that although there was a slight tendency for a rat with a higher urine volume to excrete more adrenaline than others measured at the same time, this was by no means a general rule and often the reverse was true.

Fig. 20a shows that the noradrenaline excretion level was very high for the 'old' rats after three to four weeks in the cold, after which it declined. Fig. 20b shows that the noradrenaline excretion for the control rats of experiment 2b was increased only slightly over the experimental period, for the most part being maintained between 0.20 to 0.30 $\mu$gm./24 hrs., while the rats increased in weight from 150 to 400 grams. Although the urine volumes showed wide individual variation (0.5 to 14 cc.), the noradrenaline excretion was much more constant from rat to rat. High excretion values of noradrenaline were reached within the first few days in the cold and were maintained at high levels (around 2 $\mu$gm./24 hrs.) for 60 days after which they increased still further for Groups II and III (Fig. 20b). The high value at Day 50 for Group III was undoubtedly a result of the below freezing temperature in the cold room for three days before the urine was collected. Noradrenaline excretion in the cold was approximately eight times greater than in rats at 23°C. The values obtained for the rats in experiment 1 corresponded with those of Group I of experiment 2b. On return to 23°C. the excretion of noradrenaline decreased immediately and was at four days close to the level of the controls. In general, a review of the results on individual rats shows that the noradrenaline excretion of a rat appears to vary independently of the urine volume and of the excretion of adrenaline.

The average values over the experimental period for each experimental group have been recorded in Table VI. Exposure to cold increased the urine

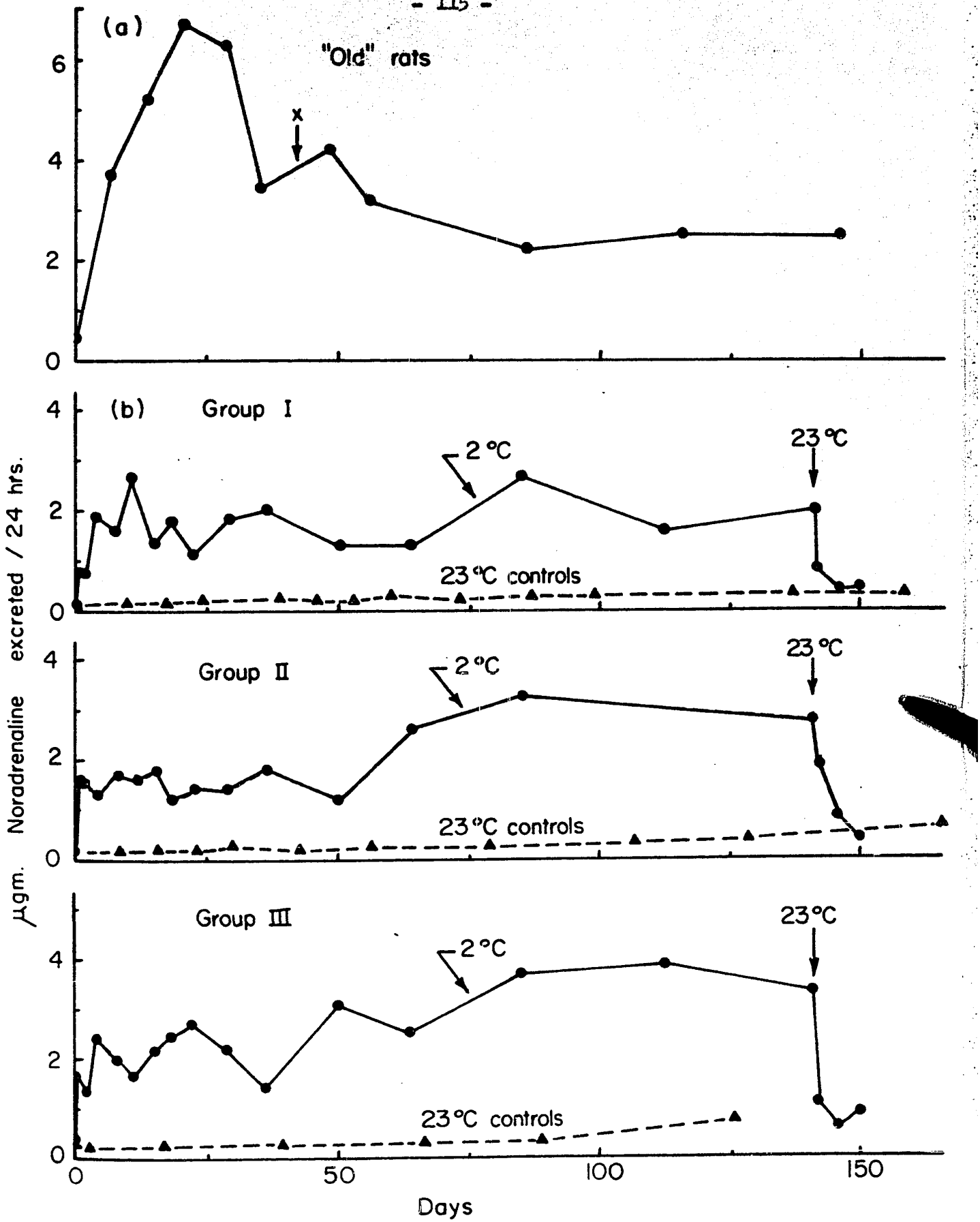



Fig. 20(a) Experiment 2a - Average noradrenaline excretion of 'old' rats at 2°C. X indicates when rats were removed from activity cages.
(b) Experiment 2b - Average noradrenaline excretion of Groups I, II and III (●) at 2°C. compared with controls (▲) at 23°C.

volume about three to four times, the adrenaline excretion about three to four times and the noradrenaline excretion about eight times on the average.

TABLE VI

Average Urine Volume and Excretion of Adrenaline and Noradrenaline for Various Experimental Groups of Rats

| Group | No. of rats initial | No. of rats final | Urine volume cc./24 hr. | Adrenaline µgm./24 hr. | Noradrenaline µgm./24 hr. |
|---|---|---|---|---|---|
| Experiment 2a | | | | | |
| Before cold | 20 | 20 | 20.6 | .184 | .486 |
| At 2°C. | 20 | 7 | 41.9 | .688 | 4.066 |
| Experiment 1 | | | | | |
| Controls 23°C. | 10 | 10 | 7.3 | .068 | .344 |
| At 2°C. | 16 | 11 | 17.7 | .198 | 1.900 |
| Experiment 2b | | | | | |
| Controls 23°C. | | | | | |
| Sprague-Dawley | 6 | 2 | 4.6 | .062 | .219 |
| Wistar | 6 | 6 | 5.8 | .059 | .242 |
| At 2°C. | | | | | |
| Group I | | | | | |
| Sprague-Dawley | 6 | 3 | 15.6 | .220 | 1.613 |
| Wistar | 6 | 2 | 17.5 | .151 | 1.597 |
| Group II | | | | | |
| Sprague-Dawley | 6 | 3 | 22.6 | .330 | 2.156 |
| Wistar | 6 | 4 | 16.5 | .150 | 1.415 |
| Group III | | | | | |
| Sprague-Dawley | 3 | 3 | 26.6 | .306 | 2.581 |
| Wistar | 3 | 3 | 24.8 | .262 | 2.241 |

## DISCUSSION

The estimation of the amounts of adrenaline and noradrenaline in rat urine by the method of Drujan et al. (1959) was quite satisfactory provided the fluorescence was read approximately one hour after the formation of the trihydroxyindole derivatives. The improved technique of Euler and Lishajko (1961) for the estimation of catechol amines, however, might be more precise. Although the calibration curves were linear, the fluorescent values for noradrenaline, but not adrenaline, were found to be lower when the assay was done at night. This is in contrast to the finding of Goldfien et al. (1961) who reported that exposing the fluorescent products of noradrenaline to daylight or other sources of light of short wavelengths appeared to cause partial destruction after which the fluorescent products remained stable.

Recoveries of catechol amines added to urine were about 50% and probably would have been higher if the pH of the extraction mixture had been kept closer to pH 8.5. The catechol amines were stable in urine left for 24 hours at room temperature; the degree of acidity did not appear to be important. Adrenaline was not stable in acidified distilled water left for 24 hours at room temperature. These findings concur with those of Mann (1953) who reported that both adrenaline and noradrenaline were more stable in urine than in water.

The average amounts of injected catechol amines excreted 24 hours after the subcutaneous injection of doses of 25, 50 or 100 $\mu$gm. at 23°C. (corrected for 100% recovery) were 2.8% for adrenaline and 2.5% for noradrenaline. These values lie within the ranges obtained by other workers. The average amounts of the catechol amines excreted after injection at 2°C. were, however, significantly greater than those at 23°C. Approximately

1.6 times as much of the injected dose was excreted by 16 cold-exposed rats (as measured at various time intervals during 60 days in the cold) as in 10 rats at room temperature. Perhaps there is a slower rate of destruction of these amines in the cold. Although LeDuc (1961b) stated that there was no change in the amount of injected adrenaline and noradrenaline excreted after injection of 100 µgm. into three rats at room temperature and after six and 30 days in the cold, his values of 2.6% for adrenaline at 3°C. is lower than that of 3.4% at room temperature. The significance of the present finding that rats at 2°C., which already excrete large amounts of catechol amines, excrete slightly more adrenaline and noradrenaline after injection than do rats at 23°C. is not clear. The injection of the catechol amines appeared to cause a diuresis at 23°C. but not at 2°C. although the diuresis was most pronounced when the rats were young. Injections of noradrenaline appeared to cause slight increase in the urinary excretion of adrenaline both at 23°C. and at 2°C. LeDuc (1961b) found no changes in the excretion of adrenaline after injection of noradrenaline and vice versa.

Exposure of animals to cold is uaually followed by loss of body weight during the first few days. As the food intake increases, the animal begins to increase in body weight but at a slower rate than in controls at room temperature (Sellers, 1957). Although there is a lower fat storage in the cold, according to Hart (1958) and Depocas (1961) the check in growth is confined to a reduction in growth of the skeletal muscles. According to Depocas (1961) a normal growth rate is not maintained because the food intake does not become adequate. In the present investigation, although growth of young rats at 2°C. was initially slowed down, they soon commenced growing again at a good rate. Weight gain of older rats was relatively

small in the cold but was similar to that of the controls at room temperature. The large and sustained drop in body weight of the 'old' rats in activity cages was unexpected. The fact that these rats began to gain weight after 50 days in the cold could have been because they had been removed from the activity cages just prior to this gain.

The average amount of urine excreted at 23°C. by the control rats of experiment 1 and 2b was 5.3 cc. per 24 hours. Although the actual volumes were quite variable (0.5 to 14 cc.), they tended to be relatively constant for any one rat. Exposure to cold caused, on the average, a three to four fold increase in the 24-hour urine volume. The average 24-hour diuresis of the rats of experiments 1 and 2b at 2°C. was 20.2 cc. Urine volumes increased immediately on exposure to cold and reached high levels during the first few days. Excretion was maintained at a high level throughout the entire period of cold exposure, the levels after 40 days in the cold being generally higher. Although the urine volumes decreased during the first eight days after the rats had been returned to room temperature at 23°C., they were still well above control levels. The average 24-hour urine volume of the 'old' rats of experiment 2a was high being 20.6 cc. at 23°C. and 41.9 cc. at 2°C. Although the urine volume increased in the cold, it did not reach its peak for these rats until the rats had been at 2°C. for three weeks. A two-fold increase in diuresis is reported for cold-exposed rats by LeDuc (1961b), 170-180 gram rats excreting on the average 8 cc. at 22°C. and 18 cc. at 3°C. per 24 hours during 36 days in the cold. LeBlanc and Nadeau (1961) reported for rats of approximately 400 grams an average 24-hour urine volume of 10 cc. for 10 rats at 25°C. and 33 cc. for 10 rats kept for seven months at 10°C. Thus the increased urine output of cold-exposed rats in the present investigation was as expected.

Although there was a tendency for the amounts of catechol amines excreted by rats maintained at room temperature to increase with the age of the rat, this increase was small when compared to the changes in excretion caused by cold. The average amount of adrenaline excreted in 24 hours (corrected for 100% recovery) for the rats maintained at 23°C. was 0.12 µgm. (0.08 to 0.22 µgm. for rats 120 to 425 grams) for experiment 2b; 0.14 µgm. (0.06 to 0.21 µgm. for rats 140 to 350 grams) for experiment 1; and 0.36 µgm. for the 'old' (456 grams) rats of experiment 2a. These values are in the same range as those reported by other workers except that the excretion values for the 'old' rats in the activity cages were very high. LeDuc (1961b) reported an increase in the absolute 24-hour excretion of adrenaline with age from 0.03 to 0.22 µgm. in rats 110 to 455 grams. Bickel et al. (1961) obtained an adrenaline excretion of 0.17 to 0.28 µgm. in 200 to 350 gram rats while LeBlanc and Nadeau (1961) reported a value of 0.13 µgm. in 400 gram rats at 25°C.

The average amount of noradrenaline excreted in 24 hours (corrected for 100% recovery) for the rats maintained at 23°C. was 0.46 µgm. (0.24 to 0.72 µgm. for 120 to 425 gram rats) for experiment 2b; 0.68 µgm. (0.41 to 0.83 µgm. for 140 to 350 gram rats) for experiment 1; and 0.97 µgm. for the 'old' (456 gram) rats in experiment 2a. These values too are in the ranges reported by others. LeDuc (1961b) reported an increase in the absolute 24-hour excretion of noradrenaline with age from 0.46 to 1.14 µgm. in rats 110 to 455 grams. Bickel et al. (1961) obtained a noradrenaline excretion of 0.47 to 0.61 µgm. in 200 to 350 gram rats while LeBlanc and Nadeau (1961) reported a higher value of 1.31 µgm. for 400 gram rats at 25°C. The normal excretion of noradrenaline at room temperature was about four times greater than for adrenaline in the present investigation. In contrast to the urine

volumes which showed marked individual variation, the adrenaline and noradrenaline values were much more constant from rat to rat.

The natural excretion of adrenaline and noradrenaline was markedly increased in rats exposed to cold at 2°C. Adrenaline excretion was increased from three to four times on the average and noradrenaline excretion approximately eight times. Although it was shown in experiment 1 that cold-exposed rats excreted 1.6 times as much injected adrenaline and noradrenaline as they did at room temperature, this slightly increased excretion is certainly insufficient to account for the markedly increased excretions of adrenaline and noradrenaline in the cold. Although the excretion of these catechol amines was increased immediately on exposure to cold, peak values were usually reached after approximately four days in the cold (experiments 1 and 2b). Excretion was maintained at a high level throughout the cold exposure and tended to increase further after 50 to 60 days in the cold. The older the rats when exposed to cold, the higher the excretion values appeared eventually to become.

As some of the present findings with Wistar rats differed from those of LeDuc (1961a,b) with Sprague-Dawley rats, it seemed possible that the two strains were different with respect to excretion of catechol amines. Indeed, Eränkö and Hopsu (1961) reported that Wistar rats required three times as much reserpine as Sprague-Dawley rats to effect equal depletion of catechol amines from the adrenal medulla. In the present parallel studies of rats of the two strains, however, although Wistar rats tended to excrete slightly smaller amounts of catechol amines in the cold, the two strains gave generally similar results. Thus strain differences cannot account for any discrepancies between the present findings and those of LeDuc (1961a,b).

The catechol amine excretion of the 'old' rats in activity cages (experiment 2a) was markedly different from that of the younger rats of experiment 1 and 2b. A very high excretion of adrenaline after one week was followed by a decline, although excretion was still at a high level. The excretion of noradrenaline was at its peak only after three to four weeks in the cold; then it also declined although it was also still at a high level.

These results contrast with those of LeDuc (1961b) who found that the excretion of noradrenaline in rats exposed to 3°C. was nearly maximal during the first 24 hours and was maintained at a level about three times as high as the of a control group at 22°C., although there was a slow and steady decline with time. This decreased excretion of noradrenaline with time in the cold obtained by LeDuc was only partly due to the weight factor. Moreover, the excretion of adrenaline was maximal only after one week in the cold and decreased afterwards much more rapidly than that of noradrenaline although it was maintained at a level one and a half to two times that of controls at 22°C. These differences cannot be explained by the fact that LeDuc's results are expressed relative to body weight and the present ones are not. This is clear because the changing trends of excretion with time in the cold were the same for the three groups of rats of experiment 2b although the average body weights varied widely. The rats of Group I increased in weight from 140 to 345 grams while the weight of the rats in Group III was fairly constant (from 376 to 409 grams) over the experimental period. The vastly different results obtained for the 'old' rats in experiment 2a are both interesting and difficult to explain. Their response could be due to the fact that they were 'old' when they were exposed to cold but may be a reflection of their presence and running activity

in activity cages.

Four days after the rats of experiment 2b were returned to 23°C. noradrenaline excretion was back at the control level. Adrenaline excretion although much reduced, tended to be above the control values after eight days. In contrast, LeDuc (1961b) reported that catechol amine excretion values returned to normal within two days after the rats were returned to room temperature from 3°C.

The average amount of adrenaline excreted (corrected for 100% recovery) in 24 hours at 2°C. by the rats of experiments 1 and 2b was 0.46 µgm. and by the 'old' rats of experiment 2a was 1.38 µgm. The average amount of noradrenaline excreted (corrected for 100% recovery) in 24 hours at 2°C. by the rats of experiments 1 and 2b was 3.86 µgm. and for experiment 2a was 8.14 µgm. Ten rats kept at 10°C. for seven months by LeBlanc and Nadeau (1961) weighed approximately 400 grams and excreted 0.64 µgm. adrenaline and 9.71 µgm. noradrenaline. These values represented a five-fold increase over values for control rats at 25°C. LeDuc (1961b) reported that the amounts of catechol amines excreted in the cold were dependent on body weight and as calculated from his graphs for rats 100 to 500 grams varied from 0.17 to 0.48 µgm. adrenaline on day one in the cold and from 0.73 to 0.98 µgm. adrenaline at the peak on day six while the noradrenaline excretion varied from 1.4 to 4.3 µgm.

Thus the present results agree with those of LeDuc (1961b) and LeBlanc and Nadeau (1961) that excretion of both catechol amines is increased in the cold. They further agree with those of LeDuc that the excretion of noradrenaline is increased about twice as much as adrenaline but not with those of LeBlanc and Nadeau who obtained equal five-fold increases for adrenaline and noradrenaline. The average increase in excretion of adren-

aline (three to four times) and of noradrenaline (eight times) in the present investigation are approximately double the averages obtained by LeDuc (one and a half to two times for adrenaline and three times for noradrenaline). The study of catechol amine excretion with time of cold exposure also yields results different from those of LeDuc. No isolated peak for adrenaline excretion after one week in the cold was found. Also the excretion of the catechol amines tended to increase slightly with time of exposure to cold rather than to decrease.

Acclimation of rats to cold is a gradual process requiring two to six weeks. It is accompanied by increased food consumption, increased metabolic rate and an increase in non-shivering heat production (Depocas, 1961). L-noradrenaline has been suggested as the mediator of this non-shivering thermogenesis. Thus the secretion of noradrenaline (as measured indirectly by its excretion in the urine) during and after acclimation of rats to cold is of great interest. There is no doubt that the excretion of noradrenaline in rats reaches high values soon after cold exposure. In fact, peak values were reached after approximately four days, the time at which the rats also began to increase in body weight. In the present experiments high excretion values were maintained throughout the 20 weeks of cold exposure. Thus acclimated rats excreted as much noradrenaline (and in some cases more) than non-acclimated rats in the cold. Adrenaline excretion followed a similar pattern. Although it also was increased in the cold, it did not increase to the same extent as the noradrenaline excretion. Sellers (1957) has stated that within a few days of removal of animals from the cold, much of the increased resistance has disappeared although it persists for some weeks in some degree. The noradrenaline excretion in the present experiments was at control levels four days after the rats

were returned to 23°C. from 2°C. while the adrenaline excretion, although much reduced, tended to be above control levels at eight days. It is perhaps possible that this raised level of adrenaline is responsible for the persistance of a slight degree of resistance to cold maintained by rats after they are returned to room temperature.

The high values both at room temperature and in the cold for the 'old' rats while they were in activity cages could possibly be due to the fact that they were able to exercise. For these rats adrenaline excretion was maximal at one week in the cold and noradrenaline excretion only after three to four weeks. The marked differences between the catechol amine excretion of these rats in activity cages and the ones maintained in the standard individual laboratory cages should be investigated further.

## SUMMARY

1. The average amount of subcutaneously injected catechol amines excreted by male white rats at 23°C. was 2.8% for adrenaline and 2.5% for noradrenaline. At 2°C. these figures were increased approximately 1.6 times.

2. There were no marked differences between the amounts of catechol amines excreted by Wistar and Sprague-Dawley rats either at 23°C. or 2°C.

3. On the average, exposure to cold increased the adrenaline excretion three to four times, the noradrenaline excretion eight times and the urine volume three to four times.

4. The excretion of both adrenaline and noradrenaline was increased immediately on exposure to cold, peak values being reached after four days and maintained throughout the 20 weeks of exposure to 2°C. There were no decreases in the excretion with increasing time of cold exposure; in fact, excretion tended to increase further after 50 to 60 days in the cold. Thus acclimated rats excreted as much of these catechol amines as non-acclimated rats. Noradrenaline excretion was at control levels four days after the cold-exposed rats were returned to 23°C. while adrenaline excretion tended to be slightly elevated even after eight days at 23°C.

5. There was some tendency for the amount of catechol amines excreted by rats at room temperature to increase with age and weight of the rat. On the average, the 24-hour excretion values of the catechol amines were 0.13 μgm. adrenaline and 0.49 μgm. noradrenaline at 23°C. and

0.46 $\mu$gm. adrenaline and 3.86 $\mu$gm. noradrenaline at 2°C.

6. 'Old' rats in activity cages excreted much more adrenaline and noradrenaline both at room temperature and in the cold than rats kept in individual laboratory cages. Their excretion of adrenaline was at a peak one week after cold exposure and their excretion of noradrenaline only after three to four weeks. It is suggested that the fact that they were allowed to exercise freely in the activity cages is responsible for this difference.

## REFERENCES

AXELROD J., WHITBY L.G., HERTTING G. & KOPIN I.L.
Circulat. Res. 9: 715-720, 1961
Studies on the metabolism of catecholamines

BEAUVALLET M., FUGAZZA J. & SOLIER M.
C. R. Soc. Biol. (Par.) 155: 2252-2254, 1961
Etude comparée du taux de la noradrénaline des surrénales et du cerveau avant et après travail musculaire

BERTLER A., HILLARP N.A. & ROSENGREN E.
Acta Physiol. Scand. 50: 124-131, 1960
Some observations on the synthesis and storage of catecholamines in the adrenaline cells of the suprarenal medulla

BICKEL M.H., CARPI A. & BOVET D.
Helv. Physiol. Pharmacol. Acta 19: 279-284, 1961
Action of reserpine and monoamine oxidase inhibitors on the urinary excretion of catecholamines in the rat

BOTTING J.H.
U.S. Army Operation Research Group Report No.3/60 1960
A method for the estimation of the urinary excretion of adrenaline and noradrenaline

BOTTING R.M. & LOCKETT M.F.
Arch. Int. Physiol. 69: 36-45, 1961
Threshold effect of subcutaneous adrenaline, noradrenaline and isoprenaline on water diuresis in rats

BOTTING R.M., FARMER J.B. & LOCKETT M.F.
Arch. Int. Physiol. 69: 203-212, 1961
The effect of subcutaneous adrenaline and isoprenaline on the excretion of electrolytes by rats

CIER J.F. & PEYRIN L.
J. Physiol. (Par) 52: 52-53, 1960
Sur la biosynthèse de l'adrénaline étudiée à l'aide des catécholamines marquées

COTTLE W.H.
Proc. Canad. Fed. biol. Soc. 3: 20 1960
Urinary noradrenaline in cold acclimated rats

DEPOCAS F.
Canad. J. Biochem. 38: 107-114, 1960
The calorigenic response of cold-acclimated white rats to infused noradrenaline

DEPOCAS F.
Brit. Med. Bull. 17: 25-31, 1961
Biochemical changes in exposure and acclimation to cold environments

DRUJAN B.D., SOURKES T.L., LAYNE D.S. & MURPHY G.F.
Canad. Jour. Biochem. & Physiol.37: 1153-1160, 1959
The differential determination of catecholamines in urine

DUNCAN D.B.
Biometrics 11: 1-42, 1955
Multiple range and multiple F tests

ERÄNKÖ O. & HARKONEN M.
Acta Physiol. Scand. 51: 247-253, 1961 (a)
Distribution and concentration of adrenaline and noradrenaline in the adrenal medulla of the rat following depletion induced by muscular work

ERÄNKÖ O. & HARKONEN M.
Endocrinology 69: 186-187, 1961 (b)
Long-term effects of muscular work on the adrenal medulla of the mouse

ERÄNKÖ O. & HOPSU V.
Acta Physiol. Scand. 51: 239-246, 1961
Distribution and concentration of adrenaline and noradrenaline in the adrenal medulla of the rat following reserpine-induced depletion

ERÄNKÖ O., KARVONEN M.J. & RÄISÄNEN L.
Acta Endocr. (Kbh.) 39: 285-287, 1962
Long-term effects of muscular work on the adrenal medulla of the rat

EULER U.S.von
Fed. Proc. (Suppl.5): 79-81, 1960
Exposure to cold and catecholamines

EULER Chr.v., EULER U.S.v. & FLODING I.
Acta Physiol. Scand. 33 Suppl. 118: 32-38, 1955
Biologically inactive catechol derivatives in urine

EULER U.S.v. & FLODING I.
Acta Physiol. Scand. 33 Suppl. 118: 45-56, 1955
A fluorimetric micromethod for differential estimation of adrenaline and noradrenaline

EULER U.S.v. & FLODING I.
Scand. J. Clin. & Lab. Invest. 8: 288-295, 1956
Diagnosis of pheochromocytoma by fluorimetric estimation of adrenaline and noradrenaline in urine

EULER U.S.v. & HELLNER S.
Acta Physiol Scand. 26: 183-191, 1952
Excretion of noradrenaline and adrenaline in muscular work

EULER U.S.v. & LISHAJKO F.
Acta Physiol. Scand. 45: 122-132, 1959
The estimation of catechol amines in urine

EULER U.S.v. & LISHAJKO F.
Acta Physiol. Scand. 51: 348-355, 1961
Improved technique for the fluorimetric estimation of catecholamines

EULER U.S.v. & ORWÉN I.
Acta Physiol. Scand. 33 Suppl 118: 1-9, 1955
Preparation of extracts of urine and organs for estimation of free and conjugated noradrenaline and adrenaline

FRANKENHAEUSSER M., JARPE G. & MATELL G.
Acta Physiol. Scand. 51: 175-186, 1961
Effects of intravenous infusions of adrenaline and noradrenaline on certain psychological and physiological functions

GOLDFIEN A., ZILELI S., GOODMAN D. & THORN G.W.
J. Clin. Endocr. 21: 281-295, 1961
The estimation of epinephrine and norepinephrine in human plasma

GOLDSTEIN M. & CONTRERA J.F.
Biochem. Pharmacol. 7: 77-78, 1961
The inhibition of norepinephrine and epinephrine synthesis in vitro

HART J.S.
Fed. Proc. 17: 1045-1054, 1958
Metabolic alterations during chronic exposure to cold

HART J.S.
Brit. Med. Bull. 17: 19-24, 1961
Physiological effects of continued cold on animals and man

HART J.S. & JANSKY L.
Fed. Proc. 21: 224, 1962
Participation of muscle and kidney in non-shivering thermogenesis, measured in vivo

HASSELMAN M., SCHAFF G.&METZ B.
C. Rend. Soc. Biol. (Par.) 154: 197-201, 1960
Influences respectives du travail, de la température ambiante et de la privation de sommeil sur l'excretion urinaire de catécholamines chez l'Homme normal

HERTTING G. & AXELROD J.
Nature (Lond.) 192: 172-173, 1961
Fate of tritiated noradrenaline at the sympathetic nerve-endings

HSIEH A.C.L. & CARLSON L.D.
Amer. J. Physiol. 190: 243-246, 1957
Role of adrenaline and noradrenaline in chemical regulation of heat production

JACOBS S.L., SOBEL C. & HENRY R.J.
J. Clin. Endocr. 21: 305-314, 1961
Specificity of the trihydroxyindole method for determination of urinary catecholamines

KÄRKI N.
Acta Physiol. Scand. 39 Suppl. 132: 1-96, 1956
The urinary excretion of noradrenaline and adrenaline in different age groups, its diurnal variation and the effect of muscular work on it

KRAMER C.Y.
Biometrics 12: 307-310, 1956
Extension of multiple range tests to group means with unequal numbers of replications

LEBLANC J.A. & NADEAU G.
Canad. J. Biochem. Physiol. 39: 215-217, 1961
Urinary excretion of adrenaline and noradrenaline in normal and cold-adapted animals

LEDUC J.
Acta Physiol. Scand. 51: 94-95, 1961 (a)
Excretion of catecholamines in rats exposed to cold

LEDUC J.
Acta Physiol. Scand. 53 Suppl.183: 1-101, 1961 (b)
Catecholamine production and release in exposure and acclimation to cold

MANN M.
J. Pharm. & Pharmacol. (Lond.) 5: 1024-1030, 1953
The stability of adrenaline and noradrenaline in human urine

MIRKIN B.L.
J. Pharmacol. Exp. Ther. 132: 218-225, 1961
Factors influencing the selective secretion of adrenal medullary hormones

OESTERLING M.J., TSE R.L. & HOLMES H.M.
Fed. Proc. 21: 192, 1962
Spectrophotometric determination of catecholamine excretion in the free and conjugated forms

PERMAN E.S.
Acta Physiol. Scand. 51: 68-74, 1961
Effect of ethanol and hydration on the urinary excretion of adrenaline and noradrenaline and on the blood sugar of rats

SELLERS E.A.
Rev. Canad. Biol. 16: 175-188, 1957
Adaptive and related phenomena in rats exposed to cold A review

SOURKES T.L. & DRUJAN B.D.
Canad. J. Biochem. & Physiol. 35: 711-719, 1957
A routine procedure for the determination of catecholamines in urine and tissues

STEEL R.G.D. & TORRIE J.H.
Principles and Procedures of Statistics. With special reference to the biological sciences. McGraw-Hill Book Co.,Inc. Toronto, 1960

TURNER G.D.
General Endocrinology. Edition 3, W.B.Saunders Co. Philadephia, 1960

# APPENDIX

## TABLE VII

Average Body Weight, Urine Volume and Excretion of Adrenaline and Noradrenaline in Rats at Room Temperature and in the Cold

Experiment 1

(a) 23°C.

| Day | No. of rats | Body weight grams | Urine volume cc./24 hr. | Adrenaline µgm./24 hr. | Noradrenaline µgm./24 hr. |
|---|---|---|---|---|---|
| 1 | 10 | 138 | 2.3 | .034 | .208 |
| 8 | 10 | 179 | 2.5 | .031 | |
| 14 | 10 | 212 | 7.5 | .065 | .367 |
| 20 | 10 | 258 | 8.0 | .073 | |
| 42,55 | 15 | 345 | 11.3 | .089 | .419 |
| 48 | 10 | 349 | 12.1 | .106 | |
| Mean | | | 7.3 | .068 | .344 |

(b) 2°C.

| Day | No. of rats | Body weight grams | Urine volume cc./24 hr. | Adrenaline µgm./24 hr. | Noradrenaline µgm./24 hr. |
|---|---|---|---|---|---|
| 5 | 16 | 139 | 11.1 | .222 | 1.774 |
| 12 | 16 | 155 | 14.4 | .214 | |
| 18 | 16 | 170 | 16.9 | .197 | 2.271 |
| 25 | 14 | 194 | 14.7 | .178 | |
| 46 | 11 | 247 | 17.7 | .174 | 1.533 |
| 53 | 11 | 253 | 21.2 | .193 | |
| Mean | | | 17.7 | .198 | 1.900 |

TABLE VIII

Average Body Weight, Urine Volume and Excretion of Adrenaline and Noradrenaline in 'Old' Rats Exposed to Cold *
Experiment 2a

| Day | No. of rats | Body weight grams | Urine volume cc./24 hr. | Adrenaline µgm./24 hr. | Noradrenaline µgm./24 hr. |
|---|---|---|---|---|---|
| 0 | 20 | 456 | 20.6 | .184 | .486 |
| 7 | 19 | 416 | 39.2 | 1.086 | 3.727 |
| 14 | 17 | 397 | 47.1 | 1.018 | 5.234 |
| 21 | 16 | 397 | 49.6 | .909 | 6.793 |
| 28 | 15 | 394 | 48.5 | .916 | 6.296 |
| 35 | 14 | 394 | 46.8 | .652 | 3.975 |
| 49 | 7 | 387 | 38.4 | .582 | 4.161 |
| 56 | 12 | 398 | 44.7 | .543 | 3.191 |
| 86 | 10 | 402 | 33.2 | .389 | 2.215 |
| 116 | 9 | 420 | 39.1 | .422 | 2.544 |
| 146 | 7 | 434 | 33.8 | .364 | 2.524 |
| Mean at 2°C. | | | 41.9 | .688 | 4.066 |

* The rats were removed from activity cages on Day 42 at 2°C.

TABLE IX

Average Body Weight, Urine Volume and Excretion of Adrenaline and Noradrenaline in Wistar and Sprague-Dawley Rats at 23°C.
Experiment 2b - Controls

(a) Wistar rats

| Day | No. of rats | Body weight grams | Urine volume cc./24 hr. | Adrenaline µgm./24 hr. | Noradrenaline µgm./24 hr. |
|---|---|---|---|---|---|
| 0 | 6 | 129 | 2.1 | .048 | .109 |
| 10 | | 170 | 5.2 | .043 | .204 |
| 17 | | 214 | 5.4 | .046 | .167 |
| 24 | | 262 | 5.5 | .046 | .192 |
| 39 | | 315 | 6.4 | .044 | .252 |
| 46 | | 339 | 5.7 | .044 | .196 |
| 53 | | 355 | 6.9 | .067 | .234 |
| 60 | | 375 | 7.1 | .070 | .345 |
| 73 | | 401 | 6.3 | .061 | .186 |
| 87 | | 423 | 5.9 | .062 | .305 |
| 109 | | 451 | 5.4 | .056 | .251 |
| 137 | | 448 | 6.1 | .078 | .349 |
| 159 | | 476 | 8.6 | .097 | .358 |
| 196 | 3 | 464 | 12.2 | .131 | .693 |
| 215 | | 465 | 12.8 | .133 | - |
| Mean (Day 10-159) | | | 5.8 | .059 | .242 |

(b) Sprague-Dawley rats

| Day | No. of rats | Body weight grams | Urine volume cc./24 hr. | Adrenaline µgm./24 hr. | Noradrenaline µgm./24 hr. |
|---|---|---|---|---|---|
| 0 | 6 | 110 | 3.7 | .038 | .124 |
| 10 | | 151 | 4.9 | .038 | .159 |
| 17 | | 177 | 2.8 | .040 | .150 |
| 24 | | 211 | 3.4 | .045 | .190 |
| 39 | | 246 | 4.1 | .056 | .215 |
| 46 | | 260 | 3.5 | .070 | .204 |
| 53 | | 272 | 3.1 | .050 | .124 |
| 60 | | 286 | 4.0 | .055 | .205 |
| 73 | | 304 | 4.2 | .052 | .209 |
| 87 | | 317 | 3.4 | .056 | .266 |
| 109 | | 332 | 2.5 | .052 | .301 |
| 137 | 5 | 329 | 7.5 | .120 | .353 |
| 159 | 4 | 369 | 13.0 | .132 | .351 |
| 196 | 2 | 378 | 4.8 | .255 | - |
| 215 | 1 | 403 | 7.0 | .302 | - |
| Mean (Day 10-159) | | | 4.6 | .062 | .219 |

TABLE X

Average Body Weight, Urine Volume and Excretion of Adrenaline and Noradrenaline in Wistar and Sprague-Dawley Rats at 2°C. and 23°C. Experiment 2b

Group I

(a) Wistar rats

| Day | No. of rats | Body weight grams | Urine volume cc./24 hr. | Adrenaline µgm./24 hr. | Noradrenaline µgm./24 hr. |
|---|---|---|---|---|---|
| 0* | 6 | 129 | 2.4 | .050 | .119 |
| 1 | | 148 | 9.1 | .076 | .902 |
| 2 | | | 8.3 | .075 | .854 |
| 4 | | 147 | 21.5 | .237 | 2.102 |
| 8 | | 157 | 13.6 | .139 | 1.631 |
| 11 | | 170 | 17.4 | .175 | 2.837 |
| 15 | | 179 | 14.0 | .118 | 1.250 |
| 18 | | 189 | 21.3 | .160 | 1.751 |
| 22 | | 200 | 14.3 | .125 | 1.088 |
| 29 | | 214 | 17.0 | .143 | 1.559 |
| 36 | | 223 | 20.8 | .155 | 2.108 |
| 50 | 5 | 255 | 21.7 | .169 | 1.445 |
| 64 | 4 | 302 | 22.8 | .169 | 1.696 |
| 85 | | 334 | 21.5 | .195 | 1.930 |
| 113 | 3 | 351 | 17.7 | .165 | 1.492 |
| 141 | 2 | 376 | 21.3 | .161 | 1.310 |
| Mean at 2°C. | | | 17.5 | .151 | 1.597 |
| 23°C. | | | | | |
| 1 | 2 | 375 | 17.0 | .131 | .584 |
| 4 | | 380 | 13.0 | .075 | .167 |
| 8 | | 382 | 11.5 | .081 | .271 |

* Measured at 23°C. three days before the rats were placed in the cold

TABLE X

(b) Sprague-Dawley rats

| Day | No. of rats | Body weight grams | Urine volume cc./24 hr. | Adrenaline µgm./24 hr. | Noradrenaline µgm./24 hr. |
|---|---|---|---|---|---|
| 0* | 6 | 116 | 2.5 | .028 | .108 |
| 1 | | 133 | 7.2 | .077 | .747 |
| 2 | | | 5.1 | .088 | .701 |
| 4 | | 125 | 16.5 | .247 | 1.644 |
| 8 | | 135 | 11.5 | .268 | 1.594 |
| 11 | | 138 | 15.6 | .317 | 2.620 |
| 15 | | 144 | 10.7 | .174 | 1.307 |
| 18 | | 146 | 19.4 | .236 | 1.851 |
| 22 | 5 | 155 | 12.6 | .174 | 1.053 |
| 29 | | 173 | 17.8 | .236 | 1.949 |
| 36 | 4 | 172 | 17.3 | .226 | 1.817 |
| 50 | 3 | 215 | 18.3 | .186 | 1.097 |
| 64 | | 246 | 14.8 | .145 | .912 |
| 85 | | 280 | 21.0 | .349 | 2.448 |
| 113 | | 298 | 20.5 | .213 | 1.743 |
| 141 | | 314 | 25.2 | .371 | 2.719 |
| Mean at 2°C. | | | 15.6 | .220 | 1.613 |
| 23°C. | | | | | |
| 1 | 3 | 309 | 18.8 | .227 | .930 |
| 4 | | 301 | 21.2 | .160 | .556 |
| 8 | | 310 | 22.2 | .159 | .632 |

* Measured at 23°C. three days before the rats were exposed to cold

TABLE XI

Average Body Weight, Urine Volume and Excretion of Adrenaline and Noradrenaline in Wistar and Sprague-Dawley Rats at 2°C. and 23°C. Experiment 2b

Group II

(a) Wistar rats

| Day | No. of rats | Body weight grams | Urine volume cc./24 hr. | Adrenaline µgm./24 hr. | Noradrenaline µgm./24 hr. |
|---|---|---|---|---|---|
| 0* | 6 | 263 | 2.8 | .034 | .108 |
| 1 | | 289 | 12.3 | .116 | 1.663 |
| 2 | | | 12.1 | .126 | 1.325 |
| 4 | | 283 | 19.3 | .192 | 1.316 |
| 8 | | 277 | 15.0 | .151 | 1.382 |
| 11 | | 282 | 14.9 | .157 | 1.775 |
| 15 | | 294 | 17.0 | .134 | 1.593 |
| 18 | | 302 | 14.9 | .118 | .945 |
| 22 | | 303 | 14.0 | .103 | 1.089 |
| 29 | | 313 | 18.7 | .152 | 1.185 |
| 36 | | 323 | 15.7 | .147 | 1.120 |
| 50 | | 336 | 15.1 | .121 | .728 |
| 64 | | 339 | 18.8 | .171 | 1.544 |
| 85 | | 350 | 25.1 | .215 | 2.264 |
| 113 | 4 | 371 | | | |
| 141 | | 373 | 18.6 | .201 | 1.875 |
| Mean at 2°C. | | | 16.5 | .150 | 1.415 |
| 23°C. | | | | | |
| 1 | 4 | 377 | 17.4 | .163 | .820 |
| 4 | | 380 | 10.6 | .114 | .506 |
| 8 | | 382 | 10.8 | .084 | .266 |

* Measured at 23°C. three days before the rats were placed in the cold

Note - Day 1 for Group II is equivalent to Day 31 for the controls

TABLE XI

(b) Sprague-Dawley rats

| Day | No. of rats | Body weight grams | Urine volume cc./24 hr. | Adrenaline µgm./24 hr. | Noradrenaline µgm./24 hr. |
|---|---|---|---|---|---|
| 0* | 6 | 229 | 4.8 | .058 | .190 |
| 1 | | 248 | 11.9 | .243 | 1.552 |
| 2 | | | 11.8 | .357 | 1.565 |
| 4 | | 235 | 16.4 | .278 | 1.363 |
| 8 | | 234 | 19.5 | .414 | 1.978 |
| 11 | | 240 | 16.5 | .250 | 1.353 |
| 15 | | 248 | 22.7 | .315 | 1.939 |
| 18 | | 244 | 18.2 | .300 | 1.526 |
| 22 | | 262 | 20.7 | .289 | 1.691 |
| 29 | | 273 | 22.5 | .278 | 1.561 |
| 36 | | 278 | 27.7 | .352 | 2.379 |
| 50 | 5 | 309 | 31.1 | .299 | 1.566 |
| 64 | 4 | 339 | 30.3 | .377 | 3.595 |
| 85 | | 335 | 32.7 | .398 | 4.310 |
| 113 | 3 | 327 | | | |
| 141 | | 351 | 34.1 | .465 | 3.803 |
| Mean at 2°C. | | | 22.6 | .330 | 2.156 |
| 23°C. | | | | | |
| 1 | 3 | 355 | 23.5 | .275 | 1.931 |
| 4 | | 345 | 22.5 | .244 | 1.212 |
| 8 | | 342 | 23.6 | .118 | .552 |

* Measured at 23°C. three days before the rats were placed in the cold

Note- Day 1 for Group II is equivalent to Day 31 for the Controls

TABLE XII

Average Body Weight, Urine Volume and Excretion of Adrenaline and Noradrenaline in Wistar and Sprague-Dawley Rats at 2°C. and 23°C. Experiment 2b

GROUP III

(a) Wistar rats

| Day | No. of rats | Body weight grams | Urine volume cc./24 hr. | Adrenaline µgm./24 hr. | Noradrenaline µgm./24 hr. |
|---|---|---|---|---|---|
| 0* | 3 | 383 | 9.3 | .091 | .478 |
| 1 | | 394 | 15.2 | .162 | 1.444 |
| 2 | | | 16.0 | .165 | 1.370 |
| 4 | | 384 | 29.3 | .304 | 2.626 |
| 8 | | 382 | 22.0 | .230 | 1.932 |
| 11 | | 379 | 19.5 | .235 | 1.596 |
| 15 | | 391 | 21.7 | .260 | 1.979 |
| 18 | | 389 | 24.0 | .259 | 2.497 |
| 22 | | 391 | 21.2 | .237 | 2.173 |
| 29 | | 396 | 24.0 | .219 | 2.130 |
| 36 | | 396 | 21.5 | .202 | 1.587 |
| 50 | | 400 | 26.2 | .297 | 2.697 |
| 64 | | 397 | 35.0 | .284 | 2.422 |
| 85 | | 413 | 29.8 | .327 | 3.247 |
| 113 | | 423 | 33.0 | .377 | 2.825 |
| 141 | | 442 | 33.5 | .378 | 3.083 |
| Mean at 2°C. | | | 24.8 | .262 | 2.241 |
| 23°C. | | | | | |
| 1 | 3 | 443 | 20.5 | .207 | .844 |
| 4 | | 451 | 20.8 | .133 | .384 |
| 8 | | 460 | 23.0 | .152 | .483 |

* Measured at 23°C. three days before the rats were placed in the cold

Note - Day 1 for Group III is equivalent to Day 71 for the Controls

TABLE XII

(b) Sprague-Dawley rats

| Day | No. of rats | Body weight grams | Urine volume cc./24 hr. | Adrenaline µgm./24 hr. | Noradrenaline µgm./24 hr. |
|---|---|---|---|---|---|
| 0* | 3 | 352 | 7.5 | .066 | .332 |
| 1 | | 358 | 16.2 | .193 | 1.802 |
| 2 | | | 12.3 | .164 | 1.146 |
| 4 | | 350 | 17.3 | .229 | 2.117 |
| 8 | | 347 | 22.7 | .308 | 2.142 |
| 11 | | 352 | 21.7 | .273 | 1.543 |
| 15 | | 365 | 23.8 | .306 | 2.314 |
| 18 | | 365 | 24.5 | .265 | 2.203 |
| 22 | | 369 | 24.0 | .278 | 3.183 |
| 29 | | 375 | 25.7 | .248 | 2.340 |
| 36 | | 367 | 22.0 | .202 | 1.173 |
| 50 | | 365 | 27.7 | .501 | 3.550 |
| 64 | | 347 | 27.7 | .335 | 2.630 |
| 85 | | 368 | 34.6 | .413 | 4.134 |
| 113 | | 379 | 41.8 | .465 | 4.980 |
| 141 | | 375 | 56.3 | .404 | 3.455 |
| Mean at 2°C. | | | 26.6 | .306 | 2.581 |
| 23°C. | | | | | |
| 1 | 3 | 374 | 40.5 | .258 | 1.336 |
| 4 | | 351 | 37.1 | .234 | .801 |
| 8 | | 366 | 42.5 | .333 | 1.288 |

* Measured at 23°C. three days before the rats were placed in the cold

Note - Day 1 for Group III is equivalent to Day 71 for the Controls