CATECHOLAMINE METABOLISM IN THE RAT UNDER VARIOUS HORMONAL CONDITIONS

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THESIS

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TABLE OF CONTENTS

INTRODUCTION

1. CATECHOLAMINE-LIKE EFFECTS OF THE THYROID HORMONE

A relationship between nervous activity and the function of the thyroid gland was observed by von Cyon as early as 1898, (1) when he noted that the increase in the pressure response and pulse rate caused by electrical stimulation of the central end of the transected depressor nerve was best obtained in euthyroids. At that time, however, the concept that some chemical substance may be needed for neuro-transmission was not yet introduced. It was Elliott, in 1904 (2) who expressed the opinion that the effects of stimulation of the sympathetic nerve might well be mediated by adrenaline. In 1908, Kraus and Friedenthal (3) established a parallelism between the effects of adrenaline and hyperthyroidism and Eppinger, Falta and Rudinger (4) demonstrated that the glycosuric action of adrenaline was dependent on the presence of the thyroid gland. In 1911, Asher and Flack (5) further observed that the pressor effect of adrenaline was augmented greatly either by stimulation of the nerves leading to the thyroid gland or by intravenous injection of thyroid gland extracts. The same authors also reported that stimulation of the thyroidal nerves increased

the excitability of the depressor nerve (vagus) of the heart. Two years later Cramer and Kraus (6) noticed that addition of thyroid gland extracts to the diet of cats and rats caused total disappearance of liver glycogen in these animals. Then in 1916 Richardson (7), working with hearts perfused with adrenaline, showed that the inotropic and chronotropic actions of this amine on the heart were enhanced in the presence of added or administered extracts of thyroid gland. Opposite results were obtained by Kakehi (1916) (8) with thyroidectomized animals. The same year, after administering iodothyroglobulin, Oswald (9) obtained an increase in the excitability of the vagus-depressor and splanchnic nerves to faradic stimulation. This increase was found to be proportional to the iodine content of the compound. However the same year Albertoni (10) presented evidence that contradicted Oswald's observations. He found that when dogs and rabbits were treated with thyroid gland extracts the effect of vagus stimulation on the heart was less evident than in normal animals. Then when Eiger (11) perfused the blood vessels of the frog, he discovered that whereas thyroid gland extract had no effect by itself, it did increase greatly the pressor response to adrenaline. Also, blood obtained from rats fed thyroid extracts and blood obtained from patients suffering from exophtalmic goitre had the property

of increasing the pressor activity of adrenaline. In contradiction to both Asher and Flack, (5) and Oswald, (9), Schafer (12) did not obtain modification of the excitability of the vagus or depressor nerve in rabbits, cats or dogs which had been previously injected with thyroid gland extracts. On the other hand this worker observed that when thyroid extracts were injected in cats and dogs during vagus nerve stimulation the resulting fall in blood pressure was greater than that caused by either of these two materials alone. One cannot speak of potentiation however, since the simultaneous effect was the sum total of the effects taken separately. Kuriyama (1918) (13) found, like Cramer and Krause, that when rats were fed thyroid extracts, they developed acidosis and their livers were depleted of their glycogen stores. Thyroid-fed rabbits similarly developed a decrease in the CO_2-combining power. Since Peters and Geyelin (14) had previously found that the hyperglycemia, caused by adrenaline in normal and diabetic subjects, was accompanied by a corresponding decrease in the alkali reserve, Kuriyama reinvestigated these effects in rabbits to see if there was a possibility that acidosis, observed in thyroid fed animals, may have been due to secretion of adrenaline. In rabbits, however, injection of adrenaline did not lead to acidosis at doses capable of producing glycosuria. Kuriyama therefore concluded that the acidosis of hyperthyroid rats and rabbits had nothing to do

with adrenaline. The author was mainly interested in this aspect of the question and did not raise the possibility that adrenaline may have been implicated in the glycogenolytic effect. In 1919, Santesson (15) reported that with rabbits either fed or injected with thyroid extract, a greater rise in blood pressure was produced by injection of adrenaline as compared to euthyroid controls.

These early investigations have encouraged and stimulated many workers in the field of catecholamine-thyroid interrelationship as can be seen from the number of subsequent papers dealing with this problem. At this point it seems appropriate to consider separately each effect of the thyroid hormone.

a) Effect on Glycogen and Blood Glucose

Bodansky (16) found that the hypoglycemic reaction to insulin is greater in thyroidectomized sheep. Collip (17) observed that injection of adrenaline in normal rats caused a 50% decrease in their liver glycogen content whereas little reduction occurred in hypophysectomized and thyroidectomized rats. After injection of adrenaline also no increase in blood glucose was noted in hypophysectomized rats whereas such was not the case for thyroidectomized animals. In hypophysectomized rats the normal glycogenolytic and hyperglycemic effects of adrenaline were restored after treatment of these animals with anterior pituitary extracts but

administration of desiccated thyroid alone failed to restore the hyperglycemic response. Houssay (18) reported that hyperthyroidism increases considerably the severity of diabetes in dog and man. Thyroidectomy improves the overall condition but diabetes is still manifest. Burn and Marks (19) made a study of the effects of the hypoglycemic action of insulin and hyperglycemic action of adrenaline in different groups of rabbits. These authors were able to show that the minimal insulin-convulsive dose was higher before thyroidectomy than after thyroidectomy. Indeed, after thyroidectomy the three rabbits studied became six to nine times more sensitive to insulin. Conversely, the adrenaline hyperglycemia was greatly reduced after thyroidectomy. Since in both cases the same animals were used, the authors could establish the following relationship; those animals in which the greatest sensitivity was observed also showed the greatest decrease to adrenaline-hyperglycemia. After the same thyroidectomized animals were fed thyroid extracts their sensitivity to insulin was greatly diminished. For example, after eight days of this treatment the animals could support five times the initial dose of insulin without entering into convulsions. After seven days of thyroid feeding in one rabbit the reactivity to standard doses of adrenaline doubled, i.e. the difference between the average blood sugar during one hour after the injection and the initial

blood sugar was twice as great after thyroid feeding. In another rabbit after fourteen days of feeding this response was tripled, indicating that the magnitude of the hyperglycemic response depended on the level of the thyroid hormone. But since their data do not include the initial value for blood glucose, it is impossible to see if the absolute increase in blood glucose observed in thyroid animals is reflected by a corresponding relative or percent increase which would be indicative of a sensitizing effect of the thyroid hormone. It is not unreasonable to suppose, on the basis of the results obtained with injections of insulin in thyroid-fed animals, that the blood glucose level of these animals may have been above normal. Indeed, it has been shown (20) that infusion of 1.5 to 3mg/kg of thyroxine into chloralosed dogs results in a gradual increase in their blood sugar level. When, after such infusions, venous blood was collected from the adrenals, it was found to contain a substance capable of inhibiting the movement and tonus of the intestine of a rabbit, and which, on this basis, was identified as adrenaline. This secretion of adrenaline was parallel to the increase in blood glucose except at higher doses of thyroxine(3mg/kg). In this case normal levels of glucose were found despite the persistence of adrenaline in the venous blood of the adrenals. The latter finding is consistent with that of Burn and Marks(21) who showed that

the hyperglycemic response to adrenaline is only diminished when, as a result of thyroid feeding, the liver glycogen of rabbits disappears.

Studies were made (21) on the various effects of metrazol, adrenaline and thyroxineon rats which were either vagotomized, thyroidectomized or vagotomized and thyroidectomized. In this series of experiments, the authors conclude that a marked increase in blood sugar, i.e. over that caused by adrenaline, could not be observed below doses of thyroxine of 0.2mg/100g . Data are also interpreted to mean that thyroid hormone increases the excitability of the centres of the sympathico-adrenal system without having much influence on the glycogenolytic action of adrenaline. According to their results the vago-insulin system under the influence of parasympathetic centres is not modified by the thyroid hormone.

With the guinea pig, Comsa (22) provided quantitative evidence that the thyroid hormone may have sensitizing effects on the hyperglycemic action of adrenaline. The first step achieved in his study was the finding of the threshold dose of adrenaline (2.8µg/100g) with respect to its effect on blood glucose. This threshold dose was found to be five times higher in thyroidectomized animals. Not only was the normal threshold dose reestablished by thyroxine treatment and the response to adrenaline found to be a

continuous function of the thyroxinedose previously injected, but it was also shown that higher doses of thyroxinelowered the threshold dose of adrenaline proportionately to the dose of thyroxine injected.

Similar conclusions were arrived at by Trendelenburg (23). In normal rabbits, a dose-response curve of adrenaline on blood sugar shows that the percent increase in blood glucose caused by adrenaline varies linearly with the amount of adrenaline given. Noradrenaline was also found to produce hyperglycemia but its potency was only 18% that of adrenaline. In all twenty rabbits which were fed thyroid for fourteen days the percent increase in blood sugar produced by adrenaline was greater than before thyroid feeding. After such treatment the dose of adrenaline necessary to bring about the same response as before treatment was reduced from 85 µg/kg to 60µg/kg and the author logically submitted, as a concept, the sensitizing effect of the thyroid treatment.

In hypophysectomized, fasting-female rats, injections of adrenaline (5µg/100g)were found to decrease the glycogen content of the rectus femoris (20% loss), abdominal (49% loss) and diaphragm (38% loss) muscles (24). These losses were comparable to those previously found in fasting intact female rats (25). Although injection of 150µg

of thyroxine increased the glycogen level in all three tested organs, there was a definite increase in their percent loss of glycogen following adrenaline administration. In the rectus femoris and abdominal muscles this sensitization of the glycogenolytic action of adrenaline was specific for thyroxine since growth hormone or cortisone acetate either inhibited this response or was without effect. In the diaphragm muscle, however, the latter hormones when given in large doses exerted a similar sensitizing effect. It may be pertinent to recall here that these effects are observed in hypophysectomized animals in which case the adrenals are not expected to function normally and hence may not be expected to contribute much to the sensitizing action of thyroxine. This is contrary to the findings of Zunz and Laberre ([20]).

Harrison ([26]), using less direct methods nevertheless supports similar conclusions regarding the effect of thyroid hormone on the hyperglycemic response to adrenaline ([26]). This worker found that although resting urinary noradrenaline was the same in normal and hyperthyroid patients, adrenaline was approximately one third that found in normal patients. The increased excretion of free catecholamines, which was found to occur in normal subjects as a result of vaso-motor-reflex stimulus (tilting) or insulin hypoglycemia, was not as great in hyperthyroid

patients. Because of previous results concerning the effect of thyroid on glycogenolysis and blood sugar, the author concludes that "Less adrenaline is required in hyperthyroidism to stimulate the glycogenolysis required to overcome insulin hypoglycemia."

b) Effect on O_2 Consumption

Although a few authors expressed the opinion that the functional state of the thyroid gland does not influence the basal metabolic rate (27, 28), much experimental work supports the contrary. In 1925 Nakayama (29) made a most extensive quantitative study on the effect of adrenaline on the metabolic rate of normal, hyperthyroid and hypothyroid rats. Essentially, the results were as follows; doses of adrenaline which caused a 25% increase in normal rats produced an increase of only 13% in thyroidectomized animals but caused an 82% increase in the metabolic rate of hyperthyroid rats. The basal metabolic rate without adrenaline gave a similar pattern, the highest being recorded for the hyperthyroid animals.

Swanson (30) observed that whereas normal rats responded to injections of adrenaline by an increase in their O_2 consumption which was proportional to the dose of adrenaline, such was not the case in thyroidectomized

animals in which adrenaline caused even a slight decrease in O_2 consumption. The data also show that when thyroid hormone was administered in sufficient quantities the effectiveness of 40µg of adrenaline was doubled.

Barker et al (31) obtained essentially the same effects, after establishing that adrenaline causes an increase of 40% in the metabolic rate, they noted that after thyroidectomy the same dose of adrenaline caused a slight fall in the metabolic rate. On the other hand, with hyperthyroid animals and still the same dose of adrenaline, the increase in metabolic rate reached 90%. The basal level of O_2 consumption reflects the thyroidal state of the animals.

Suspecting that hormonal control of temperature may be at least partially responsible for these effects, Schaffer and Thibault (32) repeated these experiments but with rats previously adapted to a constant temperature of 28° C. They could but only confirm previous results: subcutaneous injections of 0.5mg/kg of adrenaline produced an increase in O_2 consumption of 53%. The same animals when thyroidectomized and kept twenty-one days at 28° responded to the same dose of adrenaline by an increase of only 8%. The normal response was brought back by thyroid treatment. They have also obtained some evidence that the reinforcing action of thyroxine on the metabolic action of

adrenaline was not dependent on the enhancing effect of thyroxin on the basal metabolic rate (33). Noradrenaline has also been found to increase the metabolic rate of rats but to a lesser extent than adrenaline; however in thyroidectomized animals responses of both these catecholamines are comperable (34).

In rabbits, it was noticed that the total disappearance of the metabolic response to adrenaline occurred only twenty days after thyroidectomy and not before (35). Again, thyroid treatment brought back normal response and, increasing the amounts of thyroid hormone increased the stimulatory effects of adrenaline.

In accordance with previous results Ring (36) reported that in normal male rats weighing approximately 200 g ,amaximal dose of adrenaline produced an increase of 53% in the metabolic rate: in thyroidectomized animals; however, the metabolic response to adrenaline was not considerably reduced; the increase was still 43%. No mention is made at which time these measurements were made following thyroidectomy. This would seem to be an important factor in demonstrating a decrease in the metabolic effect of adrenaline (35). In normal rats, injected with thyroxine in the following amounts, 0.5, 4.0 and 8.0mg, subsequent injection of the maximal dose of adrenaline

resulted in the following respective increases - 60%, 60% and 95% (36). The basal metabolic rate revealed the same pattern. But to show that the sensitizing action of thyroid was a specific property of the hormone and did not depend on the changes induced by thyroxineon the basal levels, King introduced these same metabolic changes by other means and studied the effect of adrenaline. For example, dinitrophenol, which raised the basal metabolic rate to an average of 34%, did not modify the calorigenic action of adrenaline. Also, fasting the rats for three days, decreased the O_2 consumption but did not change the effect of adrenaline.

In vitro, it has been found (37), that frog heart obtained after perfusion with thyroxinehad an elevated rate of metabolism compared to normal animals, and several other papers support the same observation for rat heart (38, 39, 40, 41), On the other hand the results obtained by Ganjuv and Lockett (42) imply that the thyroid hormone is incapable of a direct calorigenic action and that this action is dependent on the presence of the adrenals. They have demonstrated that doses of thyroxin, which were twice the amount required to increase the O_2 consumption in normal mice, failed to do so in adrenalectomized and thyroidectomized-adrenalectomized mice. This is in direct contradiction with the in vitro findings mentioned above and also with

other *in vivo* results (43, 44, 45) showing that in rats adrenalectomy does not modify the metabolic effect of thyroxine. These discrepancies may be accounted for by species differences, or by tissue differences. However, from the foregoing considerations, it can be seen that there is general evidence that normal functioning of the thyroid gland is necessary for the calorigenic action of adrenaline.

c) Cardio-Vascular Effects

McDonald *et al* (46) showed a 75% increase in the O_2 consumption of hearts isolated from terrapins fed thyroid extract as compared with those belonging to terrapins fed a normal diet. Similar increase was also observed in the rate of hearts obtained from thyroid-fed animals. Introduction of adrenaline in the perfusion fluid circulating through the hearts of such experimental animals resulted in an 126% increase in the O_2 consumption of these hearts whereas adrenaline caused only a 45% increase in the O_2 consumption of normal hearts. This sensitization ability of thyroxineon the metabolic effect of adrenaline on the heart could not be extended to the chronotropic effect of adrenaline since this amine caused a comparable percent increase in the heart rate of both normal and hyperthyroid hearts.

In another paper, Aumann and Youmans (47) also provided data which show that thyroid-fed dogs experienced an increase in heart rate which amounted from 30 to 60 beats/min. But they also observed that injections of adrenaline caused a 60% increase in the heart rate of thyroid-fed dogs and this effect was greater than that obtained before thyroid feeding.

In a short communication (48) lacking quantitative data it is said that thyroid feeding of dogs causes, besides moderate tachycardia, an adrenaline-tachycardia which was greater than that found in normal animals and this with several doses of adrenaline. But no basal values of the heart rate are given and it is impossible to estimate the nature and extent of this increase. The pressor effect of 10 to 30µg of adrenaline was diminished after thyroid feeding: all values obtained after thyroid treatment are compared to those obtained before thyroid feeding. After these dogs were given atropine sulfate the blood pressure response to adrenaline was the same before and after thyroid feeding but greater amounts of adrenaline were necessary to induce tachycardia. In the latter case, however, the response was the same in normal and thyroid-fed animals. Such results infer that the thyroid hormone may perhaps also be involved with parasympathetic activity. Indeed, it has

been shown (49) that hearts obtained from hypothyroid cats, rats and guinea-pigs display a greater sensitivity to vagus stimulation or to the depressor effect of acetylcholine; the converse is true in the case of hyperthyroid animals, a result which is in contradiction with those reported earlier (8, 11). The same authors (49) also reported that lower doses of adrenaline were necessary to bring about an increase in the amplitude of contraction and the frequency of beats of hearts obtained from hyperthyroid cats and guinea-pigs.

Sawyer and Brown (50) observed the effects of endogenously-liberated and exogenous adrenaline on the rate of denervated heart obtained from thyroidectomized cats and thyroidectomized cats treated with thyroxine. Cold exposure and exercise were used as means of producing secretion of adrenaline. Cold exposure caused the rate of denervated hearts of normal cats to increase but with thyroidectomy this effect was reduced. In one cat which was fed orally a semi-purified preparation of thyroxine in amounts which returned the basal metabolic rate of the cat to normal levels, the percent increase in the rate response of the heart to cold stimulus was also comparable to normal. In another cat with an increased metabolic rate, the percent increase in the acceleration of the heart also was raised but only by 10% above normal. When exercise was used as a means of producing adrenal secretion the heart rate of the thyroidectomized

cats responded similarly as for cold exposure. However, in a rat given thyroxine in a sufficient amount to raise its metabolic rate to normal, the percent increase in heart rate was not restored to normal. Essentially, the same results were obtained for exogenous and for endogenous adrenaline when the latter was presumably secreted by the adrenals as a result of exposure to cold. At one point it is difficult to agree with the authors when they state that exogenous adrenaline causes a greater percent rise in the cardiac rate of cats which were given thyroxine in amounts which increased their metabolic rate above normal. This percent rise is observed to be above control only some 24 seconds after administration of adrenaline; before and after this time the percent increase is below the level of control. The results offered by this work do not firmly support a potentiating action of the thyroid hormone. In all experiments in which adrenaline was injected, the adrenal medulla was removed. Since the response of the denervated heart from thyroidectomized cats was the same with respect to secreted or exogenous adrenaline, the authors concluded a direct action of the thyroid hormone on the heart. Indeed, if the thyroid hormone exerted its effects via the adrenals by increasing the secretory activity of the catecholamine, then full response to exogenous adrenaline should be obtained

even in the absence of the thyroid gland, but such was not the case and this justifies the author's conclusion.

Brewster's (51) work reveals that the effects of both adrenaline and noradrenaline on the heart rate, cardiac output and right ventricular stroke work were enhanced in dogs by thyroid feeding. This also holds true for O_2 consumption but not for the left ventricular stroke work and blood pressure.

Beznak (52) was able to show that when rats weighing approximately 165 g were injected for 2-3 weeks with increasing doses of thyroxine varying from 0.037mg to 5.88mg, the percent increase in the cardiac output, O_2 consumption and weight of the hearts of these rats rose proportionately. This worker also studied the effect of thyroxine on the work performance of these hearts: under conditions of polyvinylpyrrolidone (PVP) loading, the hearts from thyroxine-treated rats were able to perform much better than the hearts from rats not treated so, as could be judged by the great increase in maximal cardiac output and work of the former over the latter. The greater weight of the hearts of the thyroxinized animals could not be held responsible for these effects since hearts of the same weight or hearts in which hypertrophy was induced by aortic constriction responded to PVP infusion as did normal controls.

Vandershoot et al (53) studied the effect of graded doses of adrenaline and noradrenaline on the O_2 consumption and blood pressure of hyperthyroid dogs and also on the contractile force of the hearts of such animals. Whereas a greater percent increase in the O_2 consumption is found to occur in the hyperthyroid compared to normal animals, no such increase occurred for the other effects of the catecholamines.

Experiments were also performed on the vascular responsiveness to adrenaline and noradrenaline by Page and McCubbin (54). In propylthiouracil-treated and thyroidectomized dogs the arterial blood pressure response to adrenaline and noradrenaline was diminished considerably. It may be added that the depressor action of histamine was also reduced by such treatments. Strangely enough though, when the hypothyroid animals were fed dessicated thyroid, which restored normal appearance and behaviour, normal responses to the catecholamines were not attained, and in the case of adrenaline, hypotension was noted. On the other hand, exaggerated blood pressure responses to noradrenaline were found to occur in clinical hyperthyroidism (55). The infusion of noradrenaline in four thyrotoxic patients produced a rise in systolic pressure which amounted to 47mm Hg, and in the case of the mean pressure, to 30mm Hg. When the

same patients were treated and reached the euthyroid state, the same dose of noradrenaline now caused an increase in the systolic pressure of only 14mm Hg, and in the mean pressure, of 12mm Hg. Since these data deal with only the absolute increase and do not reveal the basal pressure values, no appreciation of the percent increase can be obtained. The authors however report the case of a myxoedematous patient which, when treated with thyroxine, responded to noradrenaline by a greater percent rise in both systolic and mean blood pressure.

Wurtman et al (56) reported similar observations in hyperthyroid rats. The heart rate, mean blood pressure and pulse pressure were lower in the hypothyroid rat compared to normal whereas in the case of the hyperthyroid animals the basal level of these parameters was higher than in normals. When the different groups were submitted to doses of adrenaline ranging from 0.1 to 0.8µg/g , the mean blood pressure response of the hyperthyroid group was more than twice that obtained for the other groups. Another particularity is that the thyroidectomized group responded in a manner comparable to the normal group. No data is given for the heart rate response.

Clinical studies were also made by Murray and Kelly (57) on eleven hyperthyroid patients and twenty-two normal subjects. In these patients, the basal metabolic rate

was found to be, on the average, + 25% compared to - 3% for the normal subjects; the heart rate and pulse pressure are also reported to be elevated. It is apparent from the data that at all seven doses of adrenaline varying from 0.02 to .18μg/kg/min. there is a significant increase in the percent in O_2 consumption in the hyperthyroid patients compared to normal. Although the authors report a similar increase for the pulse pressure, they have certain reservations for the heart rate. This leads them to consider the percent change in pulse pressure X heart rate as the index of cardiac changes, in which case definite increases are recorded in the hyperthyroid patients. When normal subjects were injected triiodothyronine the same results were obtained. An interesting observation was that hypermetabolic patients with normal thyroid activity were not particularly sensitive to adrenaline and behaved as did normals. The latter finding has also been reported in another paper (58) in which it is shown that, although salicylate ingestion in human volunteers caused a rise of their metabolic rate, it did not increase their sensitivity to the catecholamines during this period of hypermetabolic activity.

A potentiating action of the thyroid hormone on the cardiovascular effects of the catecholamines has been questioned very recently by Margolius and Caffney (59) and

by Van der Schoot and Moran (40) who criticized the lack of completeness of data, lack of systematic approach and the few numbers of animals used in many of the works performed to prove such an action of the thyroid hormone. In the case of Van der Schoot and Moran (40), although thyroxine treatment in dogs and rats caused an elevated pulse pressure in dogs, and an elevated heart rate and O_2 consumption in both rats and dogs, the increase in heart rate and force of contraction of heart muscle resulting from injections of graded doses of adrenaline or noradrenaline was less in hyperthyroid dogs. These changes are reported in terms of percent change and absolute change. The blood pressure response to noradrenaline was not different in the hyperthyroid group as compared to the normal group. The blood pressure response to adrenaline was significantly decreased in the thyroxine-treated group as compared to the control group. These studies were made on seventeen hyperthyroid and seventeen normal dogs and their reactivity was measured for six doses of catecholamines which varied between .05 and 1.6µg/kg. The difference in the blood pressure response to adrenaline was attributed to a greater vasodilating effect of adrenaline in the hyperthyroid group. These results were confirmed by <u>in vitro</u> experiments in which the inotropic response to noradrenaline was tested on ventricle strips of rats and the chronotropic action of

noradrenaline was studied on the spontaneously contracting atria of rats and rabbits.

Margolius and Gaffney (59) have also systematically investigated the cardio-vascular reactivity to injected noradrenaline and to nervous stimulation in twenty-one normal, eleven hyperthyroid and six hypothyroid dogs. It is clearly evident from their results that thyroid feeding in dogs produced an increase in the heart rate, in the pulse pressure, rectal temperature and in serum iodine. However in the hypothyroid dogs no difference from the control is observed for the heart rate (when animals are anesthetized) mean arterial pressure and arterial pulse pressure. A decrease in the heart rate of awake animals was noted. When the accelerator nerve was stimulated, the absolute increase in heart rate was the same in control, hypo-or hyperthyroid groups. While the percent increase was the same in the control and hypothyroid groups, it was lower in the hyperthyroid group, because the basal heart rate of the hyperthyroid group was greatly elevated. The mean arterial blood pressure was not significantly different in the various groups of dogs when the accelerator nerve was stimulated at different frequencies, also no difference was noticed in the mean arterial blood pressure and heart rate when the different groups of dogs were injected with seven doses of noradrenaline,

varying from 0.01 to 10µg/kg, the absolute and percent increase was the same for the different groups at all doses used.

In resume, it may be said that although thyroid gland activity undoubtedly influences certain parameters of the cardio-vascular system, a potentiating effect of the thyroxine hormone on certain actions of the catecholamines, namely, on their chronotropic and pressor actions, cannot at the present time be assessed. With respect to this potentiation, several reasons may account for the contradictory reports given in the literature, some of which are already mentioned (c.f. p.zz.). It may be added that besides species differences, another factor may reasonably account for these discrepancies; there must be a limit to the potentiating action of thyroxine if there is a potentiation at all. For example, if an animal is given so much thyroxine that a maximal value has been reached for the heart rate, say, it is hard to suppose that catecholamines will produce an extra response. Of course this is an extreme case but it points out the importance that the degree of the hyperthyroidic state of the animals may have on the possible potentiating effect of the thyroid hormone. More uniform results would then probably be obtained if standard amounts of thyroxine were injected (to induce hyperthyroidism) in any given species of animal.

Because of its relevance to the work presented in this thesis and also because of the particularity of the topic of hypophysectomized animals, a separate section is reserved to survey the work dealing with hypophysectomized animals in reference to their cardio-vascular state.

2. THE EFFECT OF HYPOPHYSECTOMY ON THE CARDIO-VASCULAR STATE OF RATS

Total hypophysectomy has been shown to reduce the blood pressure of rats below normal levels (41).

It was later shown that hypophysectomy in normal dogs reduced the arterial pressure from 140/70 to 116/50 mm Hg (42). Hypophysectomy was also found to reduce experimentally-induced renal hypertension from 240/160 to 150/100 mm of Hg. Administration of thyroxine augmented this latter value to 190/120 mm Hg.

Green et al (43) observed that in rats in which hypertension was induced by administration of deoxycorticosterone (DOC) acetate, hypophysectomy caused the blood pressure to return to normal. When propylthiouracil was given to the DOC-hypertensive rats an initial drop in blood pressure occurred during the first five days but this effect did not persist and the blood pressure returned almost completely to the hypertensive levels.

Salgado (44) demonstrated that the lower level in blood pressure of rats produced by hypophysectomy was corrected by treatment with growth hormone and adrenocorticotrophic hormone. Better responses were obtained when both hormones were administered at the same time.

On the other hand, Hoelscher (45) could not prevent the hypotension and cardiac atrophy characteristic of

the hypophysectomized rats by treatment with either adrenocortical hormone or adrenocorticotrophic hormone.

White et al (66) reported that hypophysectomy as well as thyroidectomy resulted in a significant decrease in the cardiac output and O_2 consumption of dogs. In the hypophysectomized dog, normal responses were brought back by treatment with anterior pituitary extracts.

Hartly and Leblond (47) found that when thyroidectomized rats weighing approximately 150 g were given daily 3 and 6 µg of thyroxinefor 3 weeks, this dose was sufficient to restore the observed decrease in heart rate and O_2 consumption which had resulted from thyroidectomy. Both the O_2 consumption and heart rate responded proportionately to graded doses of thyroxineranging from 3µg to 12µg, but 3 and 6µg were considered by these workers to be physiological amounts. When the same doses were given to hypophysectomized animals in which the heart rate and O_2 consumption were also found to be decreased, both were restored to normal levels and with the same increments as for thyroidectomized animals. However, the response of O_2 consumption was not quite as faithful as the heart response and although the authors of this work point out that this may be due to the fact that in hypophysectomized animals the initial level of O_2 consumption is lower than in the thyroidectomized group,

the initial difference is much smaller than that observed when the different groups are treated with thyroxine. But at 12µg of thyroxine both the O_2 consumption and heart rate values are comparable in the thyroidectomized and hypophysectomized rats, the values being somewhat higher than in control rats. Hartly and Leblond (43) also reported that these effects of thyroxine could be produced in the absence of the adrenals, a finding which is of some importance in the elucidation of the mechanism of action of the thyroxine hormone. Hartly and Leblond (47) conclude that the thyroxine hormone must act on non-endocrine tissue.

Beznak (49) studied the hemodynamic effects of hypophysectomy in rats. The data show that in male albino rats hypophysectomy caused a pronounced decrease in the heart rate (from 481 beats/min. to 306 beats/min.), a decrease in cardiac output and a decrease in cardiac work. The respiratory rate and O_2 uptake were also decreased. The total peripheral resistance was slightly higher but the blood volume remained unchanged. Hypotension was attributed to a decrease in cardiac output which in turn was mainly the consequence of a decrease in heart rate, the output per beat being only slightly reduced. That these effects may simply be circumstantial is suggested by the finding that the hearts from hypophysectomized rats could increase their work

performance under conditions of PVP loading and this, in a way relatively comparable to hearts of normal rats. Beznak thus suggests that in hypophysectomy the lower metabolic demands causes a slowing in the circulation, which results in a decreased venous return, decreased cardiac output and hence hypotension.

In a subsequent paper (49) it was shown however that when the hearts of hypophysectomized rats were subjected to aortic constriction, the usual blood pressure rise and cardiac hypertrophy observed in normal animals were absent. Thyroxine treatment restored the normal level of O_2 consumption, weight of hearts, cardiac rate and blood pressure with the following respective doses injected over a four week period; 1.5mg, (O_2 consumption) 1mg, (weight of hearts) 1mg, (cardiac rate) and 2.5mg for a period of five weeks, (blood pressure). Despite such treatment the hearts from hypophysectomized rats could not reach the normal maximal values for work performance during PVP loading. Growth hormone also failed to so so in doses of 20mg given over a period of four weeks, but when this dose was given conjointly with 1.5 mg of thyroxine for the same period of time, the maximal values for work performance were above those obtained for normal hearts except for heart rate which was the same as for normal. The other values which were above normal were those obtained for blood pressure,

cardiac index minute work index, and also left ventricular weight and O_2 consumption. With these parameters the total effect obtained with both hormones also exceeded that obtained with the sum effect of both hormones taken separately. When extra work was forced upon the hearts, from hypophysectomized rats, by aortic constriction, normal responses could only be obtained when both thyroxine and growth hormone were administered in combination. In summary, the hearts from hypophysectomized rats will respond maximally to both PVP loading or aortic constriction only when the animals are administered thyroxine and growth hormone. Because appearance of the hemodynamic changes in the hypophysectomized animals coincides with the appearance of the hypermetabolic state (49) the earlier suggestion that these cardio-vascular changes may be the result of increase in O_2 demands is reinforced. But these changes may also be logically explained by a change in the catecholamine content of the hearts of hypophysectomized animals. If this was the case then thyroxine hormone should be expected to correct, in the heart the changes of catecholamine content if these changes are really responsible for the hemodynamic changes. Possibly the synergistic effect observed for thyroxine and growth hormone may be explained also in terms of catecholamines especially since certain maximal values obtained in PVP loading cannot be attributed

to increases in O_2 demands because the O_2 consumption was relatively lower. That the catecholamines may be involved, stems not only from their well known inotropic and chronotropic action on the heart but also from the relationship observed between the effects of thyroxine and those of catecholamines. Also, the catecholamine stores have a profound influence on cardiac activity (104, 105, 96).

These considerations led to our investigations dealing with the catecholamine content of hearts obtained from only hyphysectomized rats, hypophysectomized rats treated with thyroxine, hypophysectomized rats treated with growth hormone and hypophysectomized rats treated with both thyroxine and growth hormone. Catecholamine content of brain and liver was also studied.

CONCLUSION

It is apparent from the foregoing, that the diversity of action of the thyroid hormone may complicate any attempt to explain its mechanism of action. Superimposed on this is the contradictory evidence pertaining to some of the effects of the thyroid hormone. Let us separate these effects in two main categories:

1. A catecholamine-like effect for which experimental evidence is amply given. The hyperthyroid animal

is characterized by certain symptoms which mimic the effects of the catecholamines namely, increase in metabolic rate, heart rate, blood pressure and glycogenolysis. In the presence of growth hormone the thyroid hormone has also a marked influence on cardiac performance under conditions of work loading.

2. A potentiating manifestation on the action of the catecholamines. This manifestation cannot however be ascribed to all the above mentioned effects because of contradictory results given in the case of heart rate and blood pressure. On the other hand a potentiation of the O_2 consumption effect of adrenaline has repeatedly been observed and cannot be reasonably doubted. As for the glycogenolytic action of adrenaline probably more confirmation of a potentiating action of thyroxine must be awaited before it can be accepted with certainty.

If it is shown in the future that this potentiating action is particular to O_2 consumption then this would be an indication that the thyroid hormone may exert its different effects independantly. It has already been mentioned that hypermetabolism is not necessarily related to the other effects.

As may be expected several attempts have been made to explain these effects of the thyroid hormone and this will

be dealt with in the following section. Quite naturally the proposed mechanism invariably involves the catecholamines in one way or the other.

3. PHYSIOLOGICAL IMPLICATIONS AND DETAILS OF RELATIONSHIP BETWEEN THE THYROID HORMONE AND THE CATECHOLAMINES

a) The effect of thyroxine on the secretory activity of the adrenal medulla.

The contention that the thyroid hormone may exert its effect by increasing the secretion of the catecholamines from the adrenals gained strong support from an early report (20) showing that an adrenaline-like material was released in the venous blood of the adrenals of dogs which had been injected with thyroxine. This concept had been formulated much earlier in 1908 by Eppinger et al (4), and subsequently received support from the finding that the adrenals of cats fed thyroid extracts contained larger amounts of adrenaline than did cats fed normal diet (69). The latter result was confirmed histologically by the finding that the medulla was charged with chromaffin material (67). Herring later confirmed his results in male albino rats (70) but reported that with female albino rats the results were inconsistent. Kuriyama (71) failed to reproduce these effects but employed groups of rats of mixed sex. The method used for determination of catecholamines at that time was quite crude and these results cannot be taken without reservation apart from the one obtained histologically. Recently, however Hopsu (72) demonstrated that when female mice were fed thyroid there

was a decrease in the amount of chromaffin material of the adrenal medulla as judged by the intensity of the chromaffin reaction in this tissue.

Later work, in which better chemical techniques were used for the determination of catecholamines, revealed a decrease in the amount of catecholamine present in the adrenals obtained from hyperthyroid animals. Hokfelt (73) expressing his results either per gram or per kg body weight obtained lower amounts of adrenaline and noradrenaline in the adrenals of rats injected thyroxine. A converse effect was produced by ablation of the thyroid gland. Using a colorimetric method D'Iorio and Flaut (74) expressed their values in µg/mg of nitrogen and obtained a 50% decrease in the catecholamine content of adrenals of rats fed either thyroid powder or iodinated casein. Goodal (75) also obtained similar results in the thyroxine-treated sheep. Leduc et al pointed out (76) that when their results are expressed per mg of nitrogen the catecholamine content of adrenal of hyperthyroid rats is low but notdifferent from normals when the values are expressed per unit gland, an indication that the changes noted previously may simply be the result of the hypertrophic effect of thyroxineon the adrenals. Indeed Franko (77) found no significant difference in the adrenaline content of the total gland of hyperthyroid adult or young male rats compared to control. But a

significant decrease in the noradrenaline content of the adrenalsof experimental animals was found. An isolated result (78) showing increased amounts of catecholamines in the adrenals of hyperthyroid animals cannot be taken seriously in view of the number of papers proving the contrary. It does not appear that the thyroid hormone causes a state of super-secretory activity of the adrenal medulla.

Several early reports claimed that the blood of hyperthyroid animals contained more adrenaline than the blood of normal animals (79, 80, 81). The methods used by these workers however, were criticized even by their contemporaries (82, 83, 84, 85, 86). On the other hand relatively recent work by Zile and Lardy (87) reveals elevated amounts of adrenaline and noradrenaline in the serum of thyroid fed rats. But these results are counter balanced by other reports showing normal values for adrenaline and noradrenaline of thyroid-fed dogs (88) and rats (76). Also, Rogoff and Cortell (89) did not obtain any difference in the quantity of adrenaline liberated from the adrenals in hyperthyroid dog as compared to normal.

The heart rate and O_2 consumption of rats were unaffected by ablation of the adrenal glands (45), and restoration of heart rate and O_2 consumption by thyroxine treatment in hypophysectomy did not depend on the presence of the adrenals (43). Removal of the adrenal medulla or

adrenergic blockade caused by dibenzyline, in normal and thyro-parathyroidectomized rats, did not interfere with the calorigenic effect of thyroxine (45). Similar results were reported earlier (44). But there are also contradictory reports (42,90) attributing a role to the adrenals for normal calorigenic response to thyroxine.

It is hard to conceive how the thyroxine hormone could exert its effects on the isolated heart (37,38,39,40,41) if it were via the adrenals, and in the absence of more conclusive evidence this theory cannot be given unquestioned consideration.

b) <u>The effect of thyroxine on sympathetic nerve activity</u>

During recent years, attention has been directed towards the concept that thyroxine may increase the activity of the sympathetic system and therefore mimic the effects of catecholamine.

In many of the experiments, the results of which support this theory, use has been made of the drugs, guanethidine and reserpine, because of their effects on nerve activity. For instance reserpine has been shown to liberate the catecholamines from different parts of the central nervous system and from adrenal medullary glands (91,92,93,94), and to deplete the heart of its catecholamines (95,96,97). Reserpine can also interrupt adrenergic reflexes (98). Guanethidine can interrupt adrenergic reflexes but at the

neuro-effector junction specifically (99), and can deplete the myocardial noradrenaline stores (100, 101). These drugs therefore, produce a state comparable to total sympathectomy (102).

Gaffney et al (102) studied the effect of guanethidine in four volunteers in which the usual symptoms of hyperthyroidism were induced by injections of triiodothyronine. Guanethidine was found to have beneficial effects on both the basal metabolic rate and heart rate but had no effect on the low serum cholesterol level and loss of weight produced by triiodothyronine. However, tremor which had occurred as a result of such treatment was completely abolished by guanethidine.

Similar effects were also reported in one patient suffering from acute hyperthyroidism (103). Guanethidine lowered the basal metabolic rate, cardiac rate and alleviated tremor but had no effect on the cholesterol value nor did it have any effect on the weight. As with Gaffney's investigation the heart rate and O_2 consumption were not lowered to normal values. Increasing doses of guanethidine did not bring any further changes, but treatment with methylthiouracil decreased the protein-bound iodine and brought back the cardiac rate and metabolic rate to normal values.

In another publication (104), when three hyperthyroid

patients received low doses of guanethidine, cardiac rate was reduced from 111 to 88 beats per minute the stroke volume increasing from 62 to 80 ml. When three other patients were given higher doses (>40 mg) heart rate fell to 84 beats per minute, cardiac index fell from 6.7 to 4.6 liters per min. per sq meter and cardiac work decreased from 1.8 to 1.2 kg meter per min. Mean arterial pressure was also decreased but the peripheral resistance was increased.

Lee et al tested the effects of guanethidine in 27 hyperthyroid patients (107). Responses were obtained six days after the patients were given oral daily doses of 80mg of guanethidine. In this case the beneficial effect of the drug was almost complete: heart rate dropped from 116 to 81 beats per minute, pulse pressure decreased from 80 to 64 mm Hg, the main change being recorded in the systolic pressure. The body weight was increased very slightly but significantly, the blood serum cholesterol was increased from 119 to 137mg/100ml, the basal metabolic rate decreased from 54 to 39% and clinical improvements of tremor and eye lid retraction were observed. No change occurred in the protein-bound iodine or uptake of thyroid (I^{131}) which remained elevated. Finally, the definite improvement found in 90 patients suffering from hyperthyroidism and given guanethidine prompted the authors to recommend the drug for therapeutic use (108).

Reserpine has also been found to produce very similar effects in hyperthyroid patients (*109*). De Groot et al (*110*) observed the effects of guanethidine reserpine and trimetaphan camphor sulfonate (ganglionic blocking agent) on 19 hyperthyroid patients. Both guanethidine and reserpine did not affect the cardiac output but caused an 18% decrease in the heart rate which still remained high. Arfonad (trimetaphan camphor sulfonate) had no effect on the heart rate but reduced the cardiac output by 11%, the value remaining 56% above normal. The isometric contraction period as well as the duration of ventricular ejection which were noted to be short in these hyperthyroid patients were unaffected by all three drugs. All values were compared to those of 29 normal subjects. These results were found to be inconclusive and the authors do not agree that the thyroid hormone may act on sympathetic nerves to produce its effects.

When nethalide a potent β blocker was administered to thyrotoxic patients the heart rate and basal metabolic rate were still high (*111*), although this drug could prevent the inotropic and chronotropic action of isoproterenol a β receptor stimulator. It is concluded that the abnormalities of thyrotoxicosis are not mediated through adrenergic stimulation of β receptors.

Experiments on this subject have also been performed on species other than man. When dibenzyline a sympathetic blocking agent was administered in rats together with thyroxine it had the property of blocking the 35% increase in O_2 consumption occurring when thyroxine was injected alone (112). Reserpine administered to hyporthyroid dogs, significantly reduced the heart rate but arterial blood pressure and O_2 consumption were inconsistently modified (113). Interesting results are provided by Barker and Makiuchi (114). Guanethidine injected to normal rats (eleven rats) caused a decrease of cardiac beats of 40 beats per minute and a lowering of noredrenaline content by .90µg per heart (1.12µg to .26µg). In these guanethidine-treated rats thyroxine increased the heart rate to 75% of that of the control given thyroxine but had no effect on the low noradrenaline content. It is concluded that guanethidine does not interfere with the peripheral effects of thyroxine. It may also be added at least in this case that noradrenaline does not participate in the heart rate effect of thyroxine.

On the other hand, most striking effects were produced in hyperthyroid dogs when total sympathetic block was induced in these dogs by injection of procaine into the epidural space (51). "When the reflex release of adrenaline and noradrenaline was abolished by a total sympathetic block, the heart rates of the thyroid-fed animals declined to

levels that were identical to those of the euthyroid dogs with a total sympathetic block". The same effects of sympathetic blockade were observed on the cardiac index (L/min.), left and right ventricular stroke work and O_2 consumption. The serum lactate, pyruvate and glucose levels were identical in both groups of animals, hyperthyroid and euthyroid. Surgical stimulation caused greater increases in these substances in hyperthyroid dogs and this increase disappeared with sympathetic blockade. These results made Brewster and his colleagues the most ardent supporters of this theory involving thyroxinewith an increased sympathetic activity.

However impressive Brewster's work and however appealing this theory appears to be, many of these results cannot be interpreted unequivocally. First, besides contradictory reports, guanethidine and reserpine in most cases did not <u>completely</u> relieve the symptoms of hyperthyroidism and the fact that similar observations were made on euthyroid subjects must be taken into consideration (115, 116, 117, 118, 119). Second, in Brewster's work, many effects of adrenaline and noradrenaline (cardiac rate, cardiac index, right ventricular storke work, O_2 consumption) were potentiated in the hyperthyoid animals despite the fact that both the hyperthyroid and euthyroid animals were induced with sympathetic blockade. Thyroid hormone therefore could not have exerted its

potentiating action by increase in sympathetic activity.

That the action of thyroxine is a more complex one arises from the experiments of Thier et al (120). These workers made a study of the heart rate of the isolated atria obtained from differently treated rats: normal, hypothyroid, hyperthyroid, reserpinized, normal-reserpinized, hypothyroid-reserpinized and hyperthyroid-reserpinized. The heart rate was recorded at various temperatures ranging from 25° to 41°C, a peak in heart rate being recorded at the latter temperature. Over the entire range of temperature these workers found that although the atrial rate of the hyperthyroid-reserpinized rats was lower than that of the hyperthyroid, it was significantly higher than that obtained for normal rats, reserpinized or not. Thus thyroxine can affect the heart rate independently of nervous or adrenal influence and several other findings on the heart-lung preparation or denervated hearts support similar conclusions (121, 122, 123, 124, 125, 126). It may be recalled (cf. p. 13) that in vitro effects of thyroxine were noted on the O_2 consumption of hearts.

Of course the possibility of a direct action of thyroxine on this tissue without the participation of catecholamines cannot be disregarded (127) but it raises difficulties in attempting to explain all observed effects. More likely explanations stipulate that in the hyperthyroid animals more

free catecholamine is available because of a decrease in the binding capacity by certain organs necessary for its inactivation (56, 128, 129). This hypothesis possesses the advantage of explaining the potentiating effect since exogenous catecholamine could not be disposed of as readily as normally and hence the extra effect. This theory however is fairly new and it will have to stand the test of time.

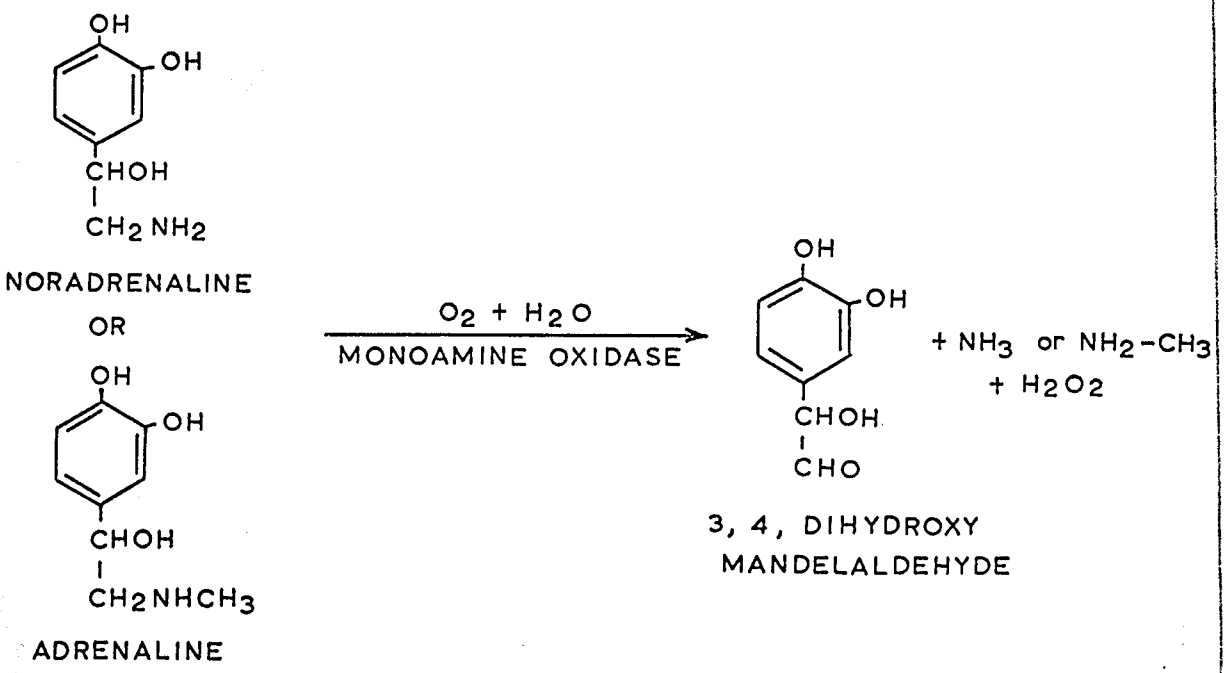
In another possibility, which has been suggested for some years now, it is maintained that the thyroxine hormone acts by inhibiting the enzymes which are responsible for inactivation of the catecholamines, a theory which also could explain the potentiating effect of thyroxine since again exogenous catecholamines would be more effective in their action. In addition in this theory local effects can be obtained without the participation of nerves or other. The experimental evidence which supports this concept will now be reviewed.

C. Effect of thyroxine on monoamine oxidase and catechol-O-methyl transferase.

a) Monoamine Oxidase

This enzyme was discovered in 1928 by Hare (130) and was suggested to be concerned with the destruction of adrenaline and noradrenaline (131, 132). It has been suggested that noradrenaline is metabolized more rapidly than adrenaline

(133,134). The importance monoamine oxidase has in the catabolism of the catecholamines has been emphasized by Zeller (135). The chemical reaction may be represented as follows:



In the presence of an aldehyde oxidase the 3,4, dihydroxy mandelaldehyde is converted to the corresponding acid. Spinks and Burn were the first to establish a relationship between the thyroid function and monoamine oxidase (136). It was found that the livers of thyroid-fed rabbits could not metabolise tyramine as efficiently as did the livers of rabbits fed normal diet. Thus a small but significant

decrease of 17% was noted in the activity of the enzyme when this activity was expressed per mg of nitrogen. When the thyroid gland was removed the liver enzyme was increased by 9%, an increase which was statistically significant when expressed per 100 mg liver. Thyroidectomized male rats showed a 25% increase in the liver monoamine oxidase which was significant when expressed both per 100mg of liver and per mg nitrogen. The authors suggest that these changes in enzymatic activity were responsible for the potentiating effect of thyroxineon the adrenalin hyperglycemia which was observed previously by Burn and Marks (9).

Trendelenburg (23) later confirmed these results. Having established that thyroid feeding of twenty rabbits potentiated the hyperglycemic effect of adrenaline, the livers of these rabbits were removed and tested for monoamine oxidase activity. They were able to detect a small (10%) but significant decrease in the enzymatic activity expressed per 100mg of liver, the values being compared to fourteen normals. He also demonstrated that when ephedrine, an inhibitor of monoamine oxidase, was injected at hourly intervals to maintain a proper concentration in the blood, the hyperglycemic effect of adrenaline was increased to the same extent as for hyperthyroid animals. Tyramine was used as a substrate for monoamine oxidase and rabbits of both sexes were

employed.

Working with male rats Zile and Lardy (87) observed no difference in the rate of oxidation of tyramine by liver mitochondrial enzyme obtained from thyroidectomized and normal animals. On the other hand, when rats were fed dessicated thyroid the ability of the mitochondrial enzyme to metabolize tyramine was reduced by half. When serotonin and tryptamine were used as substrates the reduction was not as prominent. These workers eliminated the possibility of a direct action of the hormone on the enzyme. Thyroxine, triiodothyronine, triiodothyropropionic acid and 3,5-diiodo 3' hydroxythyronine were without any effect when they were added to the incubation mixture at a final concentration of 3.3×10^{-5} M. Preincubation with these substances likewise did not affect the enzymatic activity. The presence of an inorganic inhibitor which may have been present in the liver mitochondria of hyperthyroid rats was eliminated as follows: the mitochondria obtained from thyroid fed rats was boiled and then added to an active enzymatic preparation. The activity was not affected. Finally when portions of the mitochondria obtained from normal and hyperthyroid animals were mixed together the resultant activity was the sum of that obtained separately. The authors thus concluded that the liver of the hyperthyroid

animals contained less active enzyme.

Zile (137) later reported that injections of L thyroxine, L triiodothyronine, triiodothyroacetic acid, DL-O-methyl thyroxine and thyroxamine in male rats caused a decrease in hepatic monoamine oxidase amounting to 50% in the case of the three former substances and 30% for the two others. Heart and brain monoamine oxidase were unaffected by this treatment but the specific activity was considerably less than of liver: the specific activity was five times less for brain and three times less for heart. Zile remarked that the method used was perhaps not sensitive enough to detect any changes. In this case tyramine was used as the substrate and the activity is expressed per mg of nitrogen.

Novick (138) injected rats (sex not mentioned) with 150µg/kg of L or D-triiodothyronine each day for ten to fifteen days. Another group of rats was fed thyroid powder for twelve days. The injected rats responded by an increase in the basal metabolic rate which amounted to 100% whereas the thyroid-fed rats responded by a 30% increase. In adult rats injection of L triiodothyronine produced a 70% increase in myocardial monoamine oxidase but injections of D-triiodothyronine failed to do so. The effect of thyroid feeding was similar to that of L triiodothyronine ($L\text{-}t_3$). In young rats the myocardial enzymatic activity was lower than for the adult rats and $L\text{-}t_3$ treatment of these young rats

caused a 200% increase in heart monoamine oxidase activity; D-t_3 remained without effect. In post-natal rats where practically no activity could be detected L-t_3 raised the myocardial enzymatic activity to that found in normal adult rats. When hepatic monoamine oxidase was assayed in rats fed dessicated thyroid the activity was found to be only 50% that of the control, a finding which is in accordance with previous results. Novick found that when a 100% O_2 gas phase was used in the incubation mixture instead of air the monoamine oxidase activity increased considerably and therefore this method was more apt to detect any change. Dubnock et al using male rats (139) somewhat confirmed Novick's findings when they observed that thyroidectomy caused a decrease in the monoamine oxidase activity of the heart.

Using 5-hydroxy-tryptamine as the substrate for determining enzyme activity Skillen et al (140) were able to show sex differences in myocardial monoamine oxidase activity when rats were fed dessicated thyroid. Female rats fed thyroid responded by a 70% increase in enzyme activity but male rats similarly treated showed no significant changes. Feeding propylthiouracil reduced the myocardial activity of male rats but did not affect that of female rats. The authors explain that Novick obtained an increase in myocardial

monoamine oxidase activity as a result of thyroid feeding because they had included female rats in their groups (personal communication to Skillen).

Wurtman et al (54) did not find any significant change in the amount of C^{14}-indoleacetic acid formed from C^{14}-tryptamine by livers of thyroidectomized female rats but observed a decrease in the case of male rats. The importance of the dose of thyroxine injected is stressed by the finding that injection of 100µg of thyroxine in female rats caused an increase in hepatic monoamine oxidase activity whereas administration of 500µg for five days decreased the activity, both changes being significant for $P < .05$. In male rats doses of 100µg for 7 days did not affect monoamine oxidase activity in liver but they did not try higher doses of thyroxine.

D'Iorio and Mavridès (142) injected a single dose of 2mg/kg of thyroxine into male rats and noted a 20% decrease in the ability of the liver of these rats to oxidize kynuramine. When two injections of 2mg/kg were given on two consecutive days the decrease amounted to 43%.

In female rats Utley (143) studied the effect of thyromimetic compounds on both liver and heart monoamine oxidase. In thyroidectomized female rats a decrease in myocardial monoamine oxidase activity resulted whereas hepatic activity was slightly increased. The thyromimetic compounds

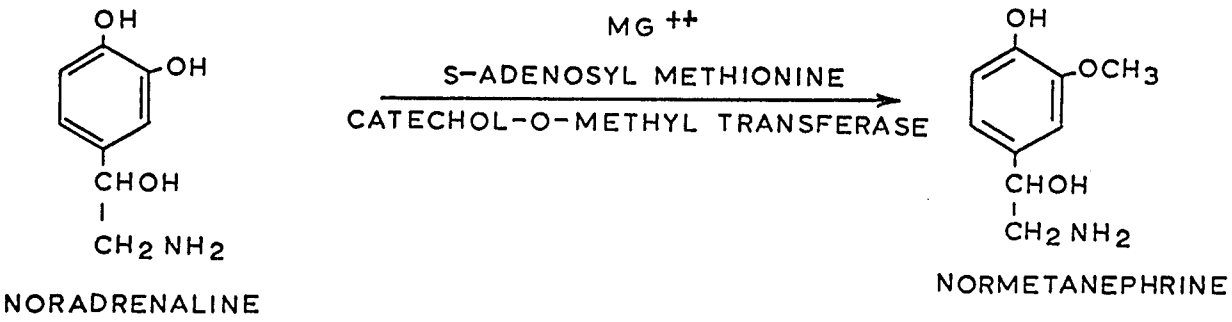
lowered liver enzyme activity but elevated that of the heart. Westerman (144) found elevated hepatic monoamine oxidase activity in rats given thyroid but no mention is made of the sex.

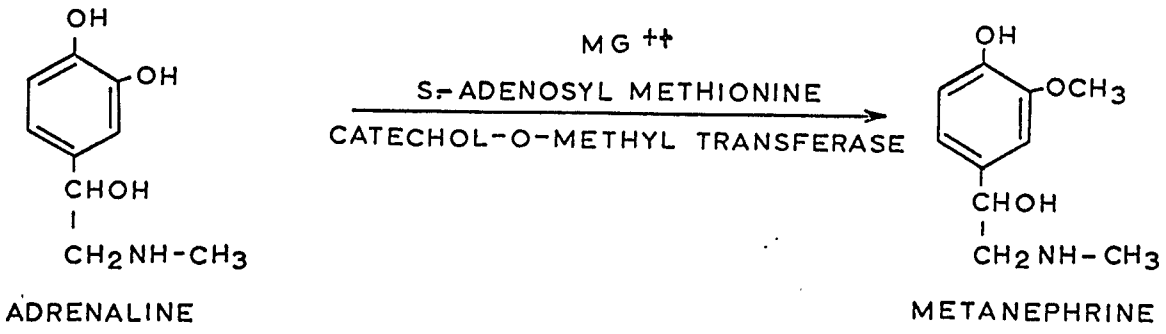
It may be generally concluded that thyroid-treated female or male rats exhibit low monoamine oxidase activity in their liver, whatever the substrate used in the determination of enzymatic activity. Myocardial activity of female rats is increased by thyroid treatment but that of male rats does not appear to be affected. Thyroidectomy seems to decrease the liver monoamine oxidase activity of male rats but to increase that of female animals. Myocardial enzyme, in hypothyroidism, is decreased in male rats but seems to be increased in female rats.

B. Catechol-O-methyl transferase.

This enzyme has been discovered recently (145, 149). In 1958, D'Iorio and Pellerin (145) reported the occurrence of a soluble enzyme present in the liver and kidney which was capable of methylating several catechol acids in the meta position. The reaction was activated by the presence of ATP, Mg reduced glutathione, and methylating activity was reported to be greater in the liver than in the kidney. These findings fitted well with the demonstration of several authors (146, 147, 148) that 3-methoxy-4-hydroxyphenolic acids

and other similar methylated products were excreted in urine. Axelrod (*149*) had described the occurrence of an enzyme in male rat liver which could transfer the methyl group of S-Adenosyl methionine to the meta position of adrenaline and noradrenaline and to other catechols also. The enzyme was present in greatest amount in the liver, less was found in the kidney and skeletal muscle did not seem to contain any. The reaction may be written as follows.





A few years before these findings, D'Iorio et al (*76*) had found that rats fed iodinated casein could not

metabolize exogenous adrenaline as well as normal animals. After normal and hyperthyroid rats were injected adrenaline the accumulation of catecholamine was observed to be greater in the spleen and liver of hyperthyroid animals. In hearts and adrenals no such difference was noted but twice as much fluorogenic material accumulated in the serum of rats fed iodinated casein than in the serum of normal rats. The probability that the thyroxine hormone acted by reducing the catabolism of the catecholamine was increased. Subsequently, similar findings were declared for noradrenaline (150). The finding of a new catabolic enzyme prompted investigation attempting to establish some relation between the thyroid hormone and catechol-O-methyl transferase. Further work confirmed this expectation. Indeed, D'Iorio and Leduc (151) recorded a 45% decrease in the methylating activity of livers of male rats weighing approximately 200g and injected with 500µg of thyroxine for three days. In agreement with these results was the finding that rats injected with thyroxine and radioactive adrenaline showed appreciable decrease in their urinary metanephrine. Since their method of assay involved the methylation of protocatechuic acid in the presence of ATP and other necessary co-factors, the possibility could not be eliminated that the S-adenosyl-methionine activating enzyme may have contributed partly or wholly to this decrease.

In a later report D'Iorio and Mavrides (142) showed that liver monoamine oxidase and catechol-O-methyl transferase activity were decreased when rats were injected thyroxine. Since S-adenosyl methionine served as the direct donor of the methyl group to noradrenaline under the influence of catechol-O-methyl transferase, the participation of other factors in this decrease could be excluded. But hepatic monoamine oxidase was more sensitive to the influence of thyroxine since it responded to one injection of thyroxine (c.f. 50) whereas the methylating enzyme responded to only two injections of 2mg/kg. given on two consecutive days. Even then the observed decrease was smaller than that found for the oxidizing enzyme. In vitro, a direct inhibiting effect of thyroxine analogues could be demonstrated on a semi-purified preparation of catechol-O-methyl transferase (142, 152). Thyroxine itself had no effect presumably because of its poor solubility at the desired pH. As for the analogues, rather high concentrations were necessary to bring about this inhibitory effect: these concentrations ranging from 2.3×10^{-3}M to 3.2×10^{-4}M. A similar observation may also be made for the in vivo results which is suggestive of a toxic effect rather than a physiologic one. Indeed, Kurtman et al (54) noted that doses of thyroxine capable of producing hyperresponsiveness to adrenaline

were incapable of modifying cardiac or hepatic catechol-O-methyl transferase as judged from the level of H^3 metanephrine in these tissues after injection of H^3 adrenaline. After administration of larger doses of thyroxine (500µg) a small but significant decrease in hepatic catechol-O-methyl transferase occurred. The authors' findings on monoamine oxidase also stressed the importance of the dose of thyroxine employed and this has already been mentioned in detail on page 50. Wurtman et al (56) cite the results of Sokoloff and Kaufmann (153) who showed that the in vivo treatment with 100ug of thyroxine increased the in vitro incorporation of leucine and valine into protein. In vitro small quantities of thyroxine increased the incorporation whereas greater amounts inhibited protein synthesis. Thus Wurtman et al suggest that the observed decreases in enzymatic activity were caused by doses of thyroxine which may have disturbed protein synthesis and hence affect indirectly the enzymes. In essence these considerations served as the starting point for the work presented in Part 1 of the Experimental Section.

From the foregoing it does not appear that the physiologic effects of thyroxine on the heart could be explained by an inhibiting effect on the catabolic enzymes. On the other hand there are some indications that thyroxine may slow down the monoamine oxidase and catechol-O-methyl

transferase activities of liver. This in turn would increase the amount of adrenaline available in the liver and could offer an explanation for the glycogenolytic effect of thyroxin and its potentiating action on adrenaline glycogenolysis. Perhaps also the effects of thyroxine on O_2 consumption may be explained by this action of thyroxine on these enzymes in liver. It has been found that noradrenaline and adrenaline increase the use of oxygen in the liver in vivo (126,187).

It seemed worthwhile therefore to reinvestigate the level of activity of both enzymes under identical-well controlled hyperthyroid conditions. That is, in order to preclude any criticism that thyroxine influences the catabolic activities via an effect on general protein synthesis it was decided to induce hyperthyroidism in male rats by feeding them a diet containing iodinated casein but also to give them a supplement of vitamin B_{12}. It has been shown that this vitamin when given in sufficient quantities may completely correct the loss in weight resulting from treatment with iodinated casein (74,111). Vitamin B_{12} has been shown to be involved in nucleic acid synthesis (155) and thus may indirectly be concerned with protein synthesis.

The experimental design also could permit one to probe into the question of whether or not vitamin B_{12} or its metabolic products are implicated in some way in the enzymatic

mechanism of catechol-O-methyl transferase. That such a possibility is not a remote one comes from the reports showing that vitamin B_{12} or its derivatives are implicated in some functional role in enzymatic systems including those representing methylating enzymes. The experimental results pertaining to this subject will now be briefly reviewed.

4. THE ENZYMATIC ROLE OF VITAMIN B_{12}

The requirements, vitamin B_{12} and its derivatives, for proper functioning of the methylmalonyl isomerase enzyme has recently been evidenced by several workers (156, 157, 158, 159, 160). This enzyme is concerned with an isomerization reaction in which the carbonyl thioester group of methyl malonyl CoA migrates to the methyl group of the same substance (161) thus producing succinyl-CoA an important intermediate of the Krebs cycle. The inhibition of this enzymatic pathway was observed in animals fed a diet deficient in vitamin B_{12} (156, 157). It was found in extensively purified preparation that the active principle is dimethyl benzimidazolyl cobamine, (DMBC) a derivative of vitamin B_{12} (161). Passage of the purified enzyme through a TEAE cellulose column yielded an inactive preparation but which could be reactivated by addition of dimethylbenzimidazolylcobamide. The enzyme has been shown to occur in bacteria (162) and in animal tissues (160, 163). The apoenzyme from animal tissue is reactivated only by the dimethylbenzimidazolyl and the benzimidazolyl (160) enzymes, whereas the apoenzyme from bacterial origin is much less specific in its requirements (164).

Vitamin B_{12} has also been shown to play an active part in the methylation of homocysteine reaction which yields methionine (165).

5 methylfolate-H_4 + homocysteine → folate-H_4 + methionine

In bacteria two kinds of enzymes are reported to activate this reaction. One enzyme has been shown to contain vitamin B_{12} and has been obtained from a mutant of E. coli. grown with B_{12} (166). The cofactors here are reduced flavin adenine dinucleotide and S-adenosyl methionine and the substrate may be either 5-methyltetrahydropteroyl-monoglutamate or triglutamate methyl-tetrapterin-H_4 (166). The other enzyme which has been obtained either from E. coli or A. aerogenes does not contain the vitamin and requires no other cofactor than magnesium ions (167, 168).

Buchanan et al (169) isolated and purified from pig liver an enzyme which had the same functional role as those described for bacteria and which contained vitamin B_{12}. The vitamin content was parallel to the enzymatic activity. The enzyme also required S-adenosyl methionine, reduced flavin adenine dinucleotide and thus resembles that obtained from a mutant of E. coli grown on B_{12}.

The possibility that methyl cobalamin may be an intermediate in these reactions arose when Guest et al (170) reported that methyl cobalamin could transfer its methyl moiety to homocysteine without the participation of any enzyme or other cofactors. The reaction however was stimulated several fold by the presence of the B_{12} enzyme.

Buchanan et al (169) rejected this possibility on the basis that if methylcobalamin was the activated form of vitamin B_{12} it should be formed from the B_{12} enzyme, reduced flavin adenine dinucleotide and S-adenosyl methionine. Then the methylcobalamin enzyme would not require these cofactors for full methylation of homocysteine from 5-methyl-tetrahydrofolate. But this was not the case, both cofactors being necessary for maximal activity. The exact role played by all these compounds is not understood at the present time.

Dickerman et al (171) observed that methionine synthesis as judged by the transfer of methyl group from N,5-methyltetrahydrofolic acid to homocysteine was impaired in liver obtained from chicks fed a diet deficient in vitamin B_{12}. The in vitro addition of vitamin B_{12}, methylcobalamin or 5,6-dimethylbenzimidazolylcobamide coenzyme failed to restore normal activity.

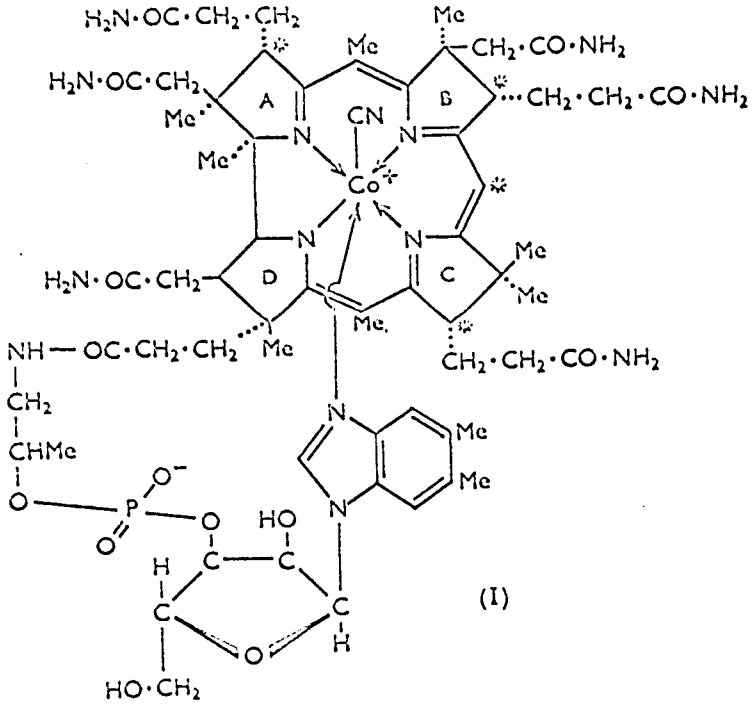
An enzyme isolated from Cl. tetanomorphum and responsible for the isomerization of β-methyl aspartate to glutamate has been shown to require a B_{12} coenzyme and a sulfhydryl reducing agent for activity (172). This enzyme was also shown not to contain vitamin B_{12} as a prosthetic group (172).

Lysine fermentation to fatty acid and ammonia has been demonstrated to be dependent on a dimethylbenzimidazole cobamide coenzyme (173).

Glycol and glycerol dehydrases have also been shown to be cobamide dependent (174, 175).

The chemical structures of vitamin B_{12} and DMBC coenzyme are represented on page 61a. It may be seen that the main structural difference resides in the absence of cyanide and the presence of a 5-deoxyadenosine group in the coenzyme (176), with methyl-cobalamin the cyanide moiety is replaced by a methyl group.

There are two common points between the catechol-O-methyl transferase and the B_{12} enzyme: first, both concern the transfer of a methyl group and second, both require S-adenosyl methionine. So far no studies have been made on the possible participation of vitamin B_{12} or its derivatives in the activity of catechol-O-methyl transferase. These substances could be concerned in this activity in two ways, first, the coenzyme form of vitamin B_{12} or methylcobalamin may be involved as a cofactor, second, vitamin B_{12} itself could be firmly attached to the enzyme so as to form the prosthetic part of the holoenzyme. In the first case this can be easily verified by the <u>in vitro</u> addition of 5,6-dimethylbenzimidazolylcobamide or methylcobalamin to the incubation mixture and by comparing the activity with another preparation not containing these cofactors. There are two ways to verify the second possibility; purify the enzyme and determine the content of the vitamin if any or compare the



(I)

FIGURE 1. Structure of vitamin B_{12}.

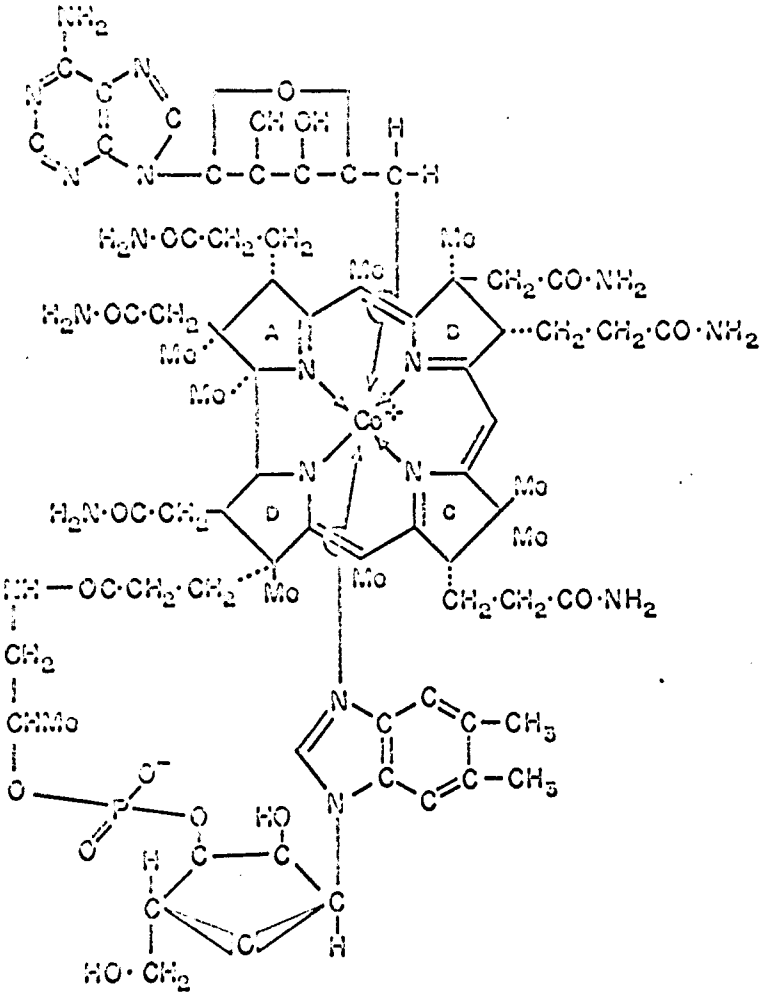


FIGURE 2. Structure of B_{12} coenzyme.

enzymatic activity in rats fed a deficient diet, one group being injected with the vitamin. The latter approach has been chosen for practical reasons one of which is apparent from the discussion on page 54 . In any event catechol-O-methyl transferase has so far resisted extensive purification (by members of our laboratory) and it would not be of decided significance to search for the presence of vitamin B_{12} in such semi-purified preparations.

5. STATEMENT OF THE PROBLEM

From the content of this introduction it is hoped that the following main points have emerged:

a) The action of the thyroid hormone is physiologically and biochemically complex and is somehow related to the catecholamines. The details of this relationship are obscure and may be complicated.

b) There is a possibility that some of the physiological effects of the thyroid hormone may be explained by an inhibitory action of the hormone on the two main enzymes which are responsible for the inactivation of the catecholamines.

The work presented in this thesis was initiated in an attempt to answer the following questions:

1) Does the liver of normally-growing hyperthyroid rats contain less monoamine oxidase and catechol-O-methyl transferase than the liver of rats with normal thyroid function?

2) In these hyperthyroid rats, is the catecholamine content of heart, liver and brain increased?

3) Can the cardio-vascular changes observed in the hypophysectomized rats and hormone-treated hypophysectomized rats be attributed partly or wholly to corresponding

changes in the catecholamine content of the hearts of these animals?

4) Are these changes, if any, specific to the hearts or are they also present in other tissues such as the liver and brain?

5) Finally, is vitamin B_{12} or its derivatives involved in catechol-O-methyl transferase activity?

EXPERIMENTAL

METHODS

1) Determination of the Basal Metabolic Rate of Rats.

The procedure used for the determination of the O_2 consumption of rats was already described ([17], [18]).

Rats were weighed to the nearest gram and were then given nembutal intraperitoneally 30mg/kg. A chamber, consisting of a glass cylinder or jar, the bottom of which was covered with fresh soda lime to absorb CO_2 and humidity, was equilibrated with oxygen for eight minutes. After equilibration, the anesthesized rat was restrained into a cylinder made of wire mesh, then placed in the chamber and the lid was screwed on the jar. After a five minute equilibration period, a calibrated glass tube was wetted and one end was dipped into liquid soap, so as to form a bubble. This tube was fitted into the lid of the jar.

When this was done and the chamber was hermetically closed the bubble moved steadily towards the chamber as oxygen was consumed. The time taken for this film seal or bubble to travel from the first mark to the second one on the tube was recorded in seconds. The volume between the two markings was five ml. When five such constant readings were made the rat was removed. The metabolic rate was

expressed as milliliters of O_2 consumed per minute per kilogram body weight. This apparatus was supplied by Phipps and Bird Co.

2) Determination of Protein.

The proteins were determined by a modified Biuret reaction (179).

Reagents:

Biuret Reagent (Stock). 45 grams of potassium tartrate reagent grade were dissolved in about 400ml of 0.2N NaOH in a 1 liter volumetric flask. 15 grams of copper sulfate pentahydrate (reagent grade) were added to the flask while stirring. 5 grams of potassium iodide (reagent grade) was then added to the mixture and dilution was made to one liter with 0.2N NaOH.

Biuret Reagent (Dilute). One volume of stock Biuret Reagent was diluted with 9 volumes of 0.2N NaOH containing 5 grams of potassium iodide per liter.

Procedure:

A certain amount of the sample to be analyzed was added to a test tube and the volume was completed to 2ml with distilled water. Then 5ml of the dilute Biuret Reagent was added and the mixture was left at room temperature for ten minutes. The blank was prepared similarly with 2ml of

water, and all tubes were clear. Readings were made at 555mμ in a Coleman Junior Spectrophotometer model 6A.

The amount of protein present in the aliquot was determined by referring to a standard curve of protein which was made using crystalline bovine serum albumin (Sigma Chemicals Co.).

3) Determination of Monoamine Oxidase in Rat Liver.

This enzymatic assay was performed according to Weissbach et al (180).

Principle:

In this method, kynuramine is used as the substrate and is oxidized by monoamine oxidase to the corresponding aldehyde. The reaction is followed by measuring the disappearance of kynuramine. This measurement was done spectrophotometrically, the decrease of absorption at the optimum wavelength of the substrate (360mμ) being taken as an index of enzyme activity.

Reagents:

Kynuramine dihydrobromide, obtained from Regis and Co., was dissolved in distilled water and refrigerated.

Phosphate Buffer 0.5M, pH 7.4, was prepared by titrating 0.5M dibasic with 0.5M monobasic phosphate solution

until a pH of 7.4 was reached. The phosphate salts were Fisher certified reagents. Lubrol, a product of C.I.L., was kindly supplied by Dr. Kraml. A 3.53% solution of this detergent was prepared with the help of a magnetic stirrer for a few hours. The solution was then refrigerated.

Preparation of the Crude Enzyme:

In preliminary experiments, liver was homogenized in five volumes of distilled water as described by Weissbach et al (180). For some unknown reason little activity was obtained. It was difficult to increase the activity by concentrating the homogenate because the resulting supernatant, being turbid, interfered with the spectrophotometric assay. At this point Lubrol was used to clarify the supernatant of the concentrated homogenate. Not only did we find that this gave a more transparent supernatant but the enzyme activity was also increased. Actually, there are some reports in the literature (191, 192) showing that solubilization of mitochondrial monoamine oxidase with certain detergents increases the activity of the enzyme.

The final procedure for enzymatic preparation was as follows:

All operations were made at 0°C except where otherwise stated. Liver tissue was homogenized with two volumes of a 3.53% solution of Lubrol. The homogenate was then centri-

fuged in a Serval centrifuge model RC 2 at 2,000 g for fifteen minutes to remove cellular debris. The reaction was carried out at 37°C in 1cm quartz cuvettes. The 3ml incubation mixture contained 150μMols of phosphate buffer, 0.3μMols of kynuramine, 0.2ml of liver supernatant and water as diluent. A blank was set up in the same manner but with no substrate. The rate of decrease of absorption at 360mμ was measured in a Bausch and Lomb recording spectrophotometer (Spectronic 505). Readings were commenced two minutes after addition of the enzyme to allow temperature equilibration, and pursued for ten minutes. During this period the decrease was linear. The temperature was kept constant by means of a water jacket surrounding the cuvettes. The water was supplied and circulated by a water bath of constant temperature. The blank in this assay procedure does not account for any effect of Lubrol on the substrate itself, however in a separate set of control experiments it was shown that Lubrol did not alter the absorption spectrum of kynuramine exposed at 37°C to this detergent for ten minutes.

Freezing the tissue for several weeks did not affect the activity of the enzyme. The results of a typical run are shown in Figure 1.

4) <u>Determination of Catechol-O-Methyl Transferase in Liver</u>.

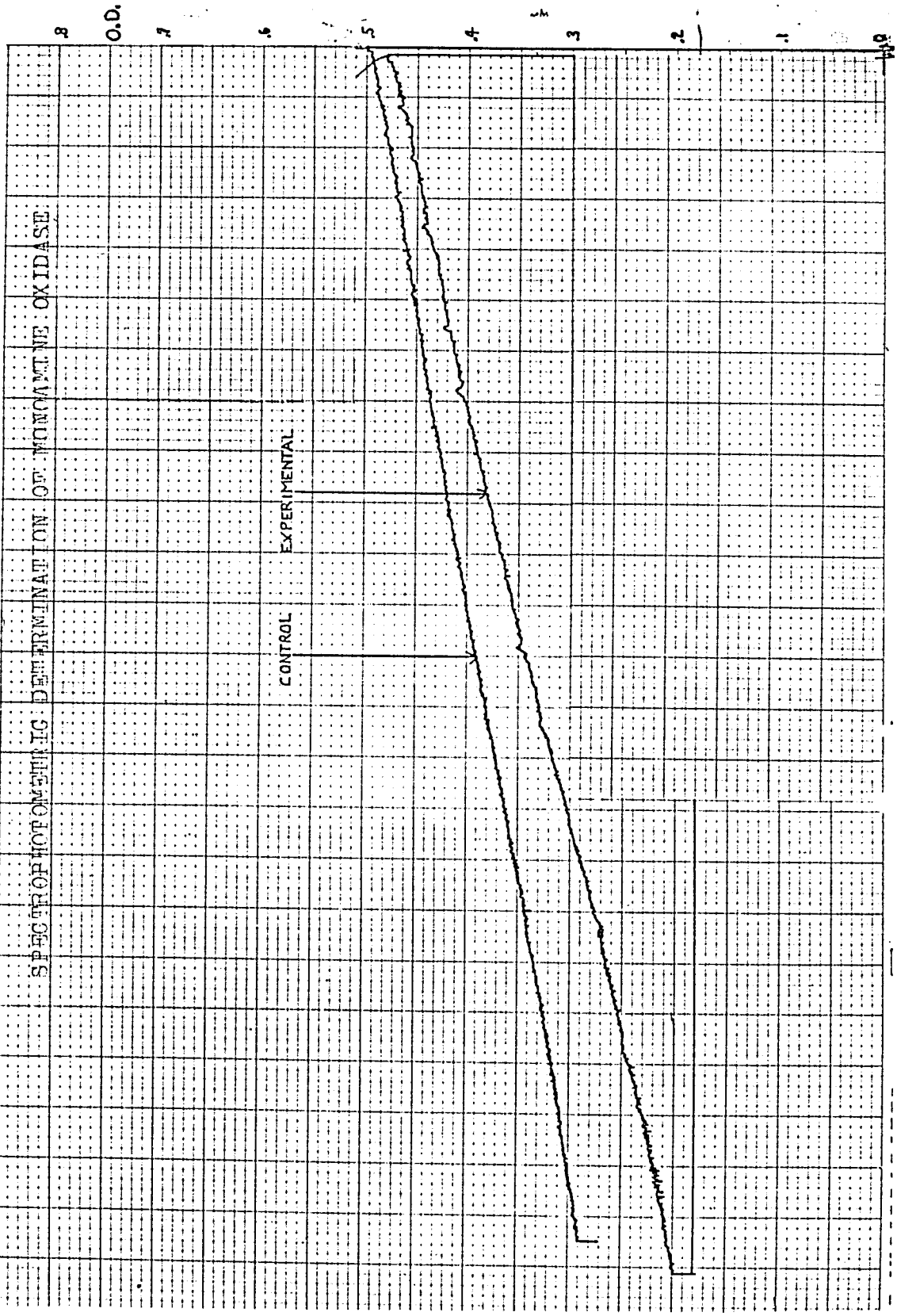


Figure I

SPECTROPHOTOMETRIC DETERMINATION OF MONOAMINE OXIDASE

The enzymatic assay procedure was essentially that described by Axelrod (183).

Principle:

After adrenaline has been converted to metanephrine by the enzyme, the product is extracted from the incubation mixture and is measured fluorimetrically.

Reagents:

Adrenaline bitartrate, procured from Winthrop Laboratories, was dissolved in glass redistilled water, divided in 1ml aliquot and frozen: fresh solutions were made after two weeks of storage.

S-adenosyl methionine, purchased from Calbiochem was dissolved in bidistilled water, the solution was separated into several portions and, when stored frozen, could be kept for several months.

Metanephrine HCl, purchased from Mann Research Laboratory, was dissolved in bidistilled water, divided into several portions and thus could be stored in the freezer for a long period of time.

Toluene-isoamylalcohol purchased from Fisher Scientific Co., was prepared by adding 300ml of toluene to 200ml of isoamyl alcohol and mixing. Although different batches of toluene invariably gave satisfactory results, this was not so with the isoamyl alcohol. Some batches of

the latter solvent caused quenching or low readings on the fluorometer and thus reduced considerably the sensitivity of the method. For this reason new batches of isoamyl alcohol were always tested before use.

0.1M Magnesium Chloride: This was prepared from the reagent grade salt obtained from Fisher Scientific Co. and was kept refrigerated.

0.5M Phosphate buffer, pH 7.9: a 0.5M solution of the disodium phosphate salt was adjusted to pH 7.9 with a 0.5M solution of the monosodium phosphate salt. The buffer was kept in the refrigerator at 4°C.

0.2M Borate buffer pH 10: This was prepared by dissolving 7.7 g of boric acid (Fisher Sci. Co.) in 250 ml, 1M solution of KCl and this solution was adjusted to pH 10 with 0.5M NaOH.

Preparation and Assay of the Liver Enzyme.

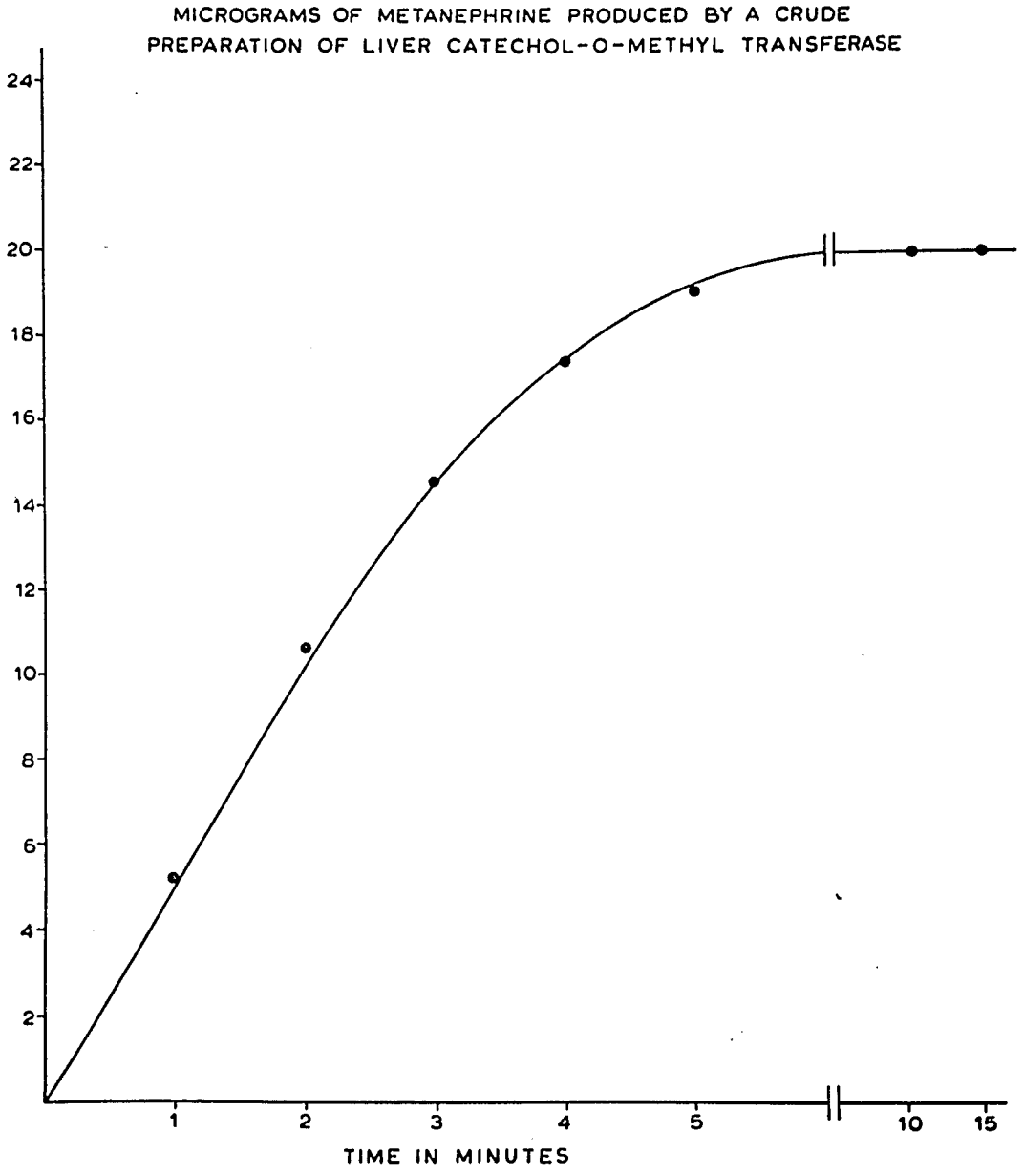
All operations were performed at 0°C except where otherwise stated. Liver tissue was homogenized in two volumes of isotonic KCl and the homogenate was centrifuged for one hour at 20,000 g. The resultant clear supernatant was used for the enzymatic assay. The incubation mixtures were as follows:

	Blank	Standard	Test
Adrenaline	1µ mol	1µ mol	1µ mol
Buffer	100µ mol	100µ mol	100µ mol
$MgCl_2$	20µ mol	20µ mol	20µ mol
S-adenosyl-methionine	0	0	1µ mol
Metanephrine	0	10-20µg	0
Enzyme	0.5ml	0.5ml	0.5ml

In each tube the volume was adjusted to 2ml with double distilled water. The incubation was carried out in stoppered 40ml centrifuge tubes immersed in a water bath maintained at 37°C. As soon as the enzyme was added, time was recorded for two minutes and the reaction was stopped by addition of 1ml of borate buffer, pH 10. This short incubation time was chosen because a time-curve study performed twice with the crude liver enzyme preparation revealed that the reaction was not linear after two minutes as can be seen from Figure II. Beyond this short interval, the non-linearity of the enzymatic rate is not due to the presence of inhibitors in the crude enzyme preparation because a similar time-curve was obtained (Ref. Anderson private communication) with a semi-purified preparation of catechol-O-methyl transferase.

After addition of the borate buffer, metanephrine was extracted by mechanically shaking the mixture in presence of 20ml of toluene-isoamyl alcohol for thirty minutes. Each mixture was then centrifuged at 2000g for

Figure II

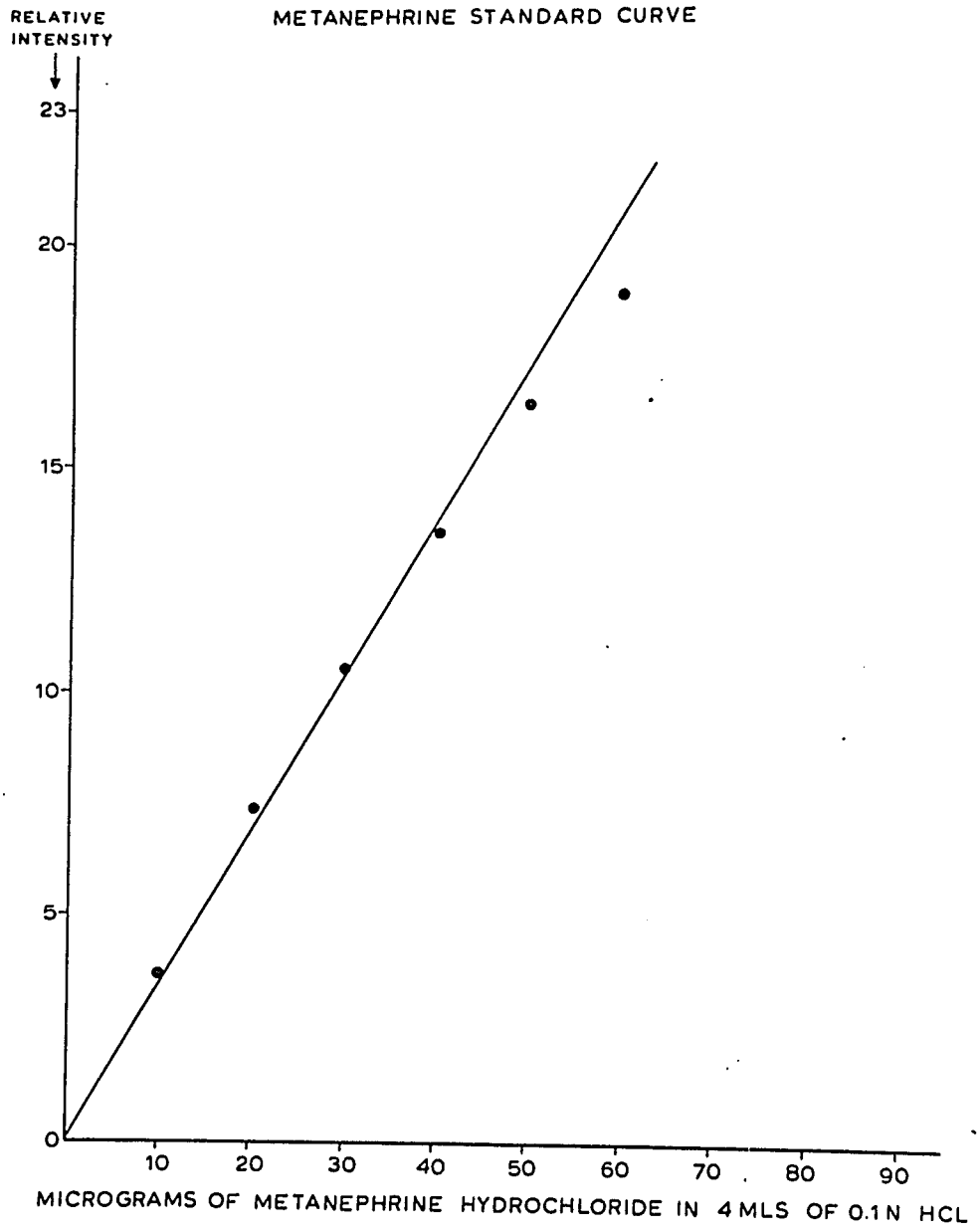

MICROGRAMS OF METANEPHRINE PRODUCED BY A CRUDE PREPARATION OF LIVER CATECHOL-O-METHYL TRANSFERASE
24
22
20
18
16
14
12
10
8
6
4
2
1
2
3
4
5
10
15
TIME IN MINUTES

fifteen minutes, after which time 15ml of the clear supernatant was transferred to another 40ml centrifuge tube containing 4ml of 0.1N HCl. The two phases were mechanically shaken for five minutes and after settling, the upper layer was removed by suction. The acid phase was read in the Aminco-Bowman spectrofluorometer using 285mμ as activation wavelength and 335mμ as emission wavelength. The instrument was adjusted to 0 with a blank sample. The amounts of metanephrine in the test samples were estimated by comparison with standards described previously. The amount of metanephrine recovered in this procedure is approximately 60% as noted by Axelrod (183). In each run two different standards (10 and 20μg of metanephrine) were prepared and the readings varied directly with the concentration. All readings for tests and standards were within the range of linearity of the instrument. These represented concentrations of metanephrine within the limits indicated in Figure 111, i.e. not greater than 40 micrograms. The freezing of tissues and storage at -20°C did not affect the enzyme activity for at least two weeks.

5) Determination of Catecholamines.

The method described by Bourkes (184) involving the precipitation of tissue proteins by trichloracetic acid,

Figure III


METANEPHRINE STANDARD CURVE
RELATIVE INTENSITY
23
20
15
10
5
0
10
20
30
40
50
60
70
80
90
MICROGRAMS OF METANEPHRINE HYDROCHLORIDE IN 4 MLS OF 0.1 N HCL

absorption of the catechloamines on alumina, elution and conversion of the catecholamines to fluorogenic material by iodine oxidation did not give satisfactory results in our hands. The recoveries were low (about 50%) and variable. The blanks were high and hardly any catecholamine could be recovered from the liver. The method proposed by Anton and Sayre (185) proved to be more sensitive, more reproducible and thus was adopted.

Principle:

Catecholamines from tissues are selectively absorbed on alumina and then eluted with perchloric acid. The differential oxidation of adrenaline and noradrenaline to the fluorescent trihydroxyindole derivatives was achieved by using ferricyanide at two different pH's. The fluorophore of catecholamines can then be measured with the fluorometer at specific wavelengths of activation and emission.

Reagents:

1) Aluminum oxide (Woelm Alupharm Chemicals, Neutral Activity, Grade 1). It was prepared as follows:

A) 100 grams of aluminum oxide were added to 500ml of 2N HCl in a 1 liter beaker which was then covered with a watch glass and heated at 90 to 100°C for forty five minutes with continuous and rapid stirring.

B) The beaker was removed from the heated stirrer and the heavier particles of aluminum oxide were allowed to settle for one and one-half minutes. The supernatant fluid was discarded with fines.

C) The precipitate was washed twice with fresh 250ml portions of 2N HCl at 70°C for ten minutes. Discarding of the supernatant with fines, was repeated each time.

D) In a final acid wash the aluminum oxide was stirred with 500ml of 2N HCl at 50°C for ten minutes.

E) After discarding the HCl the precipitate was washed repeatedly (about twenty to twenty five times) with fresh 200ml portions of distilled water until a pH of 3.4 was reached decanting the finer particles each time.

F) Finally the aluminum oxide was transferred to an evaporating dish heated at 120°C for one hour and at 200°C for two hours and then stored in a dessicator.

2) Perchloric Acid 0.4N and 0.05N was prepared by diluting 11.6N perchloric acid (Baker and Adamson quality).

3) 0.5M Phosphate buffer pH 7.0 prepared as previously described.

4) 1.6N Acetic acid prepared from glacial acetic acid.

5) Potassium ferricyanide 0.25% was prepared freshly each day.

6) Disodium ethylenediaminetetraacetate.

7) Sodium Metabisulfite.

8) Alkaline ascorbate solution was prepared immediately before use by dissolving 10mg of ascorbic acid in 0.1ml of bidistilled water and then adding 5ml of 10N NaOH.

9) All reagents were prepared with bidistilled water. The second distillation was carried out from a 0.1% solution of disodium ethylenediamine tetraacetate in an all glass still.

Preparation of Samples:

1) Frozen tissue was thawed and weighed to the nearest centigram for liver and brain and to the nearest mg for heart.

2) Homogenization was carried in ice-cold 0.4N perchloric acid. When the weight of tissue was less than 2g such as for heart and brain, 10cc of perchloric acid was used but above this weight (5g of liver was usually used) 25ml of perchloric acid was employed.

3) The homogenate was kept in ice for a least one-half hour before centrifugation to allow full precipitation of proteins.

4) Centrifugation was carried out at 0°C at 30,000g for 45 minutes in a refrigerated Serval centrifuge.

5) The supernatant was then decanted and transferred to a 100ml beaker containing:

400mg of aluminum oxide
200mg of disodium ethylenediaminetetraacetate
10mg of sodium metabisulfite

6) The mixture was stirred with a magnetic stirrer and the pH was raised to 8.6 as required for absorption of the catecholamines on the aluminum oxide. For the end-point, a few drops of phenolphthalein were added and with the help of a Pasteur pipette, first a 5N, and then a 2.5N NaOH solution was gradually added to the mixture until a very faint but persistent pink color was obtained. Anton and Sayre suggest the use of a glass stirring rod and stirring motor combination, but in our hands this procedure gave insufficient mixing and was time consuming. With the use of the water-driven magnetic stirrers connected in series, six samples could be dealt with at the same time and, moreover the grinding effect (if any) of the stirrer bar rubbing on the aluminum oxide particles did not interfere with the readings. Also, the use of the phenolphthalein end-point instead of a pH meter for indication of the pH was perhaps less precise but was time saving and, after practice gave satisfactory results.

7) The mixture was then transferred to a 40ml glass-stoppered centrifuge tube and centrifuging was performed briefly at 1500 r.p.m. to allow quick settling of the aluminum oxide particles. The supernatant was discarded by suction. 10ml of bidistilled water was added to the

original beaker which contained a small residue of aluminum oxide particles. This 10ml washing was transferred to the same centrifuge tube which was then shaken for two minutes and centrifuged again. The rinsing of the beaker as well as the washing of the aluminum oxide was done at least four times.

8) After the final operation, the supernatant wash was removed as carefully as possible and 3cc of 0.5N perchloric acid was added to the sediment. The glass stopper was secured tightly and elution was performed by shaking fifteen minutes with a mechanical agitator.

9) The eluate was then transferred to 16ml polyethylene tubes, previously cooled in ice, and centrifuged fifteen minutes in a refrigerated Serval centrifuge at 30,000g. With the aid of a Pasteur pipette, as much as possible of the clear supernatant was then slowly removed, divided equally into two tubes and stored in the freezer. One tube was used for determinations at pH 7.0 and the other, for analysis at pH 2.

With each batch of samples three internal standards of adrenaline and three internal standards of noradrenaline were run through the whole procedure. The concentration of these standards was chosen so as to give readings in the proximal range of samples. The recoveries for noradrenaline amounted to 88% ± 5% and for adrenaline 80% ± 5%.

Conversion to and Determination of the Fluorophore.

1) The eluate, prepared as just stated, was allowed to thaw. 0.2cc of the pH 7.0 buffer or 0.2cc of 1.6N acetic acid (for oxidation at pH 2.0) was added to small test tubes. To each tube 0.4cc of the eluate was added and mixed thoroughly.

2) 0.04ml of 0.25% ferricyanide solution was then added and mixed thoroughly.

3) Exactly two minutes after addition of ferricyanide, 0.4ml of alkaline ascorbate solution was added and immediately mixed.

4) After fifteen seconds exactly, 1ml of bidistilled water was added and the content was thoroughly mixed.

5) The content was transferred into cuvettes for reading in the Aminco-Bowmann spectrofluorometer. For noradrenaline, at pH 7.0, the activation and emission wavelengths were 409 and 519mμ respectively and for adrenaline, at pH 2.0, these were 422 and 529mμ respectively.

The instrument was adjusted to 0 reading with the blanks. No difference was observed between blanks obtained by omitting the ferricyanide and "faded" blanks obtained by omitting ascorbate from the alkaline solution. For practical reasons the former blank was adopted.

Although readings for samples prepared by oxidation at pH 7.0 were quite stable, this was not so for those at

lower pH, in which case readings were taken regularly, exactly four minutes after addition of ferricyanide. This instability was most apparent in determinations of the tissue samples.

All determinations were made twice for pH 7.0 samples and at least twice for pH 2 samples.

At pH 7.0 both noradrenaline and adrenaline may contribute to the amount of fluorescent material produced. As the pH is lowered the fluorescence due to noradrenaline decreases appreciably more than for adrenaline. The idea is to increase the amount of acetic acid so as to sufficiently reduce the reading due to noradrenaline without affecting too much that of adrenaline. We found, as Anton and Sayre did, that 0.2ml of 1.6N acetic acid gave adequate results. When more acid was added readings due to adrenaline decreased considerably. Under these conditions the fluorescence due to noradrenaline was approximately 5% of that obtained at pH 7.0; the fluorescence due to adrenaline at lower pH was 95% of that obtained at higher pH.

The following equations apply for the differential estimation of adrenaline and noradrenaline.

1) Noradrenaline: $\dfrac{C\left(A-\frac{B \times H}{100}\right)}{100-\frac{D \times H}{100}}$ 2) Adrenaline $\left(\frac{F \times B}{100}\right)-\left(\frac{D \times H \times A \times F}{100 \times C}\right)$

A = net fluorescence at pH 7.0

B = net fluorescence at pH 2.0

C = that concentration of noradrenaline in the cuvette which produced a net fluorescence of 100 at pH 7.0

D = percentage of noradrenaline oxidized at pH 2.0 as compared to pH 7.0 (5%)

F = that concentration of adrenaline in the cuvette which produced a net fluorescence of 100 at pH 2.0

H = net fluorescence of adrenaline (F) at pH 7.0 (120%)

ΣA = amount of adrenaline in the aliquot of the acid eluate.

The factors present in the numerator of both equations are by far the most important. In the case of noradrenaline B x H represents the amount of fluorescence contributed by adrenaline at pH 7.0 and this amount must be substracted from the total fluorescence obtained at pH 7.0. This gives the fluorescence contributed by noradrenaline alone. The resultant value is multiplied by C which is a calibration factor and which also corrects for recoveries, since an internal standard was taken to calculate C. In the case of adrenaline the net fluorescence at pH 2.0 is multiplied by F, the equivalence of C for noradrenaline. From this amount is substracted the amount of fluorescence contributed by noradrenaline at pH 2.0 and which is represented by the term $D \times \Sigma A \times F$. Thus both equations give the amount of

noradrenaline and adrenaline present in 0.4cc of eluate; in order to obtain the quantities of catecholamines present originally in the amount of tissue used, the values obtained from the equations must be multiplied by 7.5 ($\frac{3\text{ml eluate}}{0.4\text{ml aliquot}}$).

The sensitivity of the method was such that when a new Xenon lamp was used in the fluorimeter, as little as 1mμg of catecholamine could be easily detected.

It must be emphasized that the glassware had to be scrupulously clean in order to obtain low blanks and full sensitivity. This was particularly important for readings of pH 2.0 samples since the amount of adrenaline in brain, heart and especially liver is very low.

Part 1

RESULTS

IN VITRO STUDIES

ATTEMPTS TO FIND AN EFFECT OF VITAMIN B_{12} AND METHYLCOBALAMIN ON A SEMI-PURIFIED PREPARATION OF CATECHOL-O-METHYL TRANSFERASE

The semi-purified preparation of the enzyme was kindly supplied by Dr. Mavrides from this laboratory. Ten milligrams of this enzyme was used through these experiments to obtain the desired activity.

The final concentration in the incubation mixture was:

Adrenaline:	5×10^{-4}M
S-Adenosylmethionine:	5×10^{-4}M
Magnesium Chloride:	1×10^{-2}M
Phosphate Buffer:	5×10^{-2}M

When vitamin B_{12} was added in the following concentrations, 1×10^{-3}M, 1×10^{-4}M, 1×10^{-6}M the amount of metanephrine formed was exactly the same as in the control tubes.

Likewise, methylcobalamin could not modify catechol-O-methyl transferase activity at the following concentrations: 1×10^{-4}M, 1×10^{-5}M, 1×10^{-6}M. Neither could methylcobalamin replace S-adenosylmethionine at concentrations of 5.5×10^{-4}M.

We did not try the effect of 5,6-dibenzimidazolyl-cobamide on the enzyme since it was not commercially available.

ATTEMPT TO FIND AN EFFECT OF VITAMIN B_{12} ON MONOAMINE OXIDASE.

Because in vivo results showed that the liver of rats treated with B_{12} exhibited lower monoamine oxidase activity than the liver of rats not given B_{12}, it was decided to investigate on a possible direct effect of the vitamin on the enzyme.

For this purpose, a purified preparation of monoamine oxidase was obtained from Sigma Chemicals Co. (Beef plasma enzyme type 1). The method followed was described by Tabor et al (218). The procedure is essentially the same as described page 69, except that the medium was buffered with 0.2M potassium phosphate pH 7.2 and benzylamine served as the substrate. Benzylamine sulfate was prepared by dissolving 1.07 g. of redistilled benzylamine in 5ml of 2N sulfuric acid and diluted to 100ml with distilled water to a final concentration of 0.1M.

The incubation mixtures in silica cuvettes consisted of 1ml of phosphate buffer, 0.1ml of 0.1M benzylamine sulfate and water to a final volume of 3ml. The blank contained the same medium except that no substrate was added. The formation of benzaldehyde was followed by measuring the

change in optical density at 250mμ (absorption peak of benzaldehyde). The activity was measured at 30°C for ten minutes.

Vitamin B_{12} at final concentrations of 1×10^{-4}M, 1×10^{-5}M, 1×10^{-6}M did not modify the activity of this enzyme. Preincubation of the enzyme with buffer and vitamin B_{12} at a final concentration of 1×10^{-5}M for ten minutes likewise did not bring any change.

IN VIVO STUDIES

Preparation of rats

The vitamin B_{12} deficient diet was prepared as follows:

Unless otherwise stated the following ingredients were obtained from Nutritional Biochemicals Co.

First the vitamin or vitamin-like compounds were mixed in the following proportions, thiamine 0.180g, riboflavin 0.360g, nicotinic acid 1.200g, pyridoxine 0.180 g, panthotenic acid 1.500g, choline chloride 120.00g, paraaminobenzoic acid 3.00g, biotin 0.006g, folic acid 0.015g, inositol 60.00g, menadione 0.120g, total 186.861g. To this was added 600g of corn meal (from Kitchie Feed Co.) 100g of the above mixture was added to the following mixture, corn meal 2.28kg, soya meal (from Kitchie Feed Co.) 2.28kg, Salts IV (Phillips and Hard) 100g, Mazola Corn Oil 260g, cystine 15g, for a total of 5kg of diet. The iodinated casein diet was prepared by mixing 5g of iodinated casein to 5kg of the above diet thus constituting a 0.1% iodinated casein diet. Mixing was done in a mixer especially constructed for this purpose. The vitamins were first added to the corn meal and mixing was allowed several hours. The next ingredients were added one after another with thorough mixing between additions.

The whole process lasted one and one-half days.

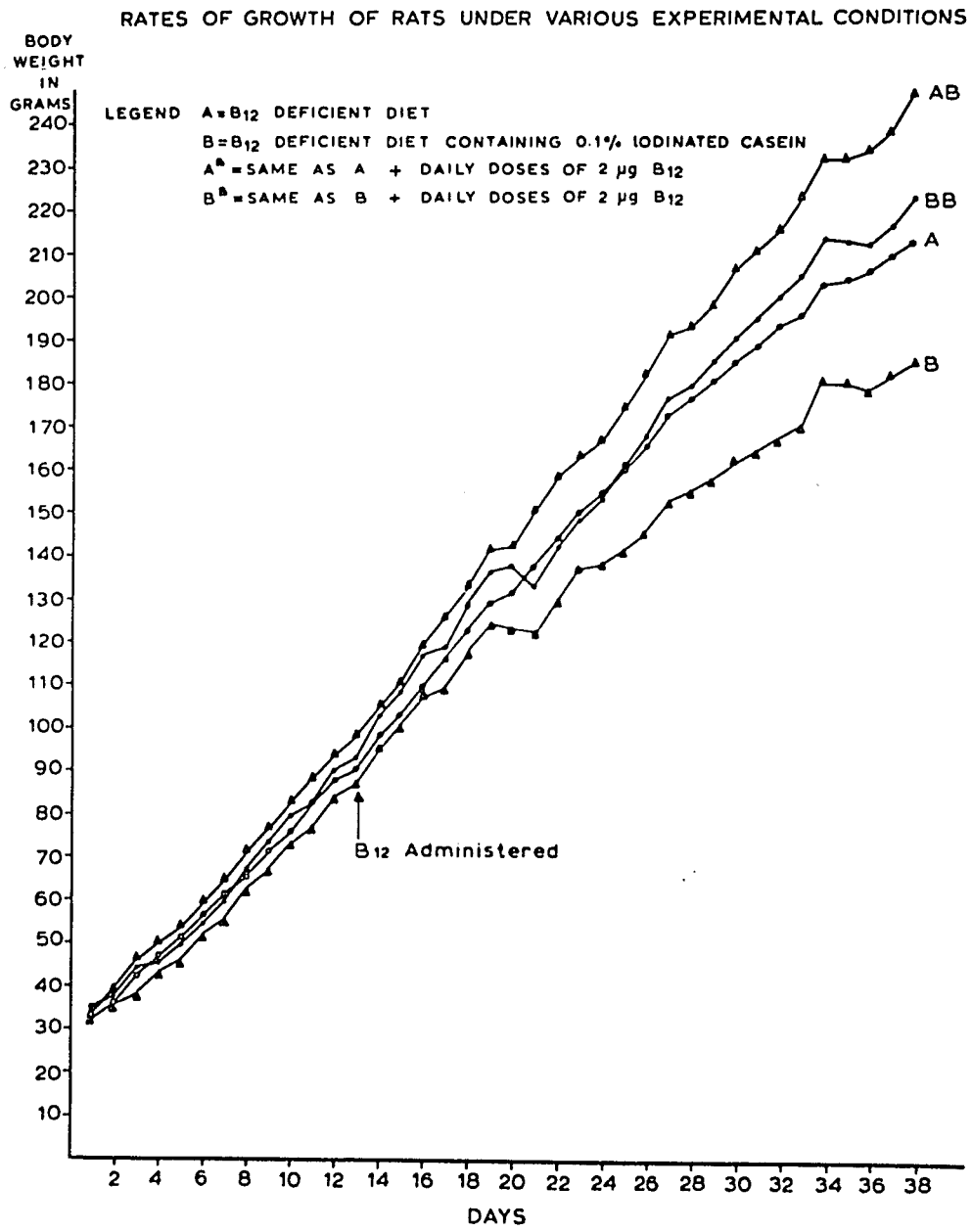
Sixty young male weanling rats (Sprague-Dawley strain) when received weighed approximately 35g . They were divided into four groups: twenty six rats were given the basal diet (deficient in vitamin B_{12}) and half of these were injected with vitamin B_{12}. The other thirty four were fed with the iodinated casein diet and half of these also received injections of vitamin B_{12}. A greater number of rats were given the iodinated casein diet because we expected a higher mortality rate in these animals.

Vitamin B_{12} was received triturated in mannitol and as such was dissolved in 0.9% NaCl. 2µg of B_{12} were given intramuscularly every day and injections were started thirteen days after the rats had been started on the diet.

Three times a week, all rats were given orally a few drops of pre-daylin (Abbott Products N°86686) which consists of a mixture of vitamin A, C and D.

The rats were placed in individual cages with raised screened bottoms and the temperature of the room was controlled. All rats were fed ad libitum and fresh water was given daily. The rats were kept on this treatment for thirty eight days and their weights were recorded daily. At the moment of sacrifice, the rats were killed by decapitation, the organs were quickly removed, rinsed in cold isotonic KCl,

Figure IV


RATES OF GROWTH OF RATS UNDER VARIOUS EXPERIMENTAL CONDITIONS
BODY WEIGHT IN GRAMS
LEGEND A=B12 DEFICIENT DIET
B=B12 DEFICIENT DIET CONTAINING 0.1% IODINATED CASEIN
A^B=SAME AS A + DAILY DOSES OF 2 µg B12
B^B=SAME AS B + DAILY DOSES OF 2 µg B12
AB
BB
A
B
B12 Administered
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
DAYS

blotted dry and stored frozen in individual plastic bags.

Figure IV represents the different growth curves of the various groups of rats. As may have been expected, the lowest curves are those representing animals not injected with B_{12}. The effect of the injected vitamin is perceivable although not very marked from the day the rats were injected. This is probably because the animals were not in a deficient state when purchased and might have had sufficient endogenous vitamin to permit normal growth for a few weeks even with a deficient diet. Beyond three weeks the lack of growth in the deficient animals became manifest and this was corrected by administration of B_{12}. It is also apparent that the animals fed iodinated casein, B_{12} deficient diet were also restricted in their growth when compared to animals fed a control B_{12} deficient diet. However, the growth of rats fed the iodinated casein diet and given B_{12} finally exceeded that of rats given the control, B_{12} deficient diet. This is more than could have been predicted from previous work (154,74) where vitamin B_{12} administration failed to completely correct the loss of weight experienced by animals given thyroid or fed a 0.1% iodinated casein diet.

A very rough estimation of food consumption was made by weighing the amount of diet remaining after thirty eight days of feeding and deducting this amount from the original weight before feeding. Thus it could be estimated that the

Table 1

FINAL BODY WEIGHTS RECORDED IN THE VARIOUS GROUPS OF RATS AT THE MOMENT OF SACRIFICE†

	Untreated		Treated with B_{12}*	
Number of rat	Diet A‡	Diet B‡	Diet A	Diet B
1	182	210	239	226
2	206	180	265	202
3	189	187	262	236
4	248	182	256	229
5	204	193	234	244
6	233	197	259	218
7	204	162	241	242
8	229	194	235	209
9	194	194	254	197
10	223	165	234	214
11	225	181	230	257
12	215	199	235	221
13	217	194	255	244
14		213		203
15		176		219
16		201		229
17		127		211
Average	213 ± 19	185 ± 20	246 ± 13.4	223 ± 17
% Change	-13%		-9.5%	
Significance	P < .01		P < .01	

‡ Diet A: Normal deficient diet.
‡ Diet B: 0.1% iodinated deficient diet.
* Rats were injected each day with 2µg/kg of vitamin B_{12}.
† All values are reported in grams.

rats given the control diet consumed an average of 623 grams of diet per rat for thirty eight days whereas the rats fed the iodinated casein diet consumed 675 grams of diet per rat for the same period of time. These results are not surprising even in terms of the restrained growth rates observed for the rats kept on the iodinated casein diet. One must consider that the hyperthyroid animals are in a constant need of energy since they are incapable of conserving whatever energy is wasted as heat.

Table 1 represents the final body weights as recorded just before the rats were sacrificed. Statistical comparison in this Table and in all others were made according to the student t test. In the groups fed a normal B_{12} deficient diet, the increase in body weight caused by administration of B_{12} was of the order of 15% and for those fed the iodinated casein diet it was 20%, both increases were statistically significant ($P < .01$). Comparing those groups with and without B_{12}, there was a comparable significant decrease of some 10% in the body weight of the animals fed the iodinated-casein ration. However this decrease was small compared to that obtained by previous workers (/54,74) with the same concentration of iodinated casein. The difference was even smaller four weeks after the animals had been given the diets and although certain symptoms of hyperthyroidism began to appear (slight tremor,

Table 2

EFFECT OF A DIET CONTAINING 0.1% IODINATED CASEIN ON THE METABOLISM OF RATS +

	Untreated		Treated with B_{12} ‡	
Number of rat	Diet A*	Diet B ⊙	Diet A	Diet B
1	20.5	38.2	23.7	40.9
2	18.5	38.1	22.9	42.4
3	26.4	36.8	20.7	36.2
4	24.3	34.5	25.3	31.9
5	20.0	36.3	24.3	31.7
6	22.3	35.8	16.0	39.2
7	27.2	39.4	25.2	32.1
8	24.0	37.7	24.1	36.4
9	21.5	37.3	16.6	43.0
10	22.4	34.7	16.6	38.4
11	21.0	39.6	25.5	33.1
12	16.2	37.5	21.1	47.7
13	19.5	34.9	26.0	36.6
14		40.1		35.3
15		39.4		41.8
16		44.5		37.2
17		43.8		37.5
18				39.8
Average	21.8 ± 3.1	38.1 ± 2.85	22.1 ± 3.6	37.8 ± 4.3
% Change	+ 75%		+ 71%	
Significance	P < .01		P < .01	

* Diet A: Normal deficient diet.
⊙ Diet B: 0.1% iodinated casein deficient diet.
‡ Rats were injected each day with 2µg/kg of vitamin B_{12}.
+ All values are reported as ml of O_2 consumed per minute per kg body weight.

Table 3

EFFECT OF HYPERTHYROIDISM ON RAT HEART WEIGHT†

Number of rat	Untreated		Treated with B_{12}*	
	Control	Hyperthyroid‡	Control	Hyperthyroid
1	.661	1.238	.852	1.540
2	.647	1.266	1.008	1.670
3	.837	1.273	.925	1.320
4	.845	.948	.999	1.310
5	.850	1.220	.899	1.410
6	.850	1.083	.866	1.270
7	.869	1.079	.852	1.358
8	.679	1.295	.859	1.392
9	.777	1.274	.865	1.463
10	.845	.890	.935	1.305
11	.799	1.212	.944	1.252
12	.835	1.274	.901	1.211
13		1.396	.810	1.378
14		1.032		1.227
Average	.791 ± .082	1.177 ± .14	.901 ± .059	1.364 ± .13
% Change	+ 48%		+ 51%	
Significance	P < .01		P < .01	

† All values are reported in grams.
* Rats were injected each day with 2µg/kg of vitamin B_{12}.
‡ Hyperthyroidism was induced by feeding the rats with 0.1% casein diet.

Table 4

EFFECT OF HYPERTHYROIDISM ON BRAIN WEIGHT†

Number of rat	Untreated		Treated with B_{12}*	
	Control	Hyperthyroid‡	Control	Hyperthyroid
1	1.39	1.57	1.68	1.69
2	1.63	1.49	1.46	1.67
3	1.38	1.56	1.34	1.69
4	1.42	1.67	1.27	1.67
5	1.53	1.62	1.73	1.52
6	1.51	1.59	1.57	1.47
7	1.59	1.58	1.59	1.57
8	1.42	1.73	1.70	1.59
Average	1.48 ± .095	1.60 ± .073	1.54 ± .17	1.60 ± .085
% Change	+ 8%		+ 3.9%	
Significance	P < .02		P > .02	

† All values are reported in grams.
‡ Hyperthyroidism was induced by feeding the rats a 0.1% iodinated casein diet.
* Rats were injected each day with 2µg/kg of vitamin B_{12}.

scarcity of fur, and greater excitability) one could not rely on these subjective signs to conclude that the rats had reached a state of hyperthyroidism. To dissipate our doubts it was decided to determine the metabolic rate of all rats.

Table 2 shows the results of such determinations. It is readily apparent that the rats fed the iodinated casein diet were hypermetabolic since their metabolic rate had increased by 75% over those which had been given a normal diet, and this whether or not they had been injected B_{12}. In fact, vitamin B_{12} did not affect the metabolic rate of rats in both control and hyperthyroid groups.

Another typical syndrome of hyperthyroidism, cardiac hypertrophy, can be observed from Table 3, where the heart weight of the hyperthyroid animals was increased by some 50% over those of controls. This increase is observed with or without B_{12}. It may be noted that the increase in weight of hearts is observed despite the loss in body weight experienced by the hyperthyroid rats. Another significant increase ($P < .01$) of 14-15% in the heart weight is observed in both control and hyperthyroid groups as a result of B_{12} administration. But in this case the increase completely paralleled the 15-20% increase in body weight caused by the vitamin.

Table 4 represents the brain weights of the various

Table 5

EFFECT OF HYPERTHYROIDISM ON LIVER WEIGHT†

Number of rat	Untreated		Treated with B_{12}*	
	Control	Hyperthyroid‡	Control	Hyperthyroid
1	9.09	13.93	10.46	11.86
2	9.87	12.06	12.18	10.84
3	9.55	11.28	11.26	12.28
4	12.91	13.31	11.16	13.98
5	9.75	13.84	10.52	12.93
6	11.93	14.48	11.70	11.79
7	10.70	10.52	10.33	12.18
8	12.20	11.06	10.13	11.27
9	9.98	13.40	11.39	12.39
10	10.29	11.05	10.81	10.08
11	11.28	13.35	10.71	12.81
12	11.46	14.35	10.63	12.40
13	11.13	13.05	11.53	14.06
14		12.63		10.72
15		10.92		12.14
16		12.38		12.89
17				11.70
Average	10.78 ± 1.1	12.6 ± 1.3	10.96 ± .66	12.13 ± 1.0
% Change	+ 16%		+ 10%	
Significance	P < .01		P < .01	

† All values are reported in grams.
‡ Hyperthyroidism was induced by feeding the rats 0.1% iodinated casein diet.
* Rats were injected each day with 2µg/kg of vitamin B_{12}.

Table 6

SOLUBLE PROTEINS OF THE LIVER OBTAINED FROM VARIOUS GROUPS OF RATS†×

Number of rat	Untreated		Treated with B_{12}*	
	Control	Hyperthyroid‡	Control	Hyperthyroid
1	.1605	.1924	.1767	.1866
2	.1717	.1665	.1764	.1706
3	.1679	.1809	.1666	.1751
4	.1561	.1776	.1826	.1869
5	.1574	.1741	.1741	.2002
6	.1671	.1883	.1736	.1722
7	.1524	.1788	.1541	.1736
8	.1785	.1921	.1545	.1791
9	.1666	.1702	.1851	.1692
10	.1834	.1809	.1777	.1809
11	.1733	.1969	.1740	.1812
12	.1761	.1760	.1598	.1910
13	.1754	.1898	.1481	.1754
14		.1824		.1933
15		.1775		.1873
16		.1692		
17		.1829		
Average	.1666±.0092	.1809 ± .0087	.1695 ± .012	.1807 ± .0086
% Change	+8.5%		+6.6%	
Significance	P < .01		P < .01	

† The protein values are obtained from the protein determination (Biuret method) performed on the clear supernatant obtained from liver homogenates.
× All values are expressed as mg protein/mg liver.
‡ Hyperthyroidism was induced by feeding the rats 0.1% iodinated casein diet.
* Rats were injected each day with 2µg/kg of vitamin B_{12}.

groups of rats. A significant 8% increase in the brain weight of the hyperthyroid animals is noted by comparing among the groups not receiving B_{12}. In the B_{12} group however this difference is smaller and not statistically significant. Within the euthyroid and hyperthyroid group B_{12} supplementation does not appear to effect the brain weight.

Table 5 shows that, in the B_{12} deficient group, the livers of hyperthyroid rats weighed more than those of euthyroid rats by 16% and this increase was 10% in the other group. B_{12} administration had no significant effects on the liver weight within the euthyroid and hyperthyroid groups.

The protein values obtained from the clear supernatant of the liver homogenate were tabulated so that the different groups could be compared. It is seen from Table 6 that a small but significant increase of 8.5%, in the group without B_{12}, occurred in the soluble protein of the liver obtained from the hyperthyroid animals. A comparable increase also occurred in the B_{12} group; no effect of B_{12} on the soluble protein of the liver can be observed.

From the results obtained on heart weight, O_2 consumption, it is evident that the rats fed the iodinated casein diet had become hyperthyroid even though these same animals were not necessarily of considerably smaller weight. In these hypermetabolic rats, apparently there is no

Table 7

CATECHOL-O-METHYL TRANSFERASE ACTIVITY IN LIVERS OBTAINED FROM VARIOUS GROUPS OF RATS

(Activity expressed as units†/mg protein×)

	Untreated		Treated with B_{12}*	
Number of rat	Control	Hyperthyroid‡	Control	Hyperthyroid
1	1.92×10^{-3}	1.72×10^{-3}	1.86×10^{-3}	1.92×10^{-3}
2	2.21×10^{-3}	1.51×10^{-3}	2.04×10^{-3}	1.63×10^{-3}
3	2.01×10^{-3}	1.95×10^{-3}	2.38×10^{-3}	1.80×10^{-3}
4	2.32×10^{-3}	1.75×10^{-3}	1.99×10^{-3}	1.69×10^{-3}
5	1.70×10^{-3}	1.73×10^{-3}	1.93×10^{-3}	1.89×10^{-3}
6	1.87×10^{-3}	1.71×10^{-3}	2.51×10^{-3}	1.77×10^{-3}
7	1.61×10^{-3}	2.03×10^{-3}	2.08×10^{-3}	1.91×10^{-3}
8	1.96×10^{-3}	1.85×10^{-3}	1.90×10^{-3}	1.48×10^{-3}
9	1.43×10^{-3}	1.58×10^{-3}	2.30×10^{-3}	1.90×10^{-3}
10	1.66×10^{-3}	1.79×10^{-3}	1.81×10^{-3}	1.86×10^{-3}
11	2.16×10^{-3}	1.89×10^{-3}	2.04×10^{-3}	2.17×10^{-3}
12	1.97×10^{-3}	1.70×10^{-3}	2.11×10^{-3}	1.84×10^{-3}
13	2.57×10^{-3}	2.25×10^{-3}	1.90×10^{-3}	1.82×10^{-3}
14		2.01×10^{-3}		2.17×10^{-3}
15		1.63×10^{-3}		1.93×10^{-3}
16		1.53×10^{-3}		2.13×10^{-3}
17		1.73×10^{-3}		1.71×10^{-3}
Average	$1.95 \times 10^{-3} \pm .31$	$1.78 \times 10^{-3} \pm .19$	$2.06 \times 10^{-3} \pm .21$	$1.86 \times 10^{-3} \pm .17$
% Change	- 8.7%		- 9.7%	
Significance	$P > .05$		$P < .01$	

× Proteins were determined on the clear supernatant of liver homogenates prepared as indicated (page 72).
† One unit is defined as the amount of enzyme which produces one micromole of metaneprine in two minutes at 37°C.
‡ Hyperthyroidism was induced by feeding the rats with 0.1% iodinated casein diet.
* Rats were injected with 2µg/kg of vitamin B_{12} each day.

Table 8

CATECHOL-O-METHYL TRANSFERASE ACTIVITY IN LIVERS OBTAINED FROM VARIOUS GROUPS OF RATS

(Activity expressed as units†/mg liver)

Number of rat	Untreated		Treated with B_{12}*	
	Control	Hyperthyroid‡	Control	Hyperthyroid
1	$.315 \times 10^{-3}$	$.334 \times 10^{-3}$	$.418 \times 10^{-3}$	$.347 \times 10^{-3}$
2	$.338 \times 10^{-3}$	$.338 \times 10^{-3}$	$.372 \times 10^{-3}$	$.371 \times 10^{-3}$
3	$.314 \times 10^{-3}$	$.325 \times 10^{-3}$	$.315 \times 10^{-3}$	$.338 \times 10^{-3}$
4	$.401 \times 10^{-3}$	$.302 \times 10^{-3}$	$.331 \times 10^{-3}$	$.355 \times 10^{-3}$
5	$.255 \times 10^{-3}$	$.393 \times 10^{-3}$	$.336 \times 10^{-3}$	$.366 \times 10^{-3}$
6	$.336 \times 10^{-3}$	$.377 \times 10^{-3}$	$.353 \times 10^{-3}$	$.362 \times 10^{-3}$
7	$.304 \times 10^{-3}$	$.271 \times 10^{-3}$	$.292 \times 10^{-3}$	$.331 \times 10^{-3}$
8	$.354 \times 10^{-3}$	$.294 \times 10^{-3}$	$.381 \times 10^{-3}$	$.307 \times 10^{-3}$
9	$.321 \times 10^{-3}$	$.294 \times 10^{-3}$	$.344 \times 10^{-3}$	$.311 \times 10^{-3}$
10	$.405 \times 10^{-3}$	$.335 \times 10^{-3}$	$.410 \times 10^{-3}$	$.268 \times 10^{-3}$
11	$.288 \times 10^{-3}$	$.311 \times 10^{-3}$	$.363 \times 10^{-3}$	$.367 \times 10^{-3}$
12	$.338 \times 10^{-3}$	$.308 \times 10^{-3}$	$.326 \times 10^{-3}$	$.344 \times 10^{-3}$
13	$.252 \times 10^{-3}$	$.359 \times 10^{-3}$	$.295 \times 10^{-3}$	$.322 \times 10^{-3}$
14		$.298 \times 10^{-3}$		$.368 \times 10^{-3}$
15		$.346 \times 10^{-3}$		$.298 \times 10^{-3}$
16		$.292 \times 10^{-3}$		$.314 \times 10^{-3}$
17		$.312 \times 10^{-3}$		$.337 \times 10^{-3}$
Average	$.324 \times 10^{-3} \pm .046$	$.322 \times 10^{-3} \pm .032$	$.349 \times 10^{-3} \pm .038$	$.335 \times 10^{-3} \pm .028$
% Change	-0.6%		-4.0%	
Significance	$P > .5$		$P > .2$	

† One unit is defined as the amount of enzyme which produces one micromole of metanephrine in two minutes at 37°C.
‡ Hyperthyroidism was induced by feeding the rats 0.1% iodinated casein diet.
* Rats were injected each day with 2μg/kg of vitamin B_{12}.

Table 9

MONOAMINE OXIDASE ACTIVITY IN LIVERS OBTAINED FROM VARIOUS GROUPS OF RATS

(Activity expressed as units†/mg protein×)

Number of rat	Untreated		Treated with B_{12}*	
	Control	Hyperthyroid‡	Control	Hyperthyroid
1	5.82	4.06	4.30	3.13
2	5.43	4.18	4.28	3.12
3	5.70	4.09	4.20	2.75
4	4.88	4.29	4.40	3.14
5	5.11	3.64	4.36	2.95
6	5.54	3.70	4.05	2.53
7	5.30	3.35	4.55	2.80
8	5.45	4.01	4.75	3.11
9	5.58	3.50	4.06	2.83
10	5.69	3.57	4.28	2.94
11	5.19	3.68	4.62	2.95
12	4.98	3.57	3.89	2.94
13	5.43	4.00	4.29	2.85
14		4.13		3.16
15		3.82		3.11
16		3.72		3.12
17		3.67		
Average	5.39 ± .27	3.82 ± .28	4.31 ± .23	2.95 ± .17
% Change	- 29%		- 31%	
Significance	P < .01		P < .01	

× Proteins were determined on the supernatant of liver homogenates.
† One unit is defined as the amount of enzyme causing a drop in the optical density of .001, in the period of time of one minute.
‡ Hyperthyroidism was induced by feeding the rats 0.1% iodinated casein diet.
* Rats were injected each day with 2µg/kg of vitamin B_{12}.

indication that they have reached a state of hyperthyroidism where protein catabolism may occur; the weight of various organs was higher than that of controls and the soluble protein content was maintained. With these results in mind, one may now consider the liver catechol-O-methyl transferase and monoamine oxidase activities of the various groups of rats.

Table 7 represents the liver catechol-O-methyl transferase activity as expressed in units per mg protein. There is comparable decrease in the enzymatic activity of the hyperthyroid rats in both groups, with and without B_{12}, which is of the order of 9% but this difference is statistically significant only in the group given B_{12}. That most of this effect is due to the increase in proteins observed in the hyperthyroid group (Table 6) becomes apparent from a furthering consideration of Table 8 in which the values are now reported per mg of liver. Then, no significant effect of hyperthyroidism can be detected on catechol-O-methyl transferase of liver.

Vitamin B_{12} did not modify the activity of this enzyme in any group whether the results are expressed per mg protein or per mg liver.

In the case of monoamine oxidase a different situation is present. When the results are expressed per mg protein (Table 9) it is readily seen that there is a

Table 10

MONOAMINE OXIDASE ACTIVITY IN LIVERS OBTAINED FROM VARIOUS GROUPS OF RATS

(Activity expressed as units†/mg liver)

	Untreated		Treated with B_{12}*	
Number of rat	Control	Hyperthyroid‡	Control	Hyperthyroid
1	1.10	.87	1.03	.88
2	1.19	.79	.99	.62
3	1.13	.95	1.02	.63
4	1.20	1.01	1.00	.86
5	1.10	.87	.91	.68
6	1.16	.96	.86	.71
7	1.15	.93	.80	.55
8	1.01	.81	.89	.60
9	1.30	.80	.95	.62
10	1.13	.83	.88	.66
11	1.12	.78	.85	.66
12	1.02	.92	.87	.62
13	1.17	.95	.97	.61
14		.83		.67
15		.92		.63
16		.74		.58
17		.85		.67
Average	1.13 ± .069	.87 ± .075	.92 ± .074	.64 ± .07
% Change	-23%		-26%	
Significance	P < .01		P < .01	

† One unit is defined as the amount of enzyme causing a drop in the optical density of 0.001, in the period of time of one minute.

‡ Hyperthyroidism was induced by feeding the rats 0.1% iodinated casein diet.

* Rats were injected each day with 2µg/kg of vitamin B_{12}.

30% decrease in liver monoamine oxidase activity of the hyperthyroid group, the decrease being comparable and statistically significant in both groups with and without B_{12}. However, examination of Table 10 where the values are reported per mg liver reveals that approximately the same differences are still present although less marked. Thus a true decrease in monoamine oxidase of the liver of hyperthyroid rats is demonstrated. Quite unexpectedly, it was observed that vitamin B_{12} exerted a similar decreasing effect on hepatic monoamine oxidase activity of both control and hyperthyroid rats. In the euthyroid rats there is a significant 20% decrease in activity caused by B_{12} (Table 9) and when the values are reported per mg liver in Table 10this decrease is 18%. In the hyperthyroid groups these differences are slightly higher being 26% when reported per mg liver (Table 10) and 22% when expressed per mg protein. All differences are statistically significant for $P < .01$. It may be noted that hyperthyroidism caused the decrease in enzymatic activity to the same extent as vitamin B_{12} since no significant difference in activity can be noted between the hyperthyroid group without B_{12} and the control group with B_{12}. This is true irrespective of how the values are expressed. Moreover the individual effects of hyperthyroidism and B_{12} are additive as can be seen from the difference in activity observed between the hyperthyroid

Table 11

NORADRENALINE CONTENT OF LIVERS OBTAINED FROM VARIOUS GROUPS OF RATS

Number of rat.	Untreated † µg/g Hyperthyroid	Untreated † µg/g Control	Untreated † µg/liver Hyperthyroid	Untreated † µg/liver Control	Treated with B_{12}* µg/g Hyperthyroid	Treated with B_{12}* µg/g Control	Treated with B_{12}* µg/liver Hyperthyroid	Treated with B_{12}* µg/liver Control
1	.0504	.0510	.3535	.3345	.0377	.0309	.2365	.1845
2	.0466	.0614	.3110	.3680	.0469	.0574	.2790	.3217
3	.0493	.0506	.2860	.2630	.0402	.0438	.2280	.2815
4	.0440	.0516	.2905	.3405	.0371	.0428	.2490	.2467
5	.0303	.0397	.1682	.2302	.0506	.0371	.2767	.2452
6	.0464	.0400	.2782	.2467	.0404	.0445	.2182	.2235
7	.0622	.0469	.3112	.2160	.0317	.0556	.1672	.2940
8	.0483	.0579	.2572	.3285	.0479	.0536	.2512	.2842
9	.0442	.0643	.2287	.3322	.0540	.0559	.2655	.2790
10	.0356	.0323	.1852	.1545	.0559	.0438	.2820	.2205
11	.0515		.2520		.0560		.2880	
12	.0570		.2887		.0582		.2880	
Mean	.0471±.0085	.0495±.010	.2692±.042	.2814±.066	.0463±.0033	.0465±.0088	.2524±.034	.2580±.039
% Change	− 4%		− 4%		− 0.4%		− 2%	
Significance	P > .5		P > .5		P > .5		P > .5	

† Hyperthyroidism was induced by feeding the rats 0.1% iodinated casein diet.
* Rats were injected each day with 2µg/kg of vitamin B_{12}.

Table 12

ADRENALINE CONTENT OF LIVERS OBTAINED FROM VARIOUS GROUPS OF RATS

Number of rat	Untreated				Treated with B_{12}*			
	µg/g		µg/liver		µg/g		µg/liver	
	Hyperthyroid‡	Control	Hyperthyroid	Control	Hyperthyroid	Control	Hyperthyroid	Control
1	.00361	.00297	.0224	.0172	.00386	.00286	.0219	.0189
2	.00287	.00265	.0172	.0162	.00174	.00286	.0117	.0165
3	.00267	.00327	.0141	.0150	.00164	.00318	.0090	.0159
4	.00255	.00265	.0132	.0150	.00361	.00251	.0195	.0141
5	.00394	.00354	.0204	.0183	.00442	.00395	.0232	.0210
6	.00402	.00417	.0197	.0200	.00357	.00363	.0187	.0181
7	.00307		.0156		.00318	.00349	.0157	.0176
8					.00256		.0126	
9					.00334		.0171	
10					.00488		.0246	
Mean	.00324±.00056	.00320±.00058	.0175±.0034	.0169±.0019	.00328±.0009	.00321±.0005	.0174±.0051	.0174±.0021
% Change	0%		+ 3%		0%		0%	
Significance	–		P > .5		–		–	

‡ Hyperthyroidism was induced by feeding the rats 0.1% iodinated casein diet.
* Rats were injected with 2µg/kg of vitamin B_{12} each day.

Table 13

NORADRENALINE CONTENT OF BRAINS OBTAINED FROM VARIOUS GROUPS OF RATS

	Untreated				Treated with B_{12}*			
	µg/g		µg/brain		µg/g		µg/brain	
Number of rat	Hyperthyroid†	Control	Hyperthyroid	Control	Hyperthyroid	Control	Hyperthyroid	Control
1	.3150	.3081	.4987	.4665	.3127	.3227	.5145	.4931
2	.3059	.3369	.5148	.4942	.3114	.3703	.5242	.6090
3	.3294	.3572	.5040	.5280	.3036	.3025	.4781	.5171
4	.3105	.3429	.5043	.5111	.3317	.3629	.5175	.4740
5	.3191		.4968		.3450		.5520	
6	.3008		.4736		.3021		.5347	
Mean	.3134±.01	.3362±.02	.4987±.014	.4999±.026	.3177±.017	.3396±.032	.5201±.045	.5233±.06
% Change	−6.8%		−0.2%		−6.4%		−0.6%	
Significance	P > .05		P > .5		P > .1		P > .5	

† Hyperthyroidism was induced by feeding the rats 0.1% iodinated casein diet.
*Rats were injected each day with 2µg/kg of vitamin B_{12}.

Table 14

ADRENALINE CONTENT OF BRAIN OBTAINED FROM VARIOUS GROUPS OF RATS

	Untreated				Treated with B_{12}*			
	µg/g		µg/brain		µg/g		µg/brain	
Number of rats	Hyperthyroid†	Control	Hyperthyroid	Control	Hyperthyroid	Control	Hyperthyroid	Control
1	.0315	.0227	.0502	.0340	.0300	.0292	.0490	.0446
2	.0225	.0227	.0378	.0333	.0238	.0187	.0401	.0283
3	.0117	.0134	.0180	.0198	.0117		.0183	
4	.0175	.0211	.0280	.0310	.0143		.0221	
5	.0183		.0288		.0199		.0318	
6	.0231		.0360		.0125		.0221	
Mean	.0207±.0682	.0199±.0044	.0331±.010	.0295±.007	.0187±.072	.0239±.0074	.0305±.013	.0344±.01
% Change	+ 4%		+ 12%		− 21%		− 11%	
Significance	P > .5		P > .5		P > .4		P > .4	

* Rats were injected each day with 2 µg/kg of vitamin B_{12}.
† Hyperthyroidism was induced by feeding the rats with a 0.1% iodinated casein diet.

group with B_{12} and the control group with no B_{12}. For example, with no B_{12}, hyperthyroidism caused a decrease in activity of 1.57 units per mg protein and with the control group B_{12} caused a decrease of 1.08 units per mg protein. The sum of these differences when substracted from the control (5.39-2.65) gives the theoritical value of 2.74 units per mg protein if the individual effects are added. Theactual value obtained, 2.95 units per mg protein, is a good approximation of the theoritical value. The same result is obtained when a similar calculation is done with values expressed per mg liver.

The catecholamine content of the brain, liver, heart has been determined in the same rats used in the foregoing experiments. Because of the difference in weight observed in these tissues between control and hyperthyroid animals it was found advisable to report the catecholamine values as µg per whole tissue weight as well as per g. tissue.

Table 11 and 12 show no difference in the noradrenaline and adrenaline content of livers obtained from control and hyperthyroid groups. Likewise vitamin B_{12} administration did not change the catecholamine content of this tissue.

The same observation may be made for the brain (Table 13 and 14).

Table 15

THYROXINE ALPHA CONTENT OF HEARTS OBTAINED FROM VARIOUS GROUPS OF RATS

	Untreated				Treated with B_{12}*			
	µg/g		µg/heart		µg/g		µg/heart	
Number of rat	Hyperthyroid†	Control	Hyperthyroid	Control	Hyperthyroid	Control	Hyperthyroid	Control
1	.6691	.9768	.7762	.7387	.5831	.7110	.7800	.6506
2	.6476	.8575	.7593	.6281	.5675	.7139	.7675	.6313
3	.6261	.9501	.6936	.4795	.4907	.9216	.6472	.7852
4	.5977	.9740	.6195	.8280	.4764	.9870	.7957	.8902
5	.6146	.9520	.8580	.7210	.5097	.8590	.6922	.7740
6	.6063	1.0980	.7725	.7055	.5256	.8933	.7612	.8925
7	.5695		.6691		.4957	.6125	.7935	.5782
8	.5960		.5789		.4918		.8250	
Mean	.5971±.054	.9347±.095	.7050±.10	.7413±.08	.5625±.075	.8140±.136	.7610±.053	.7303±.095
% Change	− 36%		− 4%		− 30%		+ 4%	
Significance	P < .01		P > .4		P < .01		P > .4	

†Hyperthyroidism was induced by feeding the rats a 0.1% iodinated casein diet.
*Rats were injected each day with 2µg/kg of vitamin B_{12}.

Table 16

ADRENALINE CONTENT OF HEARTS OBTAINED FROM VARIOUS GROUPS OF RATS

Number of rat.	Untreated † ug/g Hyperthyroid	Untreated † ug/g Control	Untreated † ug/heart Hyperthyroid	Untreated † ug/heart Control	Treated with B_{12}* ug/g Hyperthyroid	Treated with B_{12}* ug/g Control	Treated with B_{12}* ug/heart Hyperthyroid	Treated with B_{12}* ug/heart Control
1	.0329	.0437	.0312	.0349	.0244	.0264	.0322	.0225
2	.0200	.0458	.0245	.0390	.0215	.0324	.0360	.0262
3	.0194	.0405	.0270	.0352	.0208	.0292	.0285	.0292
4	.0341	.0386	.0435	.0262	.0409	.0357	.0532	.0337
5	.0371		.0449		.0280		.0367	
6	.0313		.0339		.0285		.0345	
Mean	.0291±.0076	.0421±.0032	.0341±.0084	.0338±.0054	.0273±.0074	.0309±.004	.0368±.0085	.0279±.0047
% Change	−30%		+ 0.8%		−11%		+ 31%	
Significance	P .02		P > .5		P > .3		P > .05	

† Hyperthyroidism was induced by feeding the rats 0.1% iodinated casein diet.
* Rats were injected each day with 2µg/kg of vitamin B_{12}.

Table 16a

	Untreated		Treated with B_{12}*	
	Control	Hyperthyroid†	Control	Hyperthyroid
Body Weight, in grams	213 ± 19	185 ± 20	246 ± 13.4	223 ± 17
Metabolic Rate, ml of O_2 consumed/min/kg	21.8 ± 3.1	38.1 ± 2.8	22.1 ± 3.6	37.8 ± 4.3
Liver Weight, in grams	10.78 ± 1.1	12.6 ± 1.3	10.96 ± .66	12.13 ± 1.0
Heart Weight, in grams	.791 ± .082	1.177 ± .140	.901 ± .059	1.364 ± .130
Brain Weight, in grams	1.58 ± .095	1.60 ± .073	1.54 ± .17	1.60 ± .08
Liver Soluble Proteins, mg ptn./mg liver	1166 ± .009	.181 ± .009	.169 ± .012	.191 ± .009
COMT, units/mg ptn.	1.95×10^{-3} ± .31	1.78×10^{-3} ± .19	2.06×10^{-3} ± .21	1.86×10^{-3} ± .17
COMT, mg/liver	$.324 \times 10^{-3}$ ± .046	$.322 \times 10^{-3}$ ± .032	$.349 \times 10^{-3}$ ± .038	$.335 \times 10^{-3}$ ± .028
MAO, units/mg ptn.	5.39 ± .27	3.82 ± .28	4.31 ± .23	2.95 ± .17
MAO, units/mg liver	1.13 ± .069	.87 ± .07	.92 ± .074	.64 ± .07

*Rats were injected each day with 2µg/kg of vitamin B_{12}
† Hyperthyroidism was induced by feeding the rats a 0.1% iodinated casein diet

COMT: catechol-O-methyl transferase
MAO: monoamine oxidase

Table 16b

	Untreated		Treated with B_{12}*	
	Control	Hyperthyroid†	Control	Hyperthyroid
Liver Noradrenaline, μg/g	.0495 ± .010	.0471 ± .0085	.0465 ± .0088	.0463 ± .0083
Liver Noradrenaline, μg/liver	.2814 ± .066	.2692 ± .042	.2580 ± .039	.2524 ± .034
Liver Adrenaline μg/g	.0032 ± .0006	.0032 ± .0006	.0032 ± .0005	.0033 ± .0009
Liver Adrenaline μg/liver	.0169 ± .0020	.0175 ± .0034	.0174 ± .0021	.0174 ± .0051
Heart Noradrenaline μg/g	.9347 ± .095	.5971 ± .054	.8140 ± .136	.5625 ± .075
Heart Noradrenaline μg/heart	.7413 ± .080	.7058 ± .10	.7303 ± .095	.7610 ± .058
Heart adrenaline μg/g	.0421 ± .0032	.0291 ± .0076	.0309 ± .004	.0273 ± .0074
Heart adrenaline μg/heart	.0338 ± .0054	.0341 ± .0084	.0279 ± .0047	.0368 ± .0085
Brain noradrenaline μg/g	.3362 ± .020	.3134 ± .010	.3396 ± .032	.3177 ± .017
Brain Noradrenaline μg/brain	.4999 ± .026	.4987 ± .014	.5233 ± .060	.5201 ± .025
Brain Adrenaline μg/g	.0199 ± .0044	.0207 ± .0082	.0239 ± .0074	.0187 ± .0072
Brain Adrenaline μg/brain	.0295 ± .007	.0331 ± .010	.0344 ± .010	.0305 ± .013

*Rats were injected each day with 2μg/kg of vitamin B_{12}
†Hyperthyroidism was induced by feeding the rats a 0.1% iodinated casein diet

In Table 15 it may be noted that there is a significant decrease in the noradrenaline content of the heart of hyperthyroid animals with and without B_{12}. When the results are expressed per g heart tissue. However, there is no significant difference when the values are expressed per total heart, and it seems that the decrease per g tissue may simply be the result of cardiac hypertrophy observed in the hyperthyroid group (Table 3). The same observation may be made for adrenaline (Table 16) in the hyperthyroid group with no B_{12}. Vitamin B_{12} supplementation did not significantly modify the catecholamine content of heart.

All of the above-mentioned data are summarized in Table 16*a*,b.

DISCUSSION

VITAMIN B_{12}, GROWTH, AND METABOLIC RATE.

Jaffe (188) estimated that three µg of vitamin B_{12} per kg of diet was sufficient to eliminate deficiency symptoms in rats. Assuming that the rats were eating approximately twenty grams of diet per day this requirement postulated by Jaffe would correspond to .06µg of vitamin per day. Thus the amount given to our rats, i.e., 2µg per day, was more than needed. The 15% increase in body weight of control rats caused by administration of B_{12} for 25 days is similar to that found by Bertozzoli et al (189) for 20 days with rats fed approximately the same amount of B_{12} (200µg/kg diet). The difference in weight between our B_{12} treated rats and those not so treated is also comparable to that observed by Erickson et al (190) who found similar increments in the weight of rats fed a diet containing cyanocobalamin (.003mg/100g. of diet) for 28 days. On the other hand, some other workers (191) have failed to notice such weight differences unless the animals were prevented from practicing coprophagy and this practice occurred when the rats were placed in cages with raised screened bottoms. Thus although our rats showed signs of vitamin deficiency we cannot be absolutely certain that the animals had not

ingested small quantities of the vitamin and hence had not been completely deprived of B_{12}.

Vitamin B_{12} did not modify the O_2 consumption of hyperthyroid rats and this confirms the results obtained by Ardil (192) who did not observe any difference in the O_2 consumption of rats injected with small amounts of vitamin B_{12} (2,3µg for 3 days). A 63% increase in O_2 consumption of hyperthyroid rats was caused by greater quantities of vitamin B_{12} amounting to a total of 500µg. Our rats received a total of 56 µg of vitamin and this amount did not affect O_2 consumption of hyperthyroid or control rats.

CATECHOL-O-METHYL TRANSFERASE AND MONOAMINE OXIDASE ACTIVITIES UNDER DIFFERENT EXPERIMENTAL CONDITIONS.

The growth promoting activity of the thyroid hormone is well known. On the other hand, hyperthyroidism is often associated with diminished body weight in humans (103,107) and animals (74,77,114,154, 194, 195). This apparently paradoxal observation can be reconciled on the basis that low physiological doses of thyroxinemay stimulate growth whereas higher toxic doses may have a converse effect. This same dose relationship of thyroxinehas also been noted in vitro (153) on protein synthesis. In our experiments, the growth of hyperthyroid animals was relatively well sustained when compared to their respective controls or even exceeded

that of some control groups. In both groups, with and without B_{12}, the liver weight and protein values of the hyperthyroid animals were significantly above that found for controls. Thus it appears that the hyperthyroid animals had not reached the stage of hyperthyroidism where a decrease in protein synthesis occurs. Under these conditions the hepatic catechol-O-methyl transferase of hyperthyroid rats was just as active as that of control rats. The previously reported decrease (142) in this enzyme as a result of injections of thyroxine may then have been due to a general catabolic effect of the high doses of thyroxine injected.

Under the same experimental conditions however, the liver monoamine oxidase of hyperthyroid rats was decreased by some 25%. This decrease cannot be attributed to possible diminution of food intake of the hyperthyroid rats which might have resulted in low monoamine oxidase activity, since the hyperthyroid animals consumed greater quantities of diet than the controls. Similar findings concerning the effect of thyroxine on food consumption have been obtained by others (193). It is difficult to compare the extent of this decrease in monoamine oxidase to that found by other workers since the experimental conditions were different. Some differences were the method used to induce hyperthyroidism, the extent of hyperthyroidism, the substrate employed for the enzyme assay and finally the

species of animals used. Generally speaking however, it can be said that the decrease we have observed in our experiments is of the order of that found by the others (142, 23, 136) except for a few cases where a 50% decrease in the monoamine oxidase activity of hyperthyroid animals was recorded (87, 137). In these cases however tyramine was used as a substrate and it was noted that less difference was found when serotonin or tryptamine was used as substrate (87). It may be pertinent to note here that some time ago it was observed that monoamine oxidase from different sources had different substrate specificity (196, 197) and this has been taken as an indication that there are several monoamine oxidases. Recently Gorkin (198) obtained some evidence that m-nitro-p-hydroxybenzylamine and p-nitro-phenylethylamine are oxidized by two different mitochondrial monoamine oxidases. Inhibition studies on mitochondrial monoamine oxidase by Gorkin et al (199) also indicate that mitochondrial monoamine oxidase is probably constituted by a system of multiple amine oxidases. Similar conclusions could also be derived from the work of Fuller and Walters (200). These workers found that kynuramine oxidation by rat liver monoamine oxidase was inhibited by dopamine, noradrenaline, phenethylamine, phenylbutylamine, serotonin and tyramine. Although all these amines were substrates for the enzyme there was no consistent relationship between

the degree of inhibition and the efficacy as a substrate which is an indication that these amines could not have a common point of attachment on the enzyme. The best activity was obtained when kynuramine, phenylbutylamine and phenethylamine were used as substrates for liver monoamine oxidase. Lower activities were recorded for tyramine, dopamine, noradrenaline and serotonin. Other papers also report that in vitro (201,202) monoamine oxidase activity was slow for the catecholamines. It follows from these observations that in hyperthyroidism monoamine oxidase activity may be affected differently depending on the substrate. It has been assumed by several authors that a decrease in monoamine oxidase activity can be taken as an indication that the catecholamines in hyperthyroidism are oxidized more slowly. But this conclusion, in view of the recent developments casting doubt on the homogeneity of monoamine oxidase, cannot warrant certainty unless the oxidation of the catecholamines themselves is measured and studied with livers of hyperthyroid and control animals. However, because the livers of hyperthyroid animals cannot oxidize as readily as the liver of control animals all aromatic amines so far tested (tyramine, tryptamine, serotonin and kynuramine) it is highly probable that this is also the case for the catecholamines. The mechanism by which the thyroid hormone lowers monoamine oxidase activity

in the liver is not at the present time known. It may be recalled (page 47) that Zilla and Lardy elucidated the possibility of a direct effect of thyroxine and its analogues on the enzyme. They have also showed that no inorganic inhibiter was present in the liver homogenates. But the possibility remains that some other compound for example a protein may effect monoamine oxidase and be responsible for its low activity. The profound effects of thyroxine on protein synthesis has already been mentioned and add support to this view.

The results do not demonstrate any effect of vitamin B_{12} on catechol-O-methyl transferase both *in vitro* and *in vivo*. *In vitro*, the vitamin or methylcobalamin could definitely not affect the activity of a semi-purified preparation of the enzyme. Some reservations must be made on the *in vivo* results; there is a possibility that the rats may have not been strictly deprived of vitamin B_{12} since the latter vitamin is synthesized in the intestine by bacteria and may have been absorbed therein. Also, although the rats were placed in cages with raised screened bottoms they could have practiced coprophagy (191). Generally speaking, it may be expected that very little vitamin may be required as a cofactor for the biosynthesis of enzyme macromolecules and therefore these results should not be taken as final evidence against the participation of the

vitamin in the methylating activity of catechol-O-methyl transferase.

It was a surprise to find that vitamin B_{12} supplementation significantly decreased hepatic monoamine oxidase activity by 18-20%. A direct inhibiting effect of the vitamin has to be excluded since *in vitro* B_{12} had no effect on the enzyme. The mechanism by which this effect is brought about is difficult to understand. In the literature, no report was found which dealt with the effect of vitamin B_{12} on monoamine oxidase activity but similar results were observed by Williams *et al* (203) for choline oxidase. Since they were using a manometric technique for the enzymatic assay they could attribute, at least partly, this decrease in the liver of the B_{12} treated rats to a 'competition between choline and endogenous substrate oxidation for common hydrogen transport systems'. This reason cannot hold in our case since kynuramine was the sole source of substrate. Williams *et al* (204) had also previously observed that folic acid deficiency increased the activity of D-amino acid oxidase and it appears that vitamin deficiencies are not always associated with a decrease in enzymatic activity as has often been found.

In our case one explanation which may account for the observed effect of B_{12} on monoamine oxidase activity concerns the possible involvement of sulphydryl groups in

the mechanism of action of this enzyme. In vitro, it has been found (205) that dimercaprol, thioglycollic acid, cysteine and cystine can decrease monoamine oxidase activity. Sourkes (206), has interpreted these findings to mean that sulphydryl groups are functionally concerned in the enzymatic activity through a catalytic cycle comprising alternate reduction and oxidation. Excess of -SH compounds or disulphide may then be expected to disturb this cycle. In other experiments performed by Nasbekar et al (207) it was shown that carbon tetrachloride decreased the levels of vitamin B_{12} in the liver and this was accompanied by a corresponding decrease in the amount of liver sulphydryl components. Similarly O'Dell et al (208) have reported that in the liver of new born vitamin B_{12} deficient rats there was a decrease in non-protein-SH compounds. Possibly then the accumulation of SH substances in the liver of the B_{12} treated rats may have contributed in lowering the monoamine oxidase activity of our B_{12} treated rats in a way comparable to that found in vitro. However this hypothesis is hard to reconcile with another finding of Nasbekar et al (209) that thyroid feeding in rats reduced the levels of liver and blood glutathione in which case we should have obtained an increase in the liver monoamine oxidase activity of the hyperthyroid rats if it is supposed that these compounds inhibit the enzyme. This was not the case and the

possibility that glutathione may enhance monoamine oxidase activity whereas other -SH substances may have a converse action cannot be considered seriously unless more information is obtained on the nature of the SH groups on the enzyme and the effect of glutathione on monoamine oxidase. Also thyroid hormone may exert an inhibiting action on monoamine oxidase by means which have nothing to do with the presence or absence of SH compounds. Because hyperthyroidism and vitamin B_{12} caused similar effects on monoamine oxidase it is tempting to postulate some kind of common mechanism by which this effect is brought about but the results as such do not lend support for any such hypothesis nor do they prove that this is so.

CATECHOLAMINE CONTENT OF LIVER, HEART AND BRAIN OF RATS UNDER EXPERIMENTAL CONDITIONS.

In the liver:

Despite the decrease in monoamine oxidase of the liver of hyperthyroid and B_{12} treated rats no changes in the catecholamine content of the liver of these animals was found. Not much data on the catecholamine content of liver in hyperthyroidism is given in the literature. Not much attention has been given to the catecholamine stores of the liver in hyperthyroidism. Using biological methods Holtfelt

(73) reported moderate increases in both noradrenaline and adrenaline in thyroidectomized rats injected with thyroxine, the values are reported as µg per g. tissue and as µg per kg body weight. But Leduc et al (76) did not observe any difference in the noradrenaline and adrenaline content of livers obtained from rats fed on iodinated casein diet as compared to the liver of those fed a normal diet. The latter workers employed a colorimetric method for the assay of catechol amines and expressed their results per mg of nitrogen. With our experimental conditions, we did not find any changes in the catecholamine content of the liver of hyperthyroid rats. Perhaps the methodology or experimental conditions used by Nohfelt may account for the discrepancy in results. Our method was not less sensitive than that used by Nohfelt since he had to pool several samples in order to obtain sufficient activity for assay which was not our case. It could also be that his biological method was not as specific as the one we have employed.

This absence of change in the catecholamine cannot be taken as an indication that monoamine oxidase is not concerned in the physiological effects of the thyroid hormone mentioned earlier in this thesis. It must be emphasized that the amount of catecholamine which could make the difference in physiological response between a normal and a hyperthyroid animal may be expected to be

extremely small. It may be recalled here from the experiments of Trendelenburg (page 9) that the hyperglycaemic response in normal animals which was caused by 85µg/kg of adrenaline could be reproduced in hyperthyroid rabbits with 65µg/kg, which makes a 20µg/kg difference between both groups of animals, or 2µg/100g. In the rat even a smaller amount must be effective and by the time the adrenaline has reached the target organ the effective amount could be infinitesimal. Thus, inactivation of monoamine oxidase could be responsible for the accumulation of amounts of adrenaline which are physiologically effective but which could not be detected by the present methods employed however sensitive they may appear to be. That such a possibility is likely follows from the finding of Leduc et al (76) for only when adrenaline is injected in hyperthyroid and normal animals can greater amounts of adrenaline be detected in the livers of the hyperthyroid rats.

However, an alternative explanation may account for many of the physiological and biochemical effects of thyroxine. In the adrenals the catecholamines are stored in chromaffin granules (194) and noradrenaline is also kept in sympathetic vesicles (219,220). Because these hormones are highly active it is necessary that they be kept at all times in forms which will prevent them to act randomly on their respective receptors. It is logical to assume that in the liver the

catecholamines also exist mainly in bound inactive forms inaccessible to monoamine oxidase. In hyperthyroidism then, the lower monoamine oxidase activity may not necessarily mean an increase in active free catecholamine. The usual symptoms of hyperthyroidism could be caused by some other action of thyroxinesuch as on sympathetic activity. But the potentiating action of thyroxineon the glycogenolytic effect of catecholamine that is the greater per cent increase in response to injected free catecholamine may result from a slower inactivation of these free catecholamines by monoamine oxidase of the liver. In vivo, it has been found (186,187) that adrenaline increases the metabolic rate of the liver. Because the liver has a role of central importance in the general metabolism of animals it may be expected to contribute much to the overall O_2 consumption measured in animals. Thus the well known potentiating action of thyroxineon the calorigenic effect of adrenaline could also be due to poor inactivation of the catecholamines in the liver of hyperthyroid animals.

B_{12} supplementation did not affect the catecholamine content of this tissue. D'Iorio and Flaut (74) noticed that the observed decrease in the adrenaline content of the adrenals as a result of hyperthyroidism could be corrected by administration of vitamin B_{12}. But they expressed their results per mg nitrogen and no such effects of vitamin B_{12}

(personal communication from Dr. D'Iorio) or hyperthyroidism (76) were observed when the values are expressed differently.

In the heart

Numerous reports deal with the effect of hyperthyroidism on the cardiac stores of catecholamine, the results of which are not very consistent. In those cases where a decrease in catecholamine concentration of hearts was found in hyperthyroidism perhaps not enough attention has been given to the hypertrophic action of thyroxine on the heart (114, 52) which can be noted Table 3. It follows that when the results are reported per gram, lower values are obtained in the hypertrophied hearts which is not necessarily a valid indication that thyroxine is concerned with an authentic decrease. However, a low catecholamine content in hearts of hyperthyroid animals has not been a general finding. Examination of data provided by Hokfelt (73) shows that the noradrenaline concentration of hearts obtained from thyroidectomized rats treated with thyroxine was higher than that of normal hearts. The same result was also found for adrenaline and the concentration of both catecholamines was lower in the hearts of thyroidectomized rats. Raab (210) claimed that injections of thyroxine into normal animals increased the myocardial content of "free" adrenaline, but

the method used for the estimation of "free" adrenaline was unorthodox; it depended on the relative amounts of ascorbate to adreno-cortical hormones. In sheep, Goodal (75) noted a decrease in the adrenaline content of hearts obtained from hyperthyroid animals but from thyroidectomized sheep also. Noradrenaline, in the hearts of both thyroidectomized and hyperthyroid animals was increased by 72% when a biological method was used for the estimation but this increase was only 25% when a colorimetric method was used. It is difficult to draw conclusions from these results since the values are given as µg per gram and the significance of these values is not given statistically. In the case of Leduc et al (76), the data show that the noradrenaline content of hearts of hyperthyroid rats as reported per mg nitrogen is lower by 36%, but the adrenaline is elevated by 47%.

Paradoxical results are even obtained within the same paper. Mulard et al (211) reporting their values per g tissue obtained no difference in the noradrenaline content of hearts obtained from hyperthyroid animals but demonstrated a decrease in the amount of this catecholamine in both the atria and ventricles when these were analyzed separately. Adrenaline was greatly increased in the whole heart of hyperthyroid animals but these differences were not statistically significant; adrenaline was significantly

decreased in the auricles but remained unchanged in the ventricles of hyperthyroid animals.

Goodkind et al (195) failed to detect any changes in the catecholamine content of the auricles of guinea-pigs given injections of 50µg or 100µg of triiodothyronine daily for 10 days although these animals had experienced a loss in weight as a result of those injections. The only significant increase in auricular catecholamines could be produced by injecting the guinea-pigs with 400µg of triiodothyronine for four days in which case the animals had lost much weight. Again values are expressed per g tissue. But in another paper (212) it is shown that the total cardiac catecholamines in ug per g was significantly decreased (29%) in rats which had been injected with 500µg of thyroxine daily for fourteen to twenty days. Finally, Barker and Matiuchi (114) found that when rats were injected with thyroxine (100µg/kg/day for twenty days) the size of hearts and adrenals had increased but there was no significant change in the amount of noradrenaline in the heart of these animals when the values were expressed per heart.

One cannot help but remain perplexed with this mass of contradictory information. It is noteworthy that the authors of each paper have used different methods for the determination of catecholamines. Species difference may

also account for these discrepant results. In the rat, however, there is general indication that hyperthyroidism causes a lowering of myocardial noradrenaline when this catecholamine is expressed as µg per gram tissue and this is in accordance with our own findings (36% decrease). But when the noradrenaline values are reported per heart (114) this difference disappears as we have also observed. In all probability then, these differences are brought about by cardiac hypertrophy resulting from hyperthyroidism and it is unlikely that the thyroid hormone causes any direct significant changes in the noradrenaline stores of the heart of rats. In the case of adrenaline, in the rat, increases are reported by Hokfelt and Leduc et al and obviously this effect of the thyroid hormone cannot be attributed to changes in heart size. But this finding is not generally accepted (Goodal) and our own results do not lend support to the contention that adrenaline is significantly modified in the heart of hyperthyroid rats.

Thus our experiments on the catecholamine content of hearts obtained from hyperthyroid rats do not provide evidence that the cardio-vascular effects of the thyroid hormone are due to disturbances in the total myocardial stores of catecholamines. Truly, this kind of experiments gives no information as to the relative amounts of free and bound catecholamine. During recent years, much work has been done

to demonstrate the existence of several pools of noradrenaline in the heart probably in equilibrium one with the other, (213, 214, 215, 216). There is a possibility that the thyroid hormone may affect the equilibrium of these different pools resulting in a concomitant increase in free catecholamine although the total amount of catecholamine may remain unchanged. In agreement with this idea, are the findings of Wurtman et al (56) that the hearts of hyperthyroid rats cannot bind as much free catecholamine as the hearts of euthyroid controls. Beaven et al (217) also showed that when H^3-noradrenaline was injected in euthyroid and thyrotoxic mice, 15% less radioactive noradrenaline was found in the hearts of the thyrotoxic mice. In vitro (129) Dengler et al could demonstrate that hearts slices obtained from hyperthyroid rats did not accumulate as much noradrenaline as did heart slices from normal rats. Research along these lines may perhaps provide some answer to the mechanism of action of thyroxine on the heart.

Another aspect of the question tackled by earlier workers c.f. page 2 and neglected subsequently concerns a possible effect of thyroxine on parasympathetic activity. Because the thyroid hormones mimic the effects of catecholamine all attention has been diverted towards sympathetic activity but parasympathetic activity is not unrelated to sympathetic activity and modification of the former by

thyroxine may be a means by which thyroxine exerts sympathetic effects.

We have not determined monoamine oxidase and catechol-O-methyl transferase activities in the heart but in all evidence from previous works (page 48, 55) an explanation of the cardio-vascular effects of thyroxine cannot be sought through an inhibiting action of thyroxine on these myocardial enzymes.

It then becomes very difficult to explain the effects of thyroxine on the isolated heart (37-41) and the observed potentiating effects of thyroxine in rats induced with total sympathetic block (page 42). The only resource for these local effects of thyroxine would then reside in an effect of thyroxine on the binding capacity of catecholamine or a direct action of thyroxine on the tissue (127).

In the Brain

To the author's knowledge no reports are given which deal on the brain catecholamine content of hyperthyroid animals. In view of the surmised theory that thyroid hormone increases sympathetic activity it would have been interesting to demonstrate changes in the noradrenaline content of this organ as a result of hyperthyroidism but we could not find any effect.

CONCLUSION

The results presented so far demonstrate further that the mechanism of action of the thyroid hormone is complex. A decrease in the liver monoamine oxidase activity of hyperthyroid rats was observed in conditions which were indicative of adequate protein synthesis. The hypothesis emitted by Hurtman *et al* (54) that a lowering of monoamine oxidase or catechol-O-methyl transferase in hyperthyroidism may be the result of general decrease in protein synthesis is not tenable at least in the case of the former enzyme.

Thus our results are in accordance with the concept that lowering of liver monoamine oxidase activity by thyroxine may account for the following effects of the thyroid hormone: 1) the decrease in the liver glycogen, 2) the increase in O_2 consumption, 3) the sensitization of the hyperglycemic action of adrenaline, and 4) the potentiating effect on the calorigenic action of adrenaline.

Catechol-O-methyl transferase does not appear to play an important part in these manifestations.

The cardio-vascular symptoms observed in hyperthyroidism cannot be accounted for by disturbances in the cardiac stores of catecholamine. Results from other workers on the level of the catabolic enzymes of the heart of hyperthyroid rats are inconclusive, and the mechanism by which

these effects are brought about remains obscure.

Both *in vitro* and *in vivo* results are not consistent with a possible participation of vitamin B_{12} or its derivatives in the activity of catechol-O-methyl transferase.

In vivo findings demonstrate a lowering effect of vitamin B_{12} on liver monoamine oxidase levels similar to that observed with hyperthyroid rats. *In vitro* experiments exclude a direct action of the vitamin on the enzyme and strengthens the probability of an indirect action of the vitamin.

The theory that the thyroid hormone increases sympathetic activity by increasing the amount of noradrenaline released by each impulse (59) is not supported by our results with brain where no changes in the noradrenaline content of this organ could be noticed.

Part 2

RESULTS

Preparation of rats

Black and white hypophysectomized and intact control rats were obtained from the Quebec Breeding Farm. Hypophysectomy was performed on rats weighing approximately 230 grams. One series of hypophysectomized and normal rats of the same age were sacrificed three and eight weeks after hypophysectomy. Other groups of hypophysectomized rats were injected with thyroxine, growth hormone, thyroxine plus growth hormone.

In the first series of experiments all rats were given a 5% sucrose solution as drinking fluid to increase the rate of survival of hypophysectomized rats. Rats were fed a normal diet ad libitum unless otherwise stated.

Approximately seven weeks after hypophysectomy one group of rats received subcutaneous injections of thyroxine as presently described. Thyroxine (Sigma Chemical Company) was dissolved in a slightly alkaline saline solution and injected according to the following schedule:

0.0125mg daily for 5 days, equivalent to 0.0625mg

0.0250mg daily for 7 days, equivalent to 0.1750mg

0.0500mg daily for 8 days, equivalent to 0.4000mg

0.1000mg daily for 6 days, equivalent to 0.6000mg

for a total of 1.2370mg.

Another group of hypophysectomized rats received intraperitoneal injections of growth hormone. Growth hormone was obtained from the National Institute of Health and was also dissolved in a slightly alkaline saline solution. 0.5mg was injected in the morning and 0.5mg was injected in the afternoon (daily dose 1mg) for twenty six consecutive days. Hypophysectomized rats to be given both hormones were injected with the same doses mentioned above.

Normal rats were fasted (at the time indicated in the tables) so as to have animals of weights comparable with the experimental animals. Fasting was introduced by gradually decreasing the daily supply of food from 20 grams to 5 grams, for a length of time also indicated in the tables. However, this fasting treatment did not produce marked effects because the normal rats, while fasted, still received a 5% sucrose solution. A second series of experiments was repeated omitting sucrose from the drinking water.

In the second series, thyroxinewas injected as follows:

0.0125mg daily for 3 days, equivalent to 0.0375mg

0.0250mg daily for 4 days, equivalent to 0.1000mg

0.05000mg daily for 18 days, equivalent to 0.900mg

for a total of 1.0375mg.

Growth hormone was injected as for the first series but for only sixteen days since we exhausted our limited reserve of the hormone. Also, the rats given both hormones were so treated for sixteen days only and thyroxine was administered as indicated above with a total of 0.0625mg.

All rats were given ordinary water as drinking fluid.

Approximately one week before sacrifice, an electrocardiogram was performed on each rat under light ether anaesthesia and the heart rate was calculated from this record.

WEIGHTS OF DIFFERENT ORGANS OBTAINED FROM NORMAL RATS, HYPOPHYSECTOMIZED RATS AND HYPOPHYSECTOMIZED RATS TREATED WITH THYROXINE, GROWTH HORMONE, THYROXINE PLUS GROWTH HORMONE.

The main object of this part of the work consisted in obtaining some information on the catecholamine content of certain organs obtained from rats subjected to various experimental conditions. However, hypophysectomy causes profound disturbances, in the organ weight of rats subjected to this operation. Some of these changes were corrected by administration of thyroxine, growth hormone, or both. Although many of these effects have already been acknowledged in the literature, they still constitute some point of interest especially in the context of this work where the various

Table 17

WEIGHTS OF VARIOUS ORGANS OBTAINED FROM HYPOPHYSECTOMIZED AND NORMAL RATS †

Normal Rats

Number of rat	Body Weight	Liver		Heart		Brain	Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	290	9.93	3.42	.89	.31	1.45	.50	52.0	17.93	3.19	1.10
2	287	10.82	3.77	.84	.29	1.59	.55	47.3	16.5	3.26	1.13
3	304	11.42	3.76	.85	.28	1.75	.57	43.4	14.3	3.59	1.18
4	316	12.72	4.02	1.03	.32	1.61	.51	56.6	17.9	3.34	1.06
5	290	10.02	3.45	.91	.31	1.73	.59	47.9	16.5	3.39	1.17
6	263	8.83	3.36	.80	.30	1.65	.63	33.9	12.9	3.05	1.16
Mean	291±18*	10.62±1.35	3.63±.26	.88±.08	.30±.02	1.62±.10	.56±.05	46.8±7.8	16.0±2.0	3.30±.59	1.13±.05

Three Weeks× Hypophysectomized Rats

Number of rat	Body Weight	Liver		Heart		Brain	Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	172	4.73	2.75	.47	.27	1.54	.89	13.5	7.84	0.47	.27
2	185	5.70	3.08	.47	.25	1.28	.69	12.5	6.75	0.98	.53
3	159	3.90	2.45	.45	.28	1.42	.89	12.1	7.61	0.64	.40
4	180	4.63	2.57	.45	.25	1.60	.89	9.4	5.22	0.82	.45
5	167	4.33	2.59	.46	.27	1.59	.95	12.0	7.18	0.94	.56
6	202	5.97	2.95	.53	.26	1.63	.81	11.2	5.54	0.88	.43
7	192	5.15	2.68	.51	.26	1.59	.83	14.4	7.50	1.03	.54
8	201	5.55	2.76	.51	.25	1.65	.82	14.9	7.41	1.35	.67
Mean	182±16	4.99±.72	2.72±.21	.48±.03	.26±.01	1.53±.13	.84±.08	12.5±1.4	6.88±.99	.88±.28	.48±.25
% Change	-37%	-53%	-25%	-45%	-12%	-5%	+51%	-73%	-132%	-73%	-57%
Significance	P<.01	P<.01	P<.01	P<.01	P<.01	P>.1	P<.01	P<.01	P<.01	P<.01	P<.01

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
× Time after hypophysectomy

weight changes may present some problem in the evaluation of data pertaining to the catecholamine content of tissue. For this reason, the various changes in organ weight observed in the different groups of rats will be considered first.

In the following tables all values are reported in terms of both the absolute and relative weight. The latter mode of expression refers to the weight of a particular organ in relation to body weight (g/100g body wt.). The normal rats chosen for comparison are, in all cases, of the same age as the experimental rats.

The first series will be considered. In this series all rats were given 5% sucrose as drinking fluid.

Table 17 shows the body weights and various organ weights obtained from rats three weeks after hypophysectomy compared to those of normal rats. It is readily apparent that significant decreases occurred in the body weight and various organ weights of hypophysectomized rats in all tissues except the brain. The decrease in organ weights not only accompanied the decrease in body weight but exceeded it, and consequently there is also a decrease in the relative weight of the organs affected. This decrease in the relative weight however, has not been a general finding for the liver. The adrenals and testes were the most affected by removal of the pituitary gland whereas the

Table 18

WEIGHTS OF VARIOUS ORGANS OBTAINED FROM HYPOPHYSECTOMIZED AND NORMAL RATS †

Normal Rats

Number of rat	Body Weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/kg body wt.	g	g/kg body wt.	g	g/kg body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	238	6.48	2.72	.83	.35	1.82	.76	62.4	26.2	3.28	1.38
2	275	9.47	3.44	.93	.34	1.82	.66	69.7	25.3	3.43	1.25
3	280	8.78	3.13	.81	.29	1.66	.59	63.0	22.5	3.63	1.30
4	282	8.34	2.95	.92	.33	1.68	.59	55.3	19.6	3.35	1.19
5	272			.96	.35	1.39	.51	50.5	18.6	4.27	1.57
Mean	268±18*	8.26±1.3	3.06±.30	.88±.072	.33±.02	1.66±.17	.62±.09	60.1±7.4	22.4±3.4	3.59±.40	1.33±.147

Eight Weeks× Hypophysectomized Rats

Number of rat	Body Weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	162	4.54	2.80	.48	.29	1.56	.96	8.8	5.43	0.41	.25
2	164	5.05	3.10	.51	.31	1.47	.89	10.8	6.58	0.40	.24
3	194	6.21	3.20	.49	.25	1.71	.88	12.9	6.64	0.45	.23
4	157	3.92	2.49	.47	.30	1.70	1.08	10.8	6.87	0.49	.31
5	163	5.53	3.39	.49	.30	1.59	.97	10.7	6.56	0.33	.20
Mean	169±15	5.05±.88	2.99±.38	.48±.02	.29±.02	1.60±.10	.96±.08	10[illegible]±1.46	6.41±.565	.41±.06	.24±.043
% Change	-37%	-38%	-2%	-45%	-11%	-3%	+53%	-82%	-71%	-89%	-82%
Significance	P<.01	P<.01	P>.5	P<.01	P<.01	P>.4	P<.01	P<.01	P<.01	P<.01	P<.01

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
× Nine after hypophysectomy

Table 19

WEIGHTS OF VARIOUS ORGANS OBTAINED FROM NORMAL FASTED† RATS‡

Number of rat	Body Weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	291	7.86	2.70	.83	.28	1.74	.60	40.1	13.8	3.48	1.19
2	270	8.34	3.08	.85	.31	1.77	.65	50.3	18.6	3.42	1.27
3	271	7.45	2.75	.81	.30	1.93	.71	42.5	15.7	3.31	1.23
4	314	10.26	3.26	.97	.31	1.64	.52	41.1	13.1	3.45	1.10
5	305	8.77	2.87	.69	.22	1.80	.59	43.6	14.4	3.48	1.14
6	295	8.23	2.79	.84	.28	1.74	.59	46.2	15.6	3.15	1.10
7	319	8.47	2.65	1.01	.32	1.72	.54	58.1	18.2	3.86	1.21
8	298	7.66	2.57	.87	.29	1.85	.62	44.0	14.8	3.68	1.23
9	278	8.78	3.16	.85	.30	1.82	.65	41.8	15.0	3.17	1.14
10	290	9.07	3.12	.90	.31	1.78	.61	47.6	16.4	3.36	1.16
11	312	8.73	2.79	.96	.31	1.74	.56	42.0	13.5	3.40	1.10
12	226	6.22	2.75	.90	.40	1.76	.78	35.4	15.7	2.92	1.29
13	307	8.26	2.69	.91	.29	1.68	.55	45.0	14.6	3.30	1.07
14	300	8.55	2.85	1.08	.30	1.89	.63	52.0	16.9	3.17	1.06
15	295	7.55	2.56	.87	.29	1.81	.61	50.5	17.1	4.27	1.45
16	318	9.03	2.83	.91	.29	1.78	.56	37.5	11.8	3.34	1.05
17	271	7.56	2.78	.83	.31	1.86	.63	46.0	16.9	3.01	1.11
18	276	7.39	2.67	1.03	.20			50.9	18.4	3.26	1.18
19	297	7.42	2.49					42.5	14.3	3.17	1.07
Mean	291±7*	8.18±.87	2.90±.21	.89±.09	.29±.03	1.78±.06	.61±.06	45.1±5.8	15.5±1.9	3.38±.30	1.16±.10

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
‡ All rats were fasted for 28 days

heart and liver were less affected.

It may be seen from Table 18 that eight weeks after hypophysectomy no further change occurred. The adrenals and testes were atrophied to approximately the same extent as before and a similar observation may be made for the hearts. The relative weight of the liver however was not significantly different than normal and only a decrease in the absolute weight of this tissue is apparent. The absolute weight of brain was again not significantly different than normal and fluctuations in the weight of this tissue were not observed subsequently. Thus, the observed changes in the relative weight of brain are unimportant since they merely reflect changes in body weight.

The effect of hormonal treatment will now be considered. In each table, exception made for the adrenals and testes, the values obtained in the different hormone groups are compared to two sets of values, the normal values listed in Table 19 and the values obtained from eight weeks hypophysectomized rats. The results of these comparisons are recorded at the bottom of each table. The reason for this double comparison arises from the fact that the normal rats of the same age as the experimental rats were fasted in order to facilitate comparison in catecholamine content between an experimental organ of a same weight as normal. Thus, it was felt that a better evaluation of

Table 20

WEIGHTS OF VARIOUS ORGANS OBTAINED FROM HYPOPHYSECTOMIZED RATS TREATED WITH THYROXINE†•

Number of rat	Body weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	152	5.98	3.93	.65	.43	1.58	1.04	10.7	7.00	0.28	.18
2	179	6.51	3.63	.73	.41	1.80	1.00	7.4	4.13	.31	.17
3	145	5.44	3.75	.55	.38	1.71	1.18	3.4	2.34	.25	.17
4	155	5.71	3.68	.78	.50	1.81	1.17	8.7	5.61	.29	.19
5	172	6.30	3.66	.72	.42	1.60	.93	11.0	6.39	.36	.20
6	158	6.06	3.83	.65	.41	1.76	1.11	8.0	5.06	.30	.19
7	200	6.96	3.48	.77	.38	1.68	.84	9.7	4.85	.43	.21
8	174	6.01	3.45	.76	.44	1.78	1.02	12.0	6.89	.31	.18
9	150	5.29	3.53	.63	.42	1.77	1.18	6.8	4.53	.30	.20
10	181	6.65	3.67	.75	.41			9.4	5.19	1.14	.63
Mean	170 ± 18*	6.25 ± .55	3.66 ± .15	.69 ± .07	.42 ± .03	1.72 ± .08	1.05 ± .12	8.7 ± 2.4	5.20 ± 1.44	.39 ± .26	.23 ± .11
% Change Δ	-41%	-23%	+30%	-22%	+42%	-3%	+72%	-92%	-66%	-87%	-80%
Significance	P<.01	P<.01	P<.01	P<.01	P<.01	P>.02	P<.01	P<.01	P<.01	P<.01	P<.01
% Change ▲	+1%	+23%	+15%	+43%	+56%	+8%	+22%	–	–	–	–
Significance	–	P<.01	P<.01	P<.01	P<.01	P>.02	P>.1				

* Standard deviation
Δ Compared to normal values listed Table 19
▲ Compared to eight weeks hypophysectomized rats listed Table 18
† All rats were given 5% sucrose as drinking fluid
• 1.237mg of thyroxine injected over a period of 26 days

Table 21

WEIGHTS OF VARIOUS ORGANS OBTAINED FROM HYPOPHYSECTOMIZED RATS TREATED WITH GROWTH HORMONE†°

Number of rat	Body Weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	244	9.57	3.92	.61	.25	1.65	.68	12.2	5.00	.97	.39
2	215	6.88	3.20	.70	.32	1.83	.85	5.6	2.60	.87	.40
3	207	7.76	3.74	.54	.26	1.60	.77	14.9	7.19	.41	.20
4	205	7.01	3.41	.59	.29	1.79	.87	10.1	4.92	.53	.26
5	214	7.09	3.31	.61	.28	1.76	.82	11.1	5.18	.49	.23
6	231	7.71	3.33	.57	.25	1.74	.75	11.6	5.02	.58	.25
7	193	6.69	3.46	.57	.29	1.65	.85	12.2	6.32	.34	.18
8	191	7.14	3.73	.54	.28	1.61	.84	8.8	4.61	.62	.33
Mean	219±19*	7.75±.96	3.51±.25	.59±.05	.28±.02	1.70±.09	.80±.07	10.8±3.0	5.10±1.32	.60±.22	.28±.08
% Changes	-24%	-5%	+25%	-33%	-5%	-4%	+31%	-76%	-66%	-82%	-76%
Significance	P<.01	P>.1	P<.01	P<.01	P>.05	P>.02	P<.01	P<.01	P<.01	P<.01	P<.01
% Change▲	+30%	+53%	+17%	+23%	-4%	+6%	-16%	—	—	—	—
Significance	P<.01	P<.01	P<.01	P<.01	P>.05	P>.05	P<.01				

* Standard deviation
▲ Compared to normal values listed Table 19
▲ Compared to eight weeks hypophysectomized rats listed Table 18
+ All rats were given 5% sucrose as drinking fluid
° 1mg of growth hormone injected daily for 26 days

the different hormonal treatments could be obtained by comparison with hypophysectomized rats. Comparison to normal values, on the other hand, is still of practical value.

Table 20 shows the effect of thyroxine on the weights of various organs obtained from hypophysectomized rats. In considering the percent change compared to eight weeks hypophysectomized rats it is apparent that thyroxine stimulated the growth of liver and heart, the greatest effect being noticeable on the heart. These effects also appear to be specific to these organs since thyroxine had no effect on body weight. It follows that thyroxine increased the relative as well as the absolute weights of these tissues. This increase in relative weight even exceeded the normal range as can be judged when the percent change is compared to normal values. However, the absolute weight of liver and heart of the hypophysectomized group treated with thyroxine is still below normal. No important change can be noted in the brain weight and the adrenals and testes remained atrophied as to be expected.

The effect of growth hormone may be observed in Table 21. Like thyroxine growth hormone appears to increase the liver and heart weight of hypophysectomized rats. However, this effect does not appear to be as specific as for thyroxine, at least in the case of the heart where the

Table 22

WEIGHTS OF VARIOUS ORGANS OBTAINED FROM HYPOPHYSECTOMIZED RATS TREATED WITH GROWTH HORMONE AND THYROXINE†⊙

Number of rat	Body Weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	187	7.14	3.81	.83	.44	1.89	1.01	11.0	5.88	.45	.21
2	175	6.62	3.78	.77	.44	1.65	.94	8.9	5.1	.71	.41
3	206	7.49	3.63	.97	.47	2.01	.97	11.0	5.3	1.69	.78
4	186	6.32	3.39	.83	.44	1.75	.94	12.2	6.5	.50	.27
5	184	6.65	3.61	.83	.45	1.89	1.02	9.2	5.0	1.33	.72
6	209	8.98	4.29	1.04	.49	1.75	.83	9.9	4.7	.52	.25
7	197	7.54	3.82	.96	.48	1.86	.94	13.0	6.6	.63	.32
8	170	5.94	3.49	.72	.43	1.76	1.03	7.6	4.5	.31	.18
9	229	8.67	3.78	1.03	.45	1.77	.77	8.7	3.8	1.14	.50
10	184	7.11	3.86	.84	.46	1.95	1.05	8.3	4.5	.57	.31
11	206							12.7	6.2	.44	.21
Mean	210±24*	6.83±1.3	3.75±2.5	.88±.11	.46±.023	1.83±.11	.95±.09	10.2±1.85	5.2±.94	.74±.425	.37±.21
% Change▲	-27%	-1%	+33%	-1%	+54%	+2%	+55%	-77%	-66%	-78%	-68%
Significance	P<.01	P>.5	P<.01	P>.5	P<.01	P>.1	P<.01	P<.01	P<.01	P<.01	P<.01
% Change▲	+25%	+59%	+27%	+83%	+71%	+10%	⊙	—	—	—	—
Significance	P<.01	P<.01	P<.01	P<.01	P<.01	P<.01	—				

*Standard deviation
▲Compared to normal values listed Table 19
▲Compared to eight weeks hypophysectomized rats listed Table 18
†All rats were given 5% sucrose as drinking fluid
⊙1.237mg of thyroxine and 26mg of growth hormone injected concurrently over a period of 26 days.

increase in absolute weight of this tissue parallels the increase in body weight caused by the administration of growth hormone. Thus no significant change can be noted in the relative weight of the heart. On the other hand, the stimulatory effect of growth hormone on the liver was greater than the 30% increase in body weight and this resulted in a significant increase (+17%) in the relative weight of this organ which again exceeded that obtained for normal rats (+25%). Although the absolute liver weight is not significantly different than normal the absolute heart weight is still significantly lower than normal. No change can be noted for the other organs.

When both thyroxine and growth hormone are given to hypophysectomized rats it may be seen in Table 22 that except for the heart the effects are complementary. The percent increase in body weight is approximately the same as obtained with growth hormone alone, i.e. 25%. Also, the effects on both liver weights are the sum total of the effects taken separately. However, synergism can be noted in the absolute and relative heart weights where the effect is more than the sum total of the isolated effects and an 83% and a 71% increase is recorded in the absolute and relative weights respectively. It may be noted that the absolute liver and heart weights of this group compare favorably with those obtained for normal; whereas, the

Table 23

WEIGHTS OF VARIOUS ORGANS OBTAINED FROM HYPOPHYSECTOMIZED AND NORMAL RATS +

Normal Rats

Number of rat	Body Weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	254	8.51	3.35	.84	.33	1.49	.59	41.6	16.4	2.84	1.12
2	255	9.06	3.55	.75	.29	1.63	.64	36.4	14.3	1.73	0.68
3	258	10.00	3.87	1.05	.41	1.65	..64	49.8	19.4	3.40	1.32
4	246	8.27	3.36	.79	.32	1.56	.63	35.6	15.0	3.42	1.44
5	242	8.62	3.56	.71	.29	1.30	.54	39.6	16.4	3.38	1.40
6	253	9.93	3.92	1.03	.41	1.68	.69	41.4	16.4	3.20	1.26
Mean	251±6 *	9.06 ± .23	3.60±.25	.86±.14	.34±.05	1.55±.14	.62±.05	40.7±5.1	16.1±1.9	2.99±.66	1.20 ±.28

Three Weeks× Hypophysectomized Rats

Number of rat	Body Weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	191	6.61	3.46	.55	.29	1.53	.80	9.9	5.4	.77	.42
2	185	5.94	3.21	.55	.30	1.57	.85	12.1	6.7	1.60	.89
3	172	6.99	4.06	.46	.27	1.23	.72	11.1	6.5	.68	.40
4	172	5.81	3.38	.45	.26	1.58	.92	12.2	7.3	1.18	.70
5	199	7.23	3.63	.58	.29	1.51	.76	13.4	6.8	1.25	.63
6	189	6.16	3.22	.51	.27	1.58	.83	12.4	6.6	1.15	.61
7	191							12.6	6.6	1.18	.62
Mean	184±10	6.45 ±.18	±3.49±.32	.53±.05	.28±.01	1.50±.14	.81±.07	11.9±1.1	6.5±.33	1.11±.31	.61±.16
% Change	−26%	-28%	−3%	−38%	−19%	−3%	+30%	−70%	−59%	-82%	−50%
Significance	P<.1	P<.01	P>.5	P<.01	P<.02	P>.5	P<.01	P<.01	P<.01	P<.01	P<.01

*Standard deviation
+All rats were given ordinary water as drinking fluid
×Days after hypophysectomy

Table 24

WEIGHTS OF VARIOUS ORGANS OBTAINED FROM HYPOPHYSECTOMIZED AND NORMAL FASTED RATS†

Normal Fasted Rats‡

Number of rat	Body Weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.				
1	203	5.84	2.87	.61	.30	1.64	.81				
2	200	6.02	3.01	.54	.27	1.37	.68				
3	211	7.48	3.54	.58	.27	1.44	.68				
4	207	6.88	3.32	.64	.31	1.39	.67				
5	178	5.57	3.12	.52	.29	1.50	.84				
6	191	6.05	3.16	.64	.33	1.71	.89				
Mean	199±13*	6.30±.70	3.17±.27	.58±.05	.29±.02	1.50±.14	.76±.10				

Eight Weeks× Hypophysectomized Rats

Number of rat	Body Weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	195	5.57	2.86	.46	.23	1.72	.88	9.6	4.9	1.48	0.76
2	183	5.29	2.89	.43	.23	1.67	.91	7.6	4.2	0.53	0.29
3	184	4.94	2.68	.40	.22	1.59	.86	7.4	4.2	0.79	0.56
4	169	5.38	3.18	.46	.27	1.43	.85	6.2	3.7	.37	.22
5	151	3.98	2.63	.37	.24	1.53	1.01	6.2	4.1	.38	.25
6	177	5.51	3.11	.46	.26	1.64	.92	5.5	3.2	.45	.26
7	173	4.75	2.74	.44	.25	1.57	.91	5.9	3.4	.47	.27
8	167	4.92	2.95	.41	.25	1.47	.88	7.8	4.7	.51	.31
Mean	174±13	5.04±.52	2.88±.20	.42±.03	.24±.02	1.57±.10	.90±.05	7.0±1.35	3.5±.84	.64±.39	.36±.19
% Change	-12%	-20%	-9%	-27%	-17%	+4%	+18%				
Significance	P<.01	P<.01	P>.05	P<.01	P<.01	P>.4	P<.01				

*Standard deviation †All rats were given ordinary water as drinking fluid
‡Normal rats were fasted for 20 days
×Time after hypophysectomy

relative weight is above normal. A small but significant effect of both hormones can be noted on the brain of hypophysectomized rats.

Although the liver and heart weight in this group could be compared with the weights of livers and hearts from normal rats, we had not obtained a size of normal organs which was in the weight range of other groups, for instance, the hypophysectomized group. Evidently, the ingestion of sucrose may have prevented the effect of fasting. Accordingly, the whole series of experiments was repeated with rats given ordinary water as drinking fluid. The results obtained on the various organ weights of the second series will now be presented. Table 23 shows the values obtained for three weeks hypophysectomized rats. The results are essentially the same as obtained previously. There is a 26% decrease in the body weight of hypophysectomized animals and a corresponding 28% decrease in the absolute liver weight. Thus, the relative weight of liver is not different than normal. In the case of the heart, however, there is a decrease in the relative weight as well as in the absolute weight of this tissue. No change can be noted in the brain and as usual the adrenals and testes are considerably atrophied.

In Table 24 where the values obtained for eight weeks hypophysectomized rats are compared to normal, fasted rats,

Table 25

WEIGHTS OF VARIOUS ORGANS OBTAINED FROM NORMAL FASTED RATS ††

Number of rat	Body weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt	mg	mg/100g body wt.	mg	mg/100g body wt.
1	216	4.90	2.27	.61	.32	1.69	.68	39.6	18.0	3.10	1.40
2	228	5.76	2.53	.63	.29	1.58	.78	37.4	16.1	3.38	1.46
3	236	5.56	2.35	.66	.28	1.55	.72	38.4	16.3	3.33	1.41
4	247	5.77	2.34	.74	.32	1.57	.68	38.8	15.7	3.64	1.47
5	227	5.40	2.38	.82	.37	1.55	.68	33.6	14.8	3.03	1.34
6	225	5.21	2.31			1.54	.61	35.4	15.7	3.14	1.40
7	235	5.79	2.46			1.72	.79	33.4	14.2	3.33	1.42
8	233	5.88	2.52					35.6	15.3	3.86	1.65
9	240	5.75	2.39					36.8	15.3	3.28	1.37
10	227	5.94	2.62					37.8	16.7	3.72	1.64
11	238	5.41	2.27					45.4	19.1	3.38	1.42
12	218	6.05	2.77					37.4	17.2	3.49	1.60
13	203	4.32	2.62					24.4	12.0	3.24	1.60
14	191	4.57	2.39					38.4	20.1	3.27	1.71
15	230	6.20	2.69					35.0	15.2	3.55	1.54
Mean	226±14*	5.50±.83	2.46±.25	.69±.08	.32±.03	1.60±.07	.73±.05	36.4±4.4	16.1±3.9	3.38±.73	1.49±.12

*Standard deviation
†All rats were given ordinary water as drinking fluid
‡All rats were fasted for 30 days.

Table 26

WEIGHTS OF VARIOUS ORGANS OBTAINED FROM HYPOPHYSECTOMIZED RATS TREATED WITH THYROXINE†.

Number of rat	Body Weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	162	5.64	3.48	.71	.43	1.71	1.04	8.0	4.9	.34	.20
2	163	5.29	3.24	.70	.43	1.59	.93	6.4	3.9	.36	.22
3	160	5.75	3.59	.78	.48	1.58	1.09	6.2	3.9	.25	.16
4	159	6.27	3.94	.82	.44	1.62	1.00	7.6	4.8	.29	.18
5	185	6.65	3.59	.77	.45	1.72	1.07	7.4	4.0	.35	.19
6	175	6.04	3.45	.66	.38	1.80	1.11	6.4	3.7	.34	.19
7	160	5.46	3.41	.72	.45	1.74	1.07	7.4	4.6	.37	.23
8	170	6.13	3.60			1.68	1.12	7.8	4.6	.32	.19
9	152	5.82	3.83			1.73	1.14	5.2	3.4	.32	.21
10	160	5.70	3.56					7.6	4.8	.30	.19
11	150	5.57	3.71					5.2	3.5	.34	.23
12	162	5.96	3.68					8.4	5.2	.28	.17
13	145	5.63	3.88					6.4	4.4	.28	.19
14	161	6.04	3.75					8.4	5.2	.32	.26
15	164	6.07	3.70					7.6	4.6	.33	.20
16	170	6.52	3.83					7.6	4.5	.33	.19
Mean	162±9.3*	5.90±.38	3.64±.21	.73±.05	.44±.03	1.68±.07	1.06±.06	7.1±1.0	4.3±.6	.32±.03	.19±.02
% Change▲	-28%	+7%	+48%	+5%	+39%	+5%	+45%	-80%	-73%	-90%	-88%
Significance	P<.01	P>.5	P<.01	P>.5	P<.01	P>.05	P<.01	P<.01	P<.01	P<.01	P<.01
% Change▲	-7%	+17%	+26%	+73%	+83%	+7%	+17%				
Significance	P>.02	P<.01	P<.01	P<.01	P<.01	P>.05	P<.01	—	—	—	—

* Standard deviation
▲ Compared to normal values listed Table 25
▲ Compared to values obtained from eight weeks hypophysectomized rats listed Table 24
† All rats were given ordinary water as drinking fluid
• 1.0375mg injected over a period of 25 days

the differences are generally not as marked as found previously. The reason for this, is that comparison is made with normal rats of smaller size resulting from the fasting treatment. It is interesting to note that the relative weight of heart is still lower by the same amount as that found for the first series and therefore, appears to be a specific property of the hypophysectomized rats as is, for example, atrophy of the adrenals and testes. It is evident in the latter case that no matter how small are the normal rats, when the weights of their organs are compared with hypophysectomized animals, the adrenals and testes of the normal animals will always be of relatively greater size. The adrenals and testes of normal rats were not weighed. The size of the normal rats recorded in this table was the closest we could obtain to the size of hypophysectomized rats. Still, the absolute liver and heart weights of hypophysectomized rats are significantly lower than normal despite the fasting treatment.

In Table 26 the effect of thyroxinemay be observed. When comparison is made with the eight weeks hypophysectomized rats it is apparent that thyroxinehad definitely no effect on the body growth of hypophysectomized rats. On the other hand, this hormone was capable of increasing the liver and heart weights, and these increases are significant in terms of both relative and absolute weights. These

Table 27

WEIGHTS OF VARIOUS ORGANS OBTAINED FROM HYPOPHYSECTOMIZED RATS TREATED WITH GROWTH HORMONE[†°]

Number of rat	Body Weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	195	5.76	2.95	.51	.24	1.62	.83	10.2	5.2	.65	.33
2	219	6.31	2.88	.67	.33	1.61	.79	8.6	3.9	.62	.28
3	210	5.85	2.78	.54	.26	1.85	.91	9.8	4.6	.47	.22
4	222	6.55	2.95	.46	.24	1.57	.82	10.9	4.9	.51	.23
5	211	5.52	2.62	.46	.22	1.60	.76	8.1	3.8	1.47	.70
6	203	6.02	2.96	.46	.22	1.64	.79	9.8	4.8	.73	.36
7	211	6.19	2.93	.52	.25	1.58	.75	15.2	7.2	.72	.34
8	212	6.01	2.83	.49	.22	1.70	.80	8.0	3.8	.82	.39
9	206	5.84	2.81					10.0	4.8	.54	.26
10	204	5.82	2.85					10.2	5.0	.55	.27
11	191	5.86	3.07					13.0	6.8	.84	.44
12	225	6.43	2.86					12.2	5.4	.77	.34
Mean	209±10*	6.01±.39	2.87±.11	.51±.07	.25±.04	1.64±.09	.81±.05	10.5±2.1	5.0±.8	.72±.26	.34±.13
% Change[Δ]	-7.5%	+9.2%	+17%	-26%	-22%	+2.5%	+10%	-71%	-68%	-78%	-77%
Significance	P<.01	P>.02	P<.01	P<.01	P<.01	P>.3	P>.02	P<.01	P<.01	P<.01	P<.01
% Change[▲]	+20%	+17%	-3%	+21%	0%	+4%	-10%	—	—	—	—
Significance	P<.01	P<.01	P>.5	P<.01	P =	P>.2	P<.01				

* Standard deviation
Δ Compared to normal values listed Table 25
▲ Compared to values obtained from eight weeks hypophysectomized rats listed Table 24
† All rats were given ordinary water as drinking fluid
° 1mg of growth hormone injected daily for 16 days

Table 28

WEIGHTS OF VARIOUS ORGANS OBTAINED FROM HYPOPHYSECTOMIZED RATS TREATED WITH THYROXINE AND GROWTH HORMONE † ⊙

Number of rat	Body Weight	Liver		Heart		Brain		Adrenals		Testes	
	g	g	g/100g body wt.	g	g/100g body wt.	g	g/100g body wt.	mg	mg/100g body wt.	mg	mg/100g body wt.
1	216	8.01	3.70	.89	.43	1.76	.86	10.9	5.0	.63	.29
2	209	8.29	3.97	.95	.44	1.65	.79	10.1	4.8	.50	.24
3	206	7.24	3.51	.89	.43	1.80	.81	12.1	5.9	.63	.31
4	244	10.12	4.14	.85	.41	1.77	1.03	10.9	4.5	.79	.33
5	222	7.76	3.49	.89	.36	1.83	.88	10.5	4.7	.90	.41
6	208	7.99	3.84	.78	.37	1.91	.88	12.0	5.8	.68	.33
7	213	8.24	3.86	.91	.41	1.71	.79	11.8	5.6	.56	.26
8	207	7.48	3.61					10.2	4.9	.40	.19
9	172	6.49	3.77					9.5	5.5	.47	.27
10	209	7.92	3.78					7.3	3.5	.54	.26
11	204	7.83	3.83					7.5	3.7	.30	.15
Mean	210 ±17*	7.94±.80	3.77±.19	.88±.05	.41±.03	1.76±.06	.86±.12	10.2±1.6	4.8±.79	.58±.17	.27 ±.07
% Change△	-7%	+44%	+53%	+27%	+28%	+10%	+17%	-71%	-70%	-82%	-81%
Significance	P<.02	P<.01	P<.01	P<.01	P<.01	P<.01	P>.02	P<.01	P<.01	P<.01	P<.01
% Change▲	+20%	+57%	+30%	+109%	+70%	+12%	-4%				
Significance	P<.01	P<.01	P<.01	P<.01	P<.01	P<.01	P>.2				

*Standard deviation
△Compared to normal values listed Table 25
▲Compared to values from eight weeks hypophysectomized rats listed Table 24
†All rats were given ordinary water as drinking fluid
⊙.0625mg of thyroxine and 16mg of growth hormone injected concurrently for 16 days.

effects are about four times greater on the heart. Essentially, these effects are the same as those observed previously. The absolute liver and heart weights are not significantly different than normal because the fasting treatment reduced the size of normal rats. The relative weights of liver and heart are, as found previously, higher than normal. In the case of liver, part of this increase may be attributed to a possible lowering effect of fasting on the relative weight. This is probably not the case for the heart where the relative weight in this thyroid-treated group is superior to any of the normal values obtained from rats, fasted or not.

Table 27 shows that growth hormone increased the body weight of hypophysectomized rats by 20%. But contrary to that found previously the increase in liver weight is similar to the increase in body weight and no specific effect of the growth hormone can be noted on the liver. The same observation holds for the heart, i.e. as noted before the increase in heart weight corresponds to the increase in body weight. The absolute liver weight of this group is comparable to normal but the absolute heart weight is still lower than normal. The weights of other organs are not modified.

In Table 28 the values obtained from hypophysectomized rats treated with both thyroxine and growth hormone are shown. Again, it is found that administration of these hormones

Table 29

HEART RATE VALUES‡ OBTAINED FOR VARIOUS GROUPS OF RATS†

Number of rat	Normal	Three Weeks× Hypophysectomized	Normal	Eight Weeks Hypophysectomized	Normal Fasted‡	Hypophysectomized Treated with Thyroxine•	Hypophysectomized Treated with Growth Hormone°	Hypophysectomized Treated with Thyroxine and Growth Hormone⊙
1	420	375	480	330	435	390	315	345
2	360	330	450	300	525	435	345	315
3	390	360	465	270	405	360	330	405
4	400	400	420	225	390	405	330	405
5	400	330	450	300	390	435	345	375
6	420	330			430	315	315	375
7		360			405	315	345	420
8		300			420	330		360
9					450	420		435
10					495	435		375
11					520			435
12					390			
13					420			
14					450			
15					375			
16					390			
17					435			
18					375			
Mean	398 ± 23*	348 ± 32	453 ± 22	285 ± 40	430 ± 46	384 ± 49	332 ± 15	385 ± 37
% Change		− 12%		− 37%		− 10%	− 22%	− 10%
Significance		P < .01		P < .01		P < .01	P < .01	P < .01

‡ Beats/minute
† All rats were given 5% sucrose as drinking fluid
× Time after hypophysectomy
‡ Normal rats were fasted for 28 days
* Standard deviation
• 1.237mg injected over a period of 26 days
° 1mg injected daily for 26 days
⊙ Same dose of each hormone injected concurrectly for 26 days.

Table 30

HEART RATE VALUES[‡] OBTAINED FOR VARIOUS GROUPS OF RATS[†]

Number of rat	Normal	Three Weeks × Hypophysectomized	Normal Fasted[Δ]	Eight Weeks Hypophysectomized	Normal Fasted[ǂ]	Hypophysectomized Treated with thyroxine•	Hypophysectomized Treated with Growth Hormone°	Hypophysectomized Treated with Thyroxine and Growth Hormone°
1	420	330	375	300	390	420	360	420
2	420	390	420	300	330	330	330	420
3	390	390	330	270	450	420	360	450
4	390	390	360	225	450	420	330	300
5	390	390	420	210	360	390	330	360
6	390	390	405	330	430	390	330	360
7				330	360	420	330	450
8				360	420	420	300	450
9					360	570	360	390
10					390	390	360	450
11					390	360	300	450
12					300	420	360	
13					405	420		
14					360	450		
15					330	360		
16						390		
Mean	400±15*	380±24	385 ± 37	290 ± 52	381 ± 44	410 ± 52	337 ± 24	409 ± 50
% Change		− 2%		− 24%		+ 7%	− 11%	+ 7%
Significance		P > .1		P < .01		P > .1	P < .02	P > .1

* Standard deviation
† All rats were given ordinary water as drinking fluid
Δ Normal rats were fasted for 20 days
ǂ Normal rats were fasted for 30 days
‡ Beats/min.
× Time after hypophysectomy

• 1.0375mg injected over a period of 25 day
° 1mg injected daily for 16 days
° .0625 mg of thyroxine and 16 mg of growth hormone injected concurrently for 16 days.

produced effects which are complementary and this time the heart is no exception. No synergism can be observed on either the relative or the absolute heart weights. The effects on relative heart weight are comparable to those obtained with thyroxine alone or are the sum total of the effects obtained with both hormone separately, in the case of the absolute heart weight. In this group all values except those for the adrenals and testes are significantly above normal.

The heart rate of all rats was also determined and will now be considered. In the first series, Table 29 shows the results obtained with the different groups of rats. After three weeks hypophysectomy, a small but significant reduction can be noted in the heart rate of rats subjected to this operation. After eight weeks this decrease reached 37% of normal. Thyroxine injected alone or with growth hormone increasedthe heart rate of hypophysectomized rats but not to normal levels; the heart rate of the hypophysectomized rats treated with thyroxine or thyroxine and growth hormone is still 10% lower than normal. Growth hormone also could increase the heart rate of hypophysectomized rats but this effect is small and amounts to about half of that obtained with thyroxine.

In the second series, Table 30 shows that a significant decrease in the cardiac rate of hypophysectomized rats

could be obtained only eight weeks after hypophysectomy. The 24% decrease in the heart rate of hypophysectomized rats could be completely corrected by administration of thyroxine. This effect of thyroxine on the cardiac rate was the same whether the hormone was administered in the presence of growth hormone or not. Growth hormone alone had some effect on the heart rate but could not restore the cardiac rate of hypophysectomized rats to normal levels.

The different results obtained may be summarized as follows:

1) In the hypophysectomized rats the body weight, as well as both the absolute and relative weights of the heart, adrenals and testes were significantly reduced. The size of brain was not significantly changed and only the absolute weight of liver was found to be consistently low in hypophysectomized rats. Bradycardia was also observed in these animals.

2) Growth hormone appears to have a rather general effect on the growth of hypophysectomized rats. The body weight of hypophysectomized rats was increased by administration of growth hormone and the increase in liver and heart weights seems to correspond to the increase in body weight. No effect of growth hormone could be noted for the other organs. This hormone had but only a slight effect on the heart rate.

3) Thyroxine does not appear to exert any action on the body weight of hypophysectomized rats, but could increase the weight of liver and heart. Thus thyroxine had more or less a specific action on these organs. Hypertrophy was more prominent in the heart and thyroxine had a more pronounced action on the heart rate than growth hormone. No effect of this hormone could be noted on the other tissues.

4) When both hormones were given to hypophysectomized rats there was an increase in body weight, liver and heart weight. In the latter two organs, hypertrophy could be observed since, in this group, the relative weight of these tissues were above normal. The heart rate in this group was increased to the same extent as for the thyroxine group. In all these effects, synergism of these hormones could not be consistently observed.

DISCUSSION

In all the experimental rats treated or not with hormones the adrenals and testes were clearly atrophied and this is in no way surprising since all these animals were deprived of the specific trophic hormones necessary for their maintenance. The weight values of these glands were recorded in the foregoing section for the main purpose of showing the efficiency and completeness of hypophysectomy. In fact when any doubt arose on the completeness of this operation (criteria was based on the weight of testes and adrenals) all values were automatically rejected and this also holds for the catecholamine values.

Contrary to what was observed in the other organs, the brain weight was relatively constant in all experimental groups. In this respect the brain weight seems to be independent of body weight and also does not appear to be dependent on the presence of the pituitary gland at least in the young adult rat. Possibly, a different situation may have occurred if hypophysectomy had been made in much younger rats. However, the fact that the other tissues have suffered profound changes in weight places the brain in a different category. The physiological implication of this observation alone is difficult to assess

and it could only be suggested that the brain of hypophysectomized rats in all appearances may at least potentially function as well as normal. Investigation on brain weight under these experimental conditions does not appear to have been made by other workers.

The results obtained on body weight, liver and heart weight, in general, confirm those obtained by other workers. Growth stasis in hypophysectomized rats has been reported by numerous authors (67, 221, 222, 223, 224). Our results show that growth arrest was complete and the same was also found by many others (67, 221, 224). However, Scow (222, 223) could still detect slight but significant growth in his hypophysectomized rats. He attributed this to a still possible secretion of the thyroid gland in hypophysectomized rats (222), and this brings up a much debated point. Is the thyroid hormone capable of stimulating growth of hypophysectomized rats? The growth promoting activity of the thyroid hormone can be observed clearly when growth is retarded in thyroidectomized rats (225) and then is maintained with thyroxine(67) But with hypophysectomized rats one group of workers obtained an effect of thyroxine on the body weight of hypophysectomized rats (222, 223, 230, 231) whereas others could not detect any effect (225, 227, 228, 229, 226). Scow (222) suggested that dosage is important in producing this anabolic effect of thyroxine and can be only obtained with

low doses of 2.5 - 3.0µg. However, Hartly and Leblond (67) failed to detect growth promoting activity of thyroxine in hypophysectomized rats at doses of 3µg. That age could be important is not impossible since Scow (222,223) used very young hypophysectomized rats, but this is unlikely on the basis that no effect of thyroxine on growth could be observed in very young hypophysectomized mice and this at various doses of thyroxine (232). In any case, we could not observe any growth in our hypophysectomized rats and thyroxine administered with or without growth hormone did not have any effect on the body weight of the hypophysectomized rats. On the other hand, growth hormone was capable of stimulating the growth of hypophysectomized rats and these results support the hypothesis emitted by several workers (233,234,235) that the growth promoting activity of thyroxine is due to the release of hypophyseal growth hormone by thyroxine.

The effects of growth hormone on the body weight, liver and heart weight is in agreement with other reports. Scow (223) also noted that the gain in weight of liver, heart and kidney caused by treatment with growth hormone in thyroidectomized-hypophysectomized rats was proportional to the increase in body weight. Thus the action of this hormone appears to be a general one. Along these lines, Beznak (221) showed that growth hormone could not correct the cardiac

atrophy or low relative weight of hearts resulting in hypophysectomized rats. Similar results on the lack of effect of growth hormone on cardiac atrophy of hypophysectomized rats is also reported by Whitehorn et al (224). Whitehorn et al further pointed out that the low relative heart weight of hypophysectomized rats still persists when these are compared to normal rats of the same size. This is also in accordance with our findings that the relative heart weight of hypophysectomized rats was significantly lower than that of normal fasted rats of almost the same size. Thus true cardiac atrophy can be demonstrated and appears to be a direct consequence of hypophysectomy. This does not seem to be the case for the liver since the relative weight of this tissue in hypophysectomized rats was not found to be consistently and significantly different from normal.

The effects produced by thyroxine are interesting. Despite the lack of effect of this hormone on the body weight of hypophysectomized rats, in these same rats, thyroxine could increase the weight of both the liver and heart. Thus the cardiac atrophy of hypophysectomized rats was due to the absence of normal thyroid function, As already suggested by Reznak (227). It is very difficult to decide whether there is a correlation of any kind with this

effect of thyroxine and the effect of this hormone on heart rate. The fact is that cardiac atrophy is associated with low heart rate as for example in hypophysectomized rats and hypophysectomized rats treated with growth hormone. Thyroxine treatment which caused cardiac hypertrophy also increased the heart rate. However, no strict correlation can be made between this cardiac hypertrophy and heart rate; the heart rate of hypophysectomized rats treated with thyroxine alone or with growth hormone could never be found to exceed the normal heart rate, yet, cardiac hypertrophy in these experimental groups was significantly higher than normal. In normal rats as well, no relationship could be drawn between either the absolute or relative heart weights and the heart rate. The finding that the weight effect of thyroxine is not limited to the heart and is also observable in the liver may be taken as an indication that this weight effect on the heart is probably not connected to some hemodynamic factor. Accordingly, Beznak (221) could not establish any relationship between the size of heart and heart performance. The results obtained for the heart rate on the various groups of rats are essentially the same as those obtained by Beznak (252). In the first series, thyroxine did not completely restore the heart rate to normal levels. But the heart rate of the hypophysectomized rats treated with thyroxine was only 10% lower than normal,

and in the second series, the heart rate in the thyroxine-treated groups was not different from normal.

It is tempting to suggest on the basis of the following observations that nutritional factors may possibly be involved in the observed changes in heart rate: 1) the food consumption of hypophysectomized rats is greatly reduced (236,237), 2) food consumption of hypophysectomized rats is increased by treatment with thyroxine (222) or growth hormone (personal communication from Dr. Meznak), 3) the heart rate of normal rats given 5% sucrose as drinking water was generally higher than those given ordinary water. Admittedly, the latter observation may simply be coincidental and would need more experimental support. Also no estimation can be obtained on the differential amount (if any) of food consumed between the groups treated with thyroxine and growth hormone which would be appreciated since the heart rate observed between these groups was different. On the other hand, the finding that the reduction in food intake is approximately the same in thyroidectomized rats as in hypophysectomized rats (236) is suggestive that thyroxine is mostly responsible for this anorexia. Thus a slow metabolic rate in hypophysectomized rats may cause a physiological readjustment of the animal leading to a slow cardiac rate in a manner similar to that proposed by Meznak (252) and may also be responsible for a reduction in

Table 31

NORADRENALINE CONTENT OF HEARTS OBTAINED FROM NORMAL AND HYPOPHYSECTOMIZED RATS†

Number of rat	Three weeks×				Eight weeks			
	µg/g		µg/heart		µg/g		µg/heart	
	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized
1	.7750	.9226	.6262	.4890	.7750	.8836	.6262	.4533
2	1.0190	.9552	.9532	.4957	1.0190	.9057	.9532	.4447
3	.6182	.9803	.5700	.5002	.6182	.9249	.5700	.4597
4	.3890	.9214	.3735	.4312	.3890	.8414	.3735	.3997
5	.5707	.9502	.5205	.4342	.5707	.9006	.5205	.4349
6	.7668	.1453	.6157	.3840	.7668		.6157	
7	.6140	.8494	.6307	.4035	.6140		.6307	
8	.6980		.5835		.6980		.5835	
9	.7879		.7005		.7879		.7005	
10	.7287		.6187		.7287		.6187	
Mean	.6967±.170*	.9179±.052	.6192±.145	.4482±.043	.6967±.170	.8912±.031	.6192±.145	.4393±.023
% Change		+31%		- 27%		+ 28%		- 29%
Significance		P < .01		P < .01		P < .02		P < .02

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
× Time after hypophysectomy

food intake. In turn perhaps this anorexia may contribute to the reduction in heart rate by not dispensing the amount of substances which may have some influence on cardiac rhythm.

As mentioned previously the possibility of a participation of the catecholamines in these changes of cardiac rate has been investigated and the results obtained will now be presented together with catecholamine content of other tissues.

THE CATECHOLAMINS CONTENT OF DIFFERENT ORGANS OBTAINED FROM NORMAL RATS, HYPOPHYSECTOMIZED RATS AND HYPOPHYSECTOMIZED RATS TREATED WITH THYROXINE, GROWTH HORMONE, THYROXINE PLUS GROWTH HORMONE.

In the heart

Let us now consider the results obtained in the first series of experiments where all rats were given 5% sucrose as drinking fluid. Table 31 shows the noradrenaline content of hearts obtained from rats three weeks and eight weeks after hypophysectomy. After three weeks hypophysectomy, the amount of noradrenaline per heart was decreased by 27%. However, when the values are expressed per gram a 31% increase is noted in the hearts of hypophysectomized rats since these hearts were much smaller than normal ones. After eight weeks hypophysectomy the same differences are noted and

Table 32

NORADRENALINE CONTENT OF HEARTS OBTAINED FROM VARIOUS GROUPS OF RATS †

Number of rat	Normal Fasted †		Hypophysectomized Thyroxine •		Growth Hormone ○		Thyroxine and Growth Hormone ○	
	µg/g	µg/heart	µg/g	µg/heart	µg/g	µg/heart	µg/g	µg/heart
1	.8024	.7230	.5055	.3645	.8504	.4635	.4640	.5197
2	.8055	.6847	.9354	.6052	.8107	.4597	.6639	.5497
3	.7681	.6352	.6171	.4845	.9318	.6532	.4532	.4710
4	.6358	.5767	1.0890	.5992	.7883	.4620	.5677	.5490
5	.7293	.6360	.8299	.6307	.7434	.4267	.7019	.5805
6	.5586	.5346	.8081	.5261	.7229	.4410	.4019	.4140
7	.6204	.5565	.6271	.4854	.9168	.5565	.5366	.4137
8	.6382	.4417	.5987	.4395	.9754	.5316	.6996	.5049
9	.8117	.6900					.5630	.5383
10	.7897	.7897						
11	.7250	.6307						
12	.7318	.6660						
13	.6671	.6870						
14	.7966	.6604						
15	.6426	.5385						
16	.8269	.6665						
17	.6106	.6577						
Mean	.7153±.086*	.6338±.082	.7500±.200	.5170±.092	.8424±.092	.4927±.072	.5613±.110	.5133±.066
% Change			+4.8%	-18%	+16%	-22%	-21%	-19%
Significance			P > .5	P < .01	P < .01	P < .01	P < .01	P < .01

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
‡ Normal rats were fasted for 28 days

• 1.237mg injected over a period of 26 days
○ 1mg injected daily for 26 days
○ Same dose of each hormone injected concurrently for 26 days.

Table 33

SOMATOTROPHIN CONTENT OF HEARTS OBTAINED FROM NORMAL AND HYPOPHYSECTOMIZED RATS[†Δ]

Number of rat	Three weeks[×]				Eight weeks			
	µg/g		µg/heart		µg/g		µg/heart	
	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized
1	.5700	.8322	.4762	.3720	.9684	.8538	.5617	.3945
2	.6711	.5855	.4770	.3419	1.068	.8318	.5555	.3078
3	.6043	.7370	.6285	.3419	1.369	.9697	.8354	.4461
4	.5397	.8369	.5559	.4620	1.256	.6492	.8040	.2662
5	.6580	.7649	.5178	.4230	1.443	.7534	.9195	.3315
6	.8671	.8232	.6495	.4215	1.254	.9304	.6772	.3787
7						.9104		.4252
Mean	.6517±.113*	.7633±.093	.5508±.075	.3946±.049	1.226±.173	.8427±.112	.7255±.151	.3657±.072
% Change	+ 17%		- 28%		- 31%		- 49%	
Significance	P < .01		P < .01		P < .01		P < .01	

* Standard deviation
† All rats were given ordinary water as drinking fluid
× Five after hypophysectomy
Δ The normal rats with which the eight weeks hypophysectomized rats are compared were fasted for 20 days

are quantitavely comparable to those found at three weeks hypophysectomy. All these results were statistically significant. Table 32 shows the results obtained with hypophysectomized rats administered thyroxine, growth hormone or both. Examination of this table reveals that when the values are considered per heart, hormonal treatment reduced the observed decrease in noradrenaline to a 20% difference from normals. This effect was the same in all three hormone-treated groups but the heart noradrenaline content was still significantly lower than normal in all experimental groups. Since also the noradrenaline content per heart is identical in all three groups of rats injected with the hormones the values reported per gram merely reflect the differences in heart weights respective to each group. Let us consider now the results obtained when all rats were given ordinary water. It may be observed from Table 33 that after three weeks hypophysectomy the noradrenaline values expressed per heart indicate the same 2% decrease. The increase in concentration of noradrenaline by 17% was lower than that previously obtained because of differences in heart weight. After eight weeks hypophysectomy the noradrenaline content per heart was lower by 4.9%. It must be emphasized that the normal rats with which comparison is made had been successfully fasted and consequently their heart weight was almost comparable to that of

Table 34

NORADRENALINE CONTENT OF HEARTS OBTAINED FROM VARIOUS GROUPS OF RATS†

Number of rat	Normal		Hypophysectomized					
	Fasted‡		Thyroxine•		Growth Hormone°		Thyroxine and Growth Hormone⊙	
	µg/g	µg/heart	µg/g	µg/heart	µg/g	µg/heart	µg/g	µg/heart
1	.7560	.4612	.3762	.2660	.7368	.3795	.4783	.4252
2	.8940	.5677	.5906	.4117	.4317	.2910	.4811	.4575
3	.7751	.5736			.6359	.3472	.3972	.3547
4	.7644	.5045						
5	.7836	.6426						
Mean	.7946±.054*	.9499±.071	.4834±.152	.3388±.073	.6034±.159	.3392±.045	.4522±.052	.4124±.052
% Change			-39%	-38%	-24%	-38%	-43%	-25%
Significance			P < .01	P < .02	P < .05	P < .01	P < .01	P > .02

* Standard deviation
† All rats were given ordinary water as drinking fluid
‡ Normal rats were fasted for 30 days
• 1.0375mg injected over a period of 25 days
o 1mg injected daily for 16 days
⊙ .0625mg of thyroxine and 16mg of growth hormone injected concurrently for 16 days.

hypophysectomized rats. It is important to note that despite this low heart weight the noradrenaline content per heart in normal rats was well maintained. It follows that now a decrease in the concentration of heart noradrenaline of hypophysectomized rats is also observed when the results are expressed per gram. Thus as the size of normal hearts approaches that of hearts from hypophysectomized rats a decrease in the noradrenaline content can be noted in hearts of the latter group with both means of expression. Table 34 shows the noradrenaline content of hearts obtained from hypophysectomized rats treated with thyroxine, growth hormone, or both. Unfortunately, because of technical difficulties experienced with maintaining these hearts frozen during the storage period, catecholamine determinations of more than a few experimental hearts could not be made and this makes quantitative comparison hazardous. Nevertheless, it may be remarked that despite the few number of determinations made a statistical decrease still persists in the total heart noradrenaline content in all three groups of hypophysectomized rats treated with the hormones, and this is in accordance with that previously found. It may be pertinent to draw attention to the fact that, contrary to previous comparisons, the weights of normal hearts to which these comparisons are made are the same

Table 35

ADRENALINE CONTENT OF HEARTS OBTAINED FROM NORMAL AND HYPOPHYSECTOMIZED RATS†

Number of rat	Three weeks×				Eight weeks			
	µg/g		µg/heart		µg/g		µg/heart	
	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized
1	.0238	.0285	.0192	.0151	.0238	.0545	.0192	.0280
2	.0221	.0143	.0207	.0074	.0221	.0334	.0207	.0164
3	.0172	.0277	.0166	.0141	.0172	.0369	.0166	.0181
4	.0229	.0184	.0209	.0086	.0229	.0364	.0209	.0173
5	.0177	.0223	.0142	.0120	.0177	.0316	.0142	.0154
6	.0195	.0262	.0200	.0119	.0195		.0200	
7	.0133		.0111		.0133		.0111	
8	.0179		.0159		.0179		.0159	
9	.0184		.0156		.0184		.0156	
Mean	.0172±.0039*	.0229±.0056	.0171±.0034	.0115±.0030	.0172±.0039	.0385±.0092	.0171±.0034	.0190±.0051
% Change	+33%		-32%		+123%		+11%	
Significance	P > .02		P < .01		P < .01		P > .5	

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
× Time after hypophysectomy

Table 36

ADRENALINE CONTENT OF HEARTS OBTAINED FROM VARIOUS GROUPS OF RATS†

Number of rat	Normal Fasted‡		Hypophysectomized Thyroxine		Hypophysectomized Growth Hormone		Hypophysectomized Thyroxine and Growth Hormone	
	µg/g	µg/heart	µg/g	µg/heart	µg/g	µg/heart	µg/g	µg/heart
1	.0253	.0210	.0380	.0274	.0114	.0062	.0237	.0183
2	.0116	.0105	.0098	.0064	.0033	.0018	.0169	.0189
3	.0168	.0147	.0108	.0087	.0004	.0003	.0157	.0130
4	.0170	.0162	.0160	.0104	.0126	.0074	.0027	.0028
5	.0114	.0103	.0186	.0149	.0145	.0083	.0152	.0145
6	.0324	.0225	.0097	.0072	.0189	.0115	.0179	.0130
7	.0216	.0183			.0061	.0022	.0336	.0346
8	.0297	.0297					.0185	.0154
9	.0137	.0125						
10	.0207	.0213						
11	.0269	.0223						
12	.0142	.0119						
13	.0178	.0143						
14	.0120	.0129						
Mean	.0194±.007*	.0170±.006	.0171±.011	.0125±.006	.0093±.007	.0053±.004	.0180±.009	.0163±.009
% Change			-11%	-26%	-52%	-68%	-7.2%	-4.1%
Significance			P > .5	P > .1	P < .01	P < .01	P > .5	P > .5

* Standard deviation
† All rats given 5% sucrose as drinking fluid
‡ Normal rats were fasted for 28 days

• 1.237mg injected over a period of 26 days
o 1mg injected daily for 26 days
⊙ Same dose of each hormone injected concurrently for 26 days.

(consult Table 26) as that observed for the thyroxine group and are some 30% lower than that of the thyroxine plus growth hormone (Table 25) group, thus the results as expressed per heart are essentially the same despite changes in size of heart. On the other hand, when the results are expressed as concentrations the values seem to reflect changes in weight. Thus, as seen in Table 34, the noradrenaline content per gram in all experimental groups is lower compared to normal mainly because the size of normal hearts to which they are compared is smaller.

Similar observations can be made for adrenaline except that the results obtained with this catecholamine were not as consistent as for noradrenaline. In the first series, Table 35 shows that after three weeks hypophysectomy, the adrenaline content of hearts is 32% lower than normal. The observed 33% increase in the concentration of adrenaline is not statistically significant. After eight weeks hypophysectomy, the myocardial adrenaline content per heart is not significantly different from normal but the adrenaline concentration is significantly above normal. This increase can be completely accounted for by the smaller size of hearts from hypophysectomized rats as compared to normal. In Table 36 is shown the effect of various hormonal treatments on the adrenaline content. No significant change is noted except in the group of hypophysectomized

Table 37

ALKALINE CONTENT OF HEARTS OBTAINED FROM NORMAL AND HYPOPHYSECTOMIZED RATS[†Δ]

Number of rat	Three weeks[×]				Eight weeks			
	μg/g		μg/heart		μg/g		μg/heart	
	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized
1	.0086	.0084	.0072	.0037	.0301	.0251	.0174	.0103
2	.0324	.0142	.0231	.0083	.0843	.0116	.0438	.0051
3	.0166	.0179	.0174	.0083	.0280	.0183	.0171	.0079
4	.0160	.0080	.0165	.0044	.0565	.0073	.0362	.0030
5	.0254	.0171	.0200	.0102	.0216	.0192	.0138	.0090
6	.0109	.0219	.0075	.0112	.0224	.0356	.0121	.0132
7						.0237		.0109
Mean	.0182±.0092[*]	.0145±.0055	.0153±.0065	.0076±.0030	.0404±.0240	.0201±.0093	.0234±.0135	.0085±.0035
% Change	-20%		-49%		-50%		-63%	
Significance	P < .5		P ≥ .01		P > .05		P ≥ .01	

* Standard deviation
† All rats were given ordinary water as drinking fluid
× Time after hypophysectomy
Δ Normal rats with which eight weeks hypophysectomized rats are compared were fasted for 20 days

Table 38

ADRENALINE CONTENT OF HEARTS OBTAINED FROM VARIOUS GROUPS OF RATS †

Number of rat	Normal		Hypophysectomized					
	Fasted ‡		Thyroxine •		Growth Hormone ○		Thyroxine and Growth Hormone ⊙	
	µg/g	µg/heart	µg/g	µg/heart	µg/g	µg/heart	µg/g	µg/heart
1	.0481	.0294	.0343	.0243	.0244	.0126	.0169	.0150
2.	.0422	.0268	.0195	.0135	.0219	.0148	.0205	.0195
3	.0545	.0403			.0180	.0098	.0232	.0208
4	.0371	.0245						
5	.0407	.0333						
Mean	.0445±.008*	.0308±.006	.0269±.0097	.0189±.0077	.0214±.003	.0124±.0025	.0202±.003	.0184±.0031
% Change			-39%	-38%	-51%	-59%	-54%	-39%
Significance			P > .02	P > .05	P < .01	P < .01	P < .01	P ≤ .01

* Standard deviation
† All rats were given ordinary water as drinking fluid
‡ Normal rats were fasted for 30 days
• 1.0375mg injected over a period of 25 days
○ 1mg injected daily for 16 days
⊙ .0625 mg of thyroxine and 16mg of growth hormone injected concurrently for 16 days.

Table 39

CATECHOLAMINE CONTENT, WEIGHTS AND RATES OF HEARTS OBTAINED FROM VARIOUS GROUPS OF RATS†

	Time after hypophysectomy	Group	Noradrenaline		Adrenaline		Heart Rate	Heart Weight
	Weeks		µg/g	µg/heart	µg/g	µg/heart	beats/min.	g
Series A*	-	Normal	.6967±.17	.6192±.145	.0172±.0039	.0171±.0039	398 ± 23.5	.88 ± .08
	3	Hypophysectomized	.9179±.052	.4482±.043	.0229±.0056	.0115±.0030	348 ± 32	.48 ± .03
	-	Normal	.6967±.17	.6192±.145	.0172±.0039	.0171±.0039	453 ± 22.3	.88 ± .07
	8	Hypophysectomized	.8912±.031	.4393±.023	.0385±.0092	.0190±.0051	285 ± 39.7	.48 ± .02
	-	Normal △	.7153±.086	.6338±.082	.0194±.007	.0170±.0056	430 ± 46.3	.89 ± .09
	11	Thyroxine 1	.7500±.20	.5170±.092	.0171±.011	.0125±.0079	384 ± 49.4	.69 ± .07
	11	Growth Hormone 1	.8424±.092	.4927±.072	.0093±.0066	.0053±.004	315 ± 54	.59 ± .05
	11	Thyroxine + Growth Hormone 1	.5613±.11	.5133±.066	.0180±.0086	.0163±.0089	385 ± 37.5	.88 ± .11
Series B×	-	Normal	.6517±.113	.5508±.075	.0182±.0092	.0153±.0065	400 ± 15.5	.86 ± .14
	3	Hypophysectomized	.7633±.093	.3946±.099	.0145±.0055	.0076±.0030	380 ± 24.5	.53 ± .05
	-	Normal ▲	1.2260±.173	.7255±.151	.0404±.024	.0234±.0135	385 ± 37	.58 ± .05
	8	Hypophysectomized	.8427±.112	.3657±.072	.0201±.0093	.0085±.0035	290 ± 52	.42 ± .03
	-	Normal ▽	.7946±.055	.5499±.071	.0445±.0068	.0308±.006	381 ± 44	.69 ± .08
	10	Thyroxine 2	.4834±.152	.3388±.073	.0269±.0097	.0189±.0077	410 ± 52	.73 ± .05
	10	Growth Hormone 2	.6014±.159	.3392±.045	.0214±.0032	.0124±.0025	337 ± 24	.51 ± .07
	10	Thyroxine + Growth Hormone 2	.4522±.052	.4124±.052	.0202±.003	.0184±.0031	409 ± 50	.88 ± .05

† Mean values are given with standard deviation
* All rats were given 5% sucrose as drinking fluid
× All rats were given ordinary water as drinking fluid
△ Fasted for 28 days
▲ Fasted for 20 days
▽ Fasted for 30 days
1 See footnotes Table 32
2 See footnotes Table 34

rats given growth hormone where there is a 68% decrease in the total heart adrenaline and a similar decrease in the concentration of adrenaline. In the other series of experiments, Table 37 shows that three weeks after hypophysectomy rats exhibit a 49% decrease in their total myocardial adrenaline whereas the adrenaline content per gram was not significantly modified. These results are essentially the same as found previously. However, this decrease extends to eight weeks-hypophysectomized rats and amounts to 63%; similar decreases in total adrenaline are also noted (Table 38) in the hormone-treated rats. These differences are significant in the growth hormone groups despite the small number of determinations made. As for noradrenaline, the adrenaline content per gram is lower in the hearts of hypophysectomized rats treated with the hormones mainly because of weight differences between these experimental and normal hearts.

All the above mentioned data together with the results obtained with absolute heart weight and heart rate are summarized in Table 39.

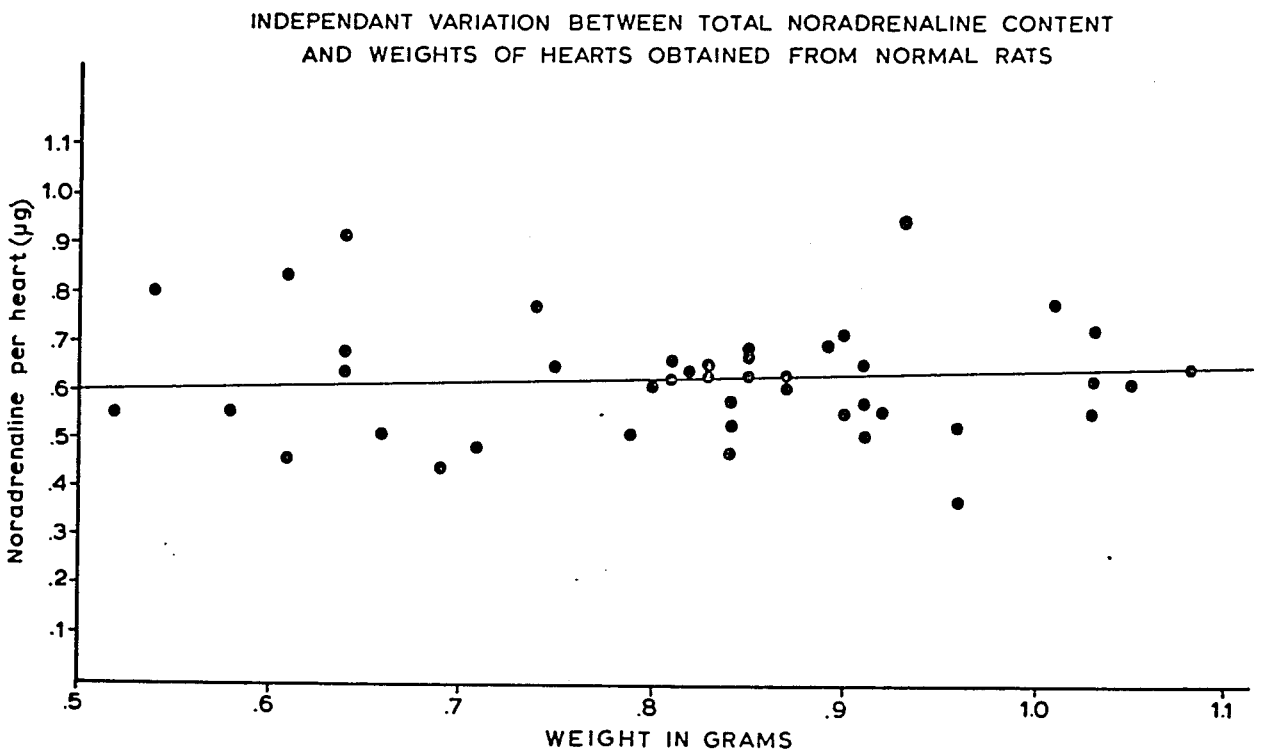
In conclusion it may be said that in both series the total noradrenaline content of all experimental hearts was significantly lower than normal irrespective of changes in weight. It appears that this reduction in total noradrenaline was not as marked in the hearts of hypophysectomized

rats treated with thyroxine, growth hormone or both. When the results are expressed on a per gram basis variable results were obtained depending on the size of hearts.

In the case of adrenaline, in the second series a reduced total adrenaline content in hearts of both three and eight weeks hypophysectomized rats was found and hormonal treatment did not appear to raise the adrenaline content to a normal level. In the first series the total adrenaline content of hearts from eight weeks hypophysectomized rats was not significantly different from normal and in the presence of this result if is difficult to attribute some beneficial effect of thyroxine on the total adrenaline content of hearts from hypophysectomized rats. Although it seems probable that the adrenaline content per heart is lower in hypophysectomized rats this finding has to be taken with reservations and no definite conclusion can be drawn on the effects of hormonal treatment.

It may have been felt during the foregoing discussion that more emphasis was put on the results obtained from catecholamine values expressed per heart. The reason for this preference will be apparent from what follows. It is realised that in considering the results by this means of expression the author becomes exposed to criticism. For example, in considering a decrease in the total catecholamine content of such small hearts as those of hypophy-

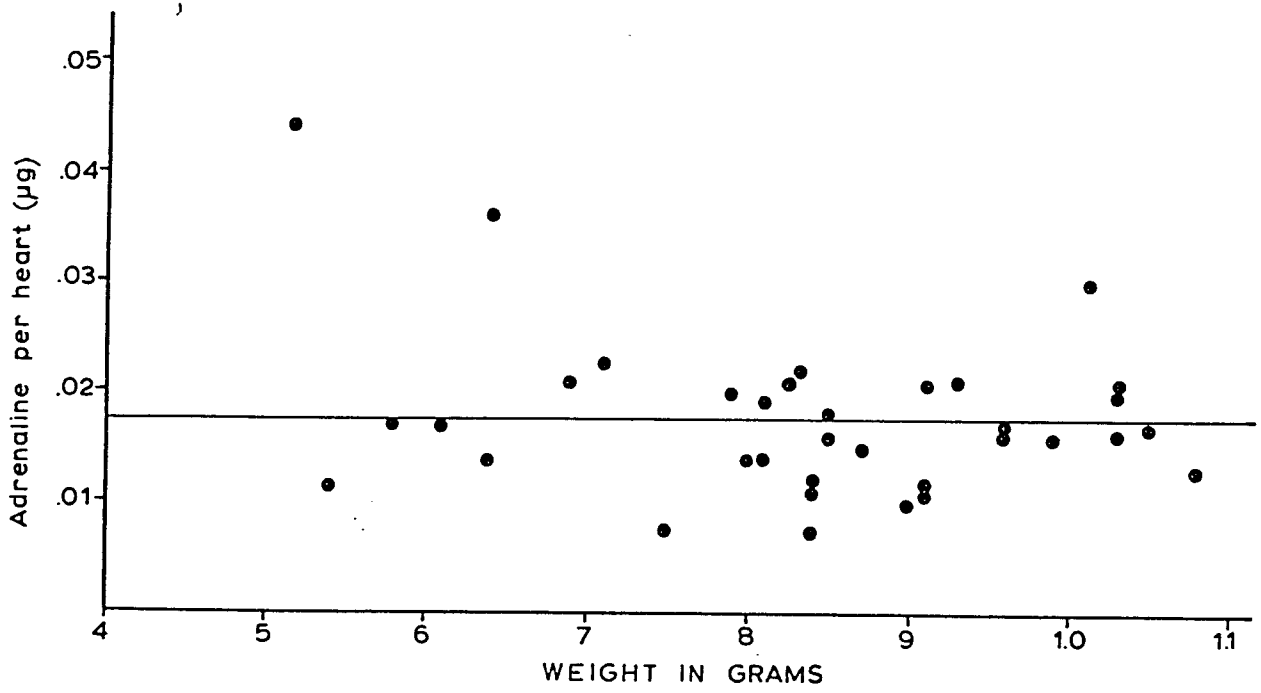
Figure V



Line plotted by the method of least squares

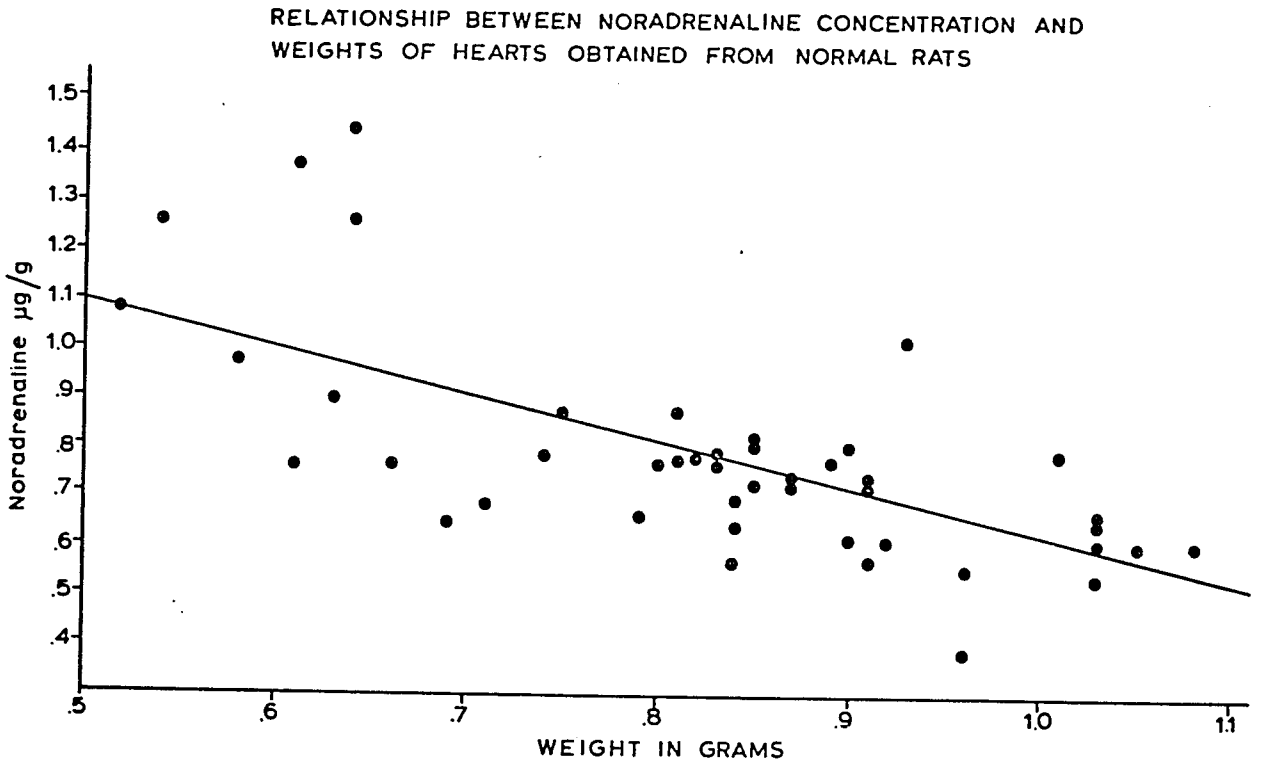
Figure VI

INDEPENDANT VARIATION BETWEEN TOTAL ADRENALINE CONTENT AND WEIGHTS OF HEARTS OBTAINED FROM NORMAL RATS



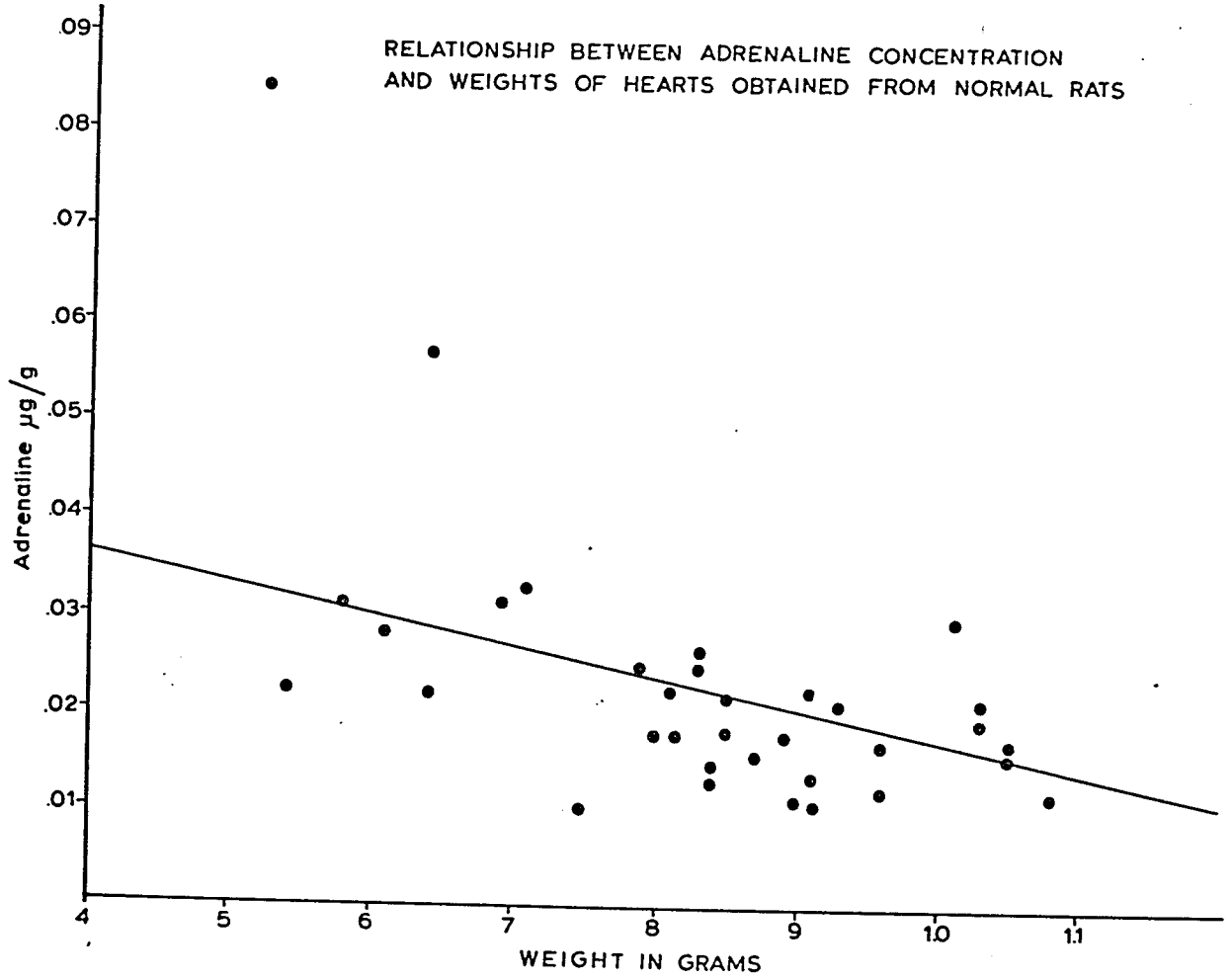
Line plotted by the method of least squares

Figure VII



Line plotted by the method of least squares

Figure VIII



Line plotted by the method of least squares

sectomized rats one cannot help but think that this decrease may have been occasioned indirectly by a weight factor and is not a true direct effect of hypophysectomy. That such an objection is probably not valid follows from the observation, made during the course of these experiments, that the catecholamine content per normal heart is relatively constant and does not depend on the size of the heart. This is represented statistically in Figure V for noradrenaline and in Figure VI for adrenaline. It is readily seen that for a range of weight varying from .5 to 1.0 gram the amount of catecholamine per heart is independent of heart weight. A direct consequence of this constancy in the amount of total catecholamine in hearts of various weights is that the concentration of catecholamine is inversely proportional to the weights of hearts. The quantitative demonstration of this relationship can be observed in Figure VII for noradrenaline and Figure VIII for adrenaline. This relationship is quantitavely comparable with both noradrenaline and adrenaline; normal hearts weighing 0.5 gram contain approximately twice as much catecholamine on a per gram basis than hearts weighing 1 gram. A very similar relationship between catecholamine concentration and heart weight has been noted by Wegmann et al (277) in dogs. It is evident from these figures that in considering results solely on a per gram basis one

is liable to introduce a second variable of weight effect and may lead to erroneous interpretations. This point is of capital importance in our case where the different experimental conditions used resulted in variation of weight of wide range; if comparisons are to be made on a per gram basis reliable results can only be obtained when the hearts of experimental animals do not differ in weight from those of normals. This presented a problem since, as mentioned, a wide variety of weights were obtained for the experimental hearts. On the other hand, the fasting of normal rats permitted us to obtain hearts of a relatively wide range of weights and the above mentioned relationship between catecholamine concentration and weight of heart could be established. It was emphasized in presenting the tables that whenever normal hearts had sizes similar to experimental hearts the results were the same with both means of expression. In fact, when correction is made for the weight effects, the results considered per gram are in all aspects the same as those obtained per heart. This can easily be verified by comparing each experimental value for catecholamine concentration with their respective heart weight to the normal regression line of Figure VII and VIII. Because of these considerations it seems only reasonable to consider the values of catecholamine per heart a more valid means of expression. It gives a more faithful picture of

Table 40

NORADRENALINE CONTENT OF LIVERS OBTAINED FROM NORMAL AND HYPOPHYSECTOMIZED RATS†

Number of rat	Three weeks×				Eight weeks			
	µg/g		µg/liver		µg/g		µg/liver	
	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized
1	.0539	.0790	.5831	.4384	.0778	.0656	.5041	.3627
2	.0378	.0698	.4316	.4167	.0693	.0642	.6084	.2914
3	.0557	.0923	.5531	.3996	.0465	.0665	.3878	.4129
4	.0632	.0372	.6332	.1916	.0606	.0562	.5738	.2838
5	.0612	.0699	.5403	.3309		.0801		.3139
6	.0332	.0676	.4223	.3853				
7		.0678		.2644				
8		.1046		.4842				
Mean	.0508±.012*	.0735±.020	.5272±.084	.3638±.096	.0635±.013	.0665±.009	.5195±.097	.3329±.054
% Change	+ 44%		- 30%		+ 4.7%		- 35%	
Significance	P > .02		P < .01		P > .5		P < .01	

* Standard deviation
× Time after hypophysectomy
† All rats were given 5% sucrose as drinking fluid

Table 41

NORADRENALINE CONTENT OF LIVERS OBTAINED FROM VARIOUS GROUPS OF RATS†

Number of rat	Normal Fasted† µg/g	Normal Fasted† µg/liver	Hypophysectomized Thyroxine• µg/g	Hypophysectomized Thyroxine• µg/liver	Hypophysectomized Growth Hormone° µg/g	Hypophysectomized Growth Hormone° µg/liver	Hypophysectomized Thyroxine and Growth Hormone° µg/g	Hypophysectomized Thyroxine and Growth Hormone° µg/liver
1	.0863	.7370	.0857	.5124	.0488	.4670	.0690	.4296
2	.0864	.6402	.0647	.4211	.0898	.6178	.0822	.5441
3	.0882	.6517	.0754	.4101	.0628	.4873	.0661	.4950
4	.0742	.6514	.0766	.4373	.0610	.4276	.0918	.5801
5	.0903	.5607	.0688	.4334	.0921	.6529	.0572	.3803
6	.0573	.4331	.0893	.5411	.0754	.5813	.0672	.6034
7	.0806	.6625	.0744	.5178	.0721	.4823	.0599	.4516
8	.0810	.6034	.0975	.5859	.0685	.4890	.1110	.6593
9	.0600	.5262	.0641	.3390			.0971	.8418
10	.0794	.5998	.0787	.5233			.0781	.5552
11	.1051	.8681					.0790	.7726
12	.0590	.4519						
13	.0807	.7045						
14	.0649	.5860						
15	.0635	.6515						
16	.1105	.8685						
17	.0986	.8223						
Mean	.0803±.016	.6481±.125	.0775±.011	.4721±.075	.0713±.012	.5236±.081	.0780±.016	.5739±.142
% Change			- 3%	- 27%	- 11%	- 18%	- 2%	- 11%
Significance			P > .05	P < .01	P > .1	P < .02	P > .5	P > .1

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
‡ Normal rats were fasted for 28 days

• 1.237mg injected over a period of 26 days
° 1mg injected daily for 26 days
• Same dose of each hormone injected concurrently for 26 days.

the effects caused in the catecholamine content by the different experimental conditions used and by-passes difficulties arising from weight effects.

In the liver

Let us now consider the results obtained in the first series of experiments. Table 40 shows the noradrenaline content of livers obtained from three weeks and eight weeks-hypophysectomized rats. The only significant differences are those occurring when the results are expressed per liver. Thus it may be observed that after three weeks the livers of hypophysectomized rats contained 30% less noradrenaline than normals. After eight weeks there is still a significant 35% decrease in the livers of hypophysectomized rats. The weights of livers from hypophysectomized rats were some 39-50% lower than normal.

Upon considering Table 41 it may be deduced that administration of both thyroxine and growth hormone together reduces the observed decrease in total noradrenaline content of livers of hypophysectomized rats to insignificant differences. Growth hormone is less effective and thyroxine does not bring much change. It may be noted however, that these changes follow those obtained with liver weight. Indeed it may be recalled that the weights of livers from hypophysectomized rats treated with both hormones were comparable to

Table 42

ADRENOCORTICOTROPIC CONTENT OF LIVERS OBTAINED FROM NORMAL AND HYPOPHYSECTOMIZED RATS[†Δ]

Number of rat	Three weeks[×]				Eight weeks			
	μg/g		μg/liver		μg/g		μg/liver	
	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized
1	.0228	.0787	.2264	.4680	.0428	.0732	.2942	.4033
2	.0522	.0772	.5229	.5106	.0622	.0764	.3972	.4041
3	.0684	.0746	.4995	.5392	.0691	.0653	.4140	.3228
4	.0563	.0804	.5280	.4663	.1071	.0626	.8011	.3486
5	.0643	.0572	.5472	.3528	.0863	.0631	.5039	.3104
6	.0678	.0717	.5844	.5012		.0342		.1840
7						.0553		.2626
Mean	.0543±.016*	.0733±.008	.4847±.130	.4730±.065	.0735±.024	.0614±.014	.4822±.190	.3194±.080
% Change	+35%		-2%		-16%		-33%	
Significance	P > .02		P > .5		P > .2		P > .05	

* Standard deviation
† All rats were given ordinary water as drinking fluid
× Time after hypophysectomy
Δ Normal rats with which eight weeks hypophysectomized rats are compared were fasted for 20 days

Table 43

NO. ADRENALINE CONTENT OF LIVERS OBTAINED FROM VARIOUS GROUPS OF RATS†

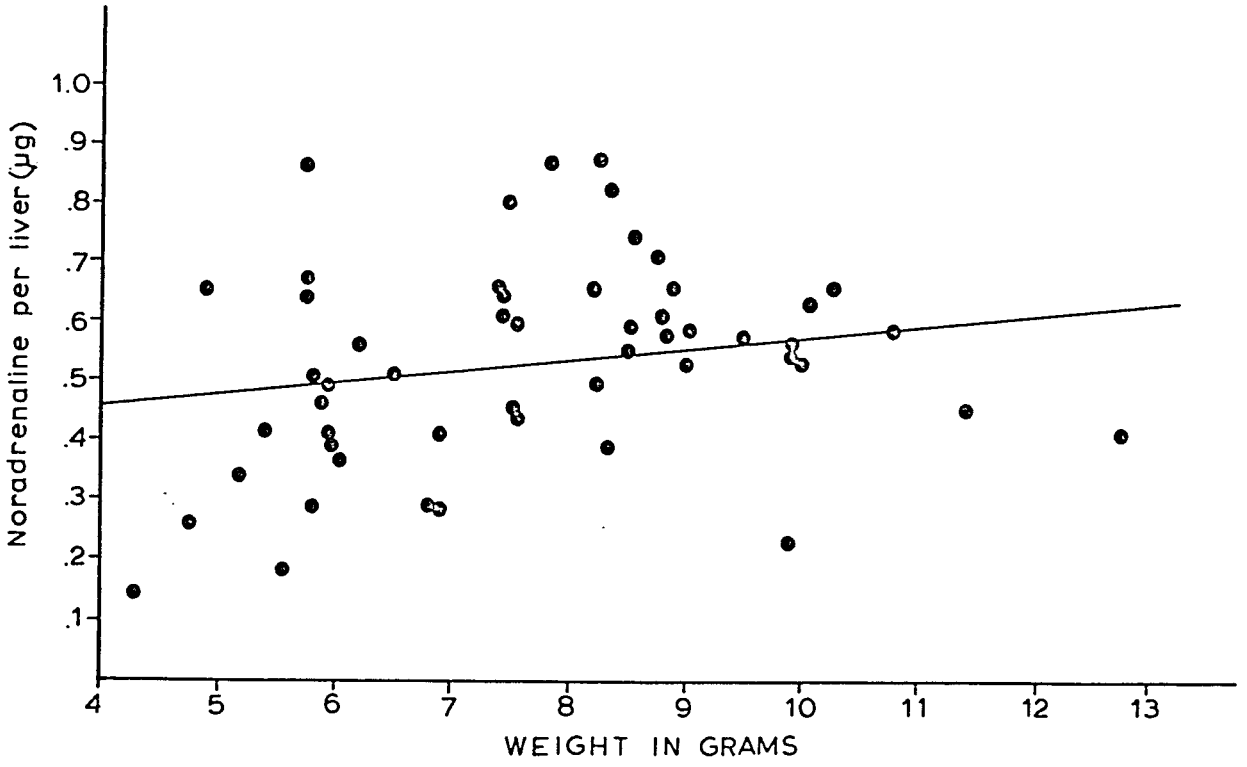
Number of rat	Normal Fasted‡ µg/g	Normal Fasted‡ µg/liver	Hypophysectomized Thyroxine• µg/g	Hypophysectomized Thyroxine• µg/liver	Hypophysectomized Growth Hormone° µg/g	Hypophysectomized Growth Hormone° µg/liver	Hypophysectomized Thyroxine and Growth Hormone⊙ µg/g	Hypophysectomized Thyroxine and Growth Hormone⊙ µg/liver
1	.1490	.0582	.0871	.4912	.0586	.3375	.0646	.5179
2	.1331	.6522	.0274	.1449	.0780	.4564	.0771	.5582
3	.1162	.6704	.0339	.1949	.0587	.3704	.0286	.2371
4	.0313	.1740	.0776	.4865	.0822	.5384	.0215	.1718
5	.0758	.4093	.0265	.1762	.0479	.2883	.0486	.4920
6	.0657	.3423	.0707	.3863	.0879	.5282	.0669	.5191
7	.0796	.4680	.0860	.5194	.0576	.3565	.0688	.5146
8	.1115	.6411	.0722	.4426	.0815	.4776	.0172	.1417
9	.0498	.2883	.0303	.1687	.0694	.4039	.0800	.6256
10	.0611	.4817	.0732	.4260	.0849	.4958	.0549	.4348
11	.0471	.2849	.0545	.3106	.0543	.3491	.0791	.6193
12	.0747	.4041	.0173	.0974				
13	.0318	.1453	.0377	.2247				
14	.0843	.3645	.0497	.3016				
15	.0410	.2542	.0643	.3884				
16	•		.0345	.1725				
Mean	.0761±.037*	.4292 ±.200	.0551±.024	.3082 ±.140	.0691±.014	.4183 ±.085	.0552±.020	.4392±.170
% Change			- 28%	- 28%	- 11%	- 2%	- 29%	+2%
Significance			P > .1	P > .1	P > .4	P > .5	P > .05	P > .5

* Standard deviation
† All rats were given ordinary water as drinking fluid
‡ Normal rats were fasted for 30 days

• 1.0375mg injected over a period of 25 days
° 1mg injected daily for 16 days
⊙ .0625mg of thyroxine and 16mg of growth hormone injected concurrently for 16 days

Figure IX

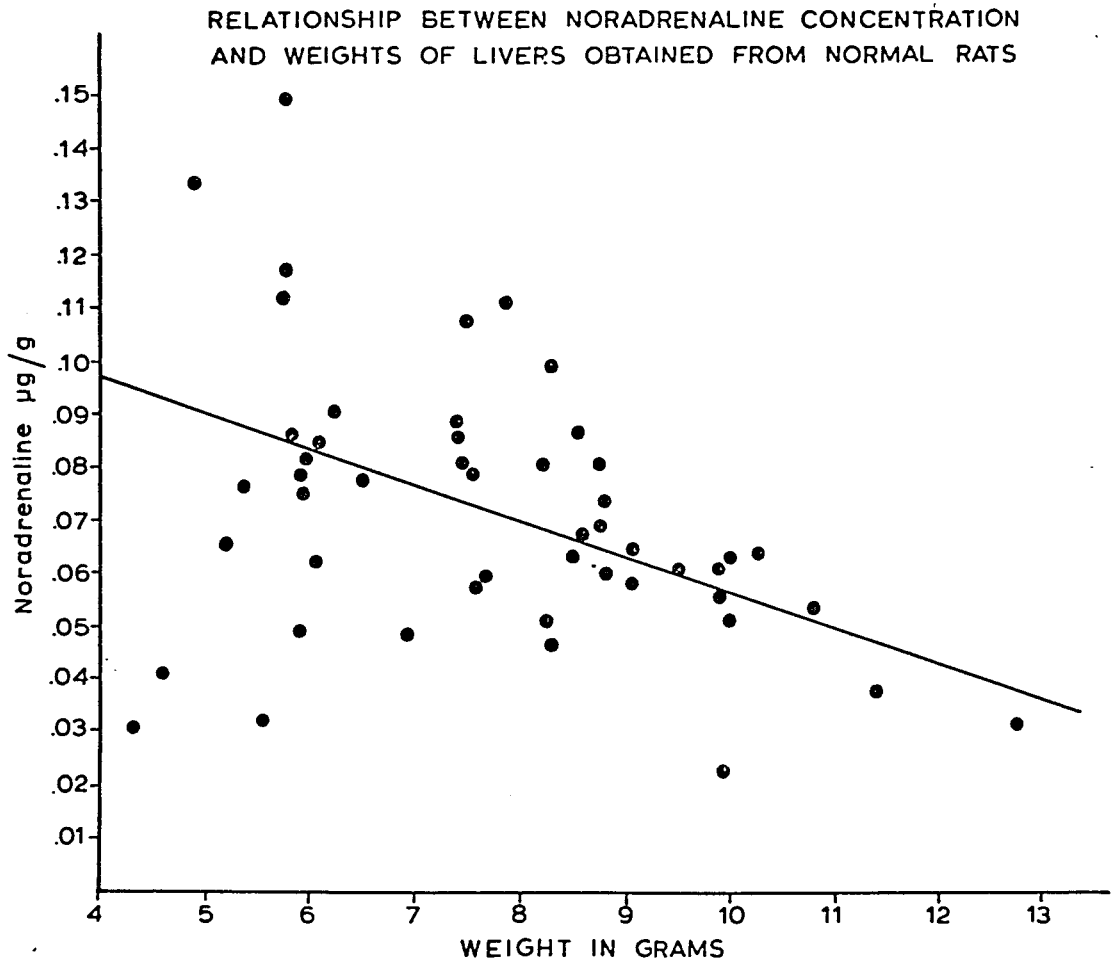
RELATIONSHIP BETWEEN TOTAL NORADRENALINE CONTENT AND WEIGHTS OF LIVERS OBTAINED FROM NORMAL RATS



Line plotted by the method of least squares

normal, whereas the livers of thyroxine-treated hypophysectomized rats were still 23% lower. In the growth hormone group the weights of livers were in an intermediate level between the other two hormone groups. With the other series, Table 42 shows no significant differences in the noradrenaline content of either three or eight weeks-hypophysectomized rats. The difference in liver weight between the experimental and normal group was much less in this series. In Table 43 also, no significant difference is observed in the noradrenaline content of the different groups of rats treated with the hormones, the weights of normal livers being in this case lower or comparable to the other experimental groups. It may be apparent from these results that there may be a certain dependance of the noradrenaline content per liver on the weight of this tissue. Indeed, it may be observed from Figure IX that the total noradrenaline content of normal livers was not strictly independent of liver weight, contrary to that found for the heart. This effect in the liver was not very marked as can be judged from the slope of the regression line. The difference in the total noradrenaline content of livers weighing between 5 and 10 grams is of the order of 0.1µg. However, this difference is such that it could reduce to insignificant levels the decrease in total noradrenaline

Figure X



Line plotted by the method of least squares

Table 44

ADRENALINE CONTENT OF LIVERS OBTAINED FROM NORMAL AND HYPOPHYSECTOMIZED RATS†

Number of rat	Three weeks×				Eight weeks			
	µg/g		µg/liver		µg/g		µg/liver	
	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized
1	.0021	.0002	.0181	.0008	.0053	.0033	.0343	.0182
2	.0011	0	.0145	0	.0042	.0036	.0368	.0163
3	.0013	.0003	.0139	.0011	.0061	.0042	.0508	.0212
4	.0011	.0008	.0108	.0040		.0033		.0129
5	.0015	0	.0167	0				
6	0	.0009	0	.0052				
7		.0020		.0079				
8		0		0				
Mean	.0011±.0007*	.0005±.0007	.0123±.0065	.0023±.0029	.0052±.0009	.0036±.0004	.0406±.0089	.0171±.0035
% Change	- 50%		- 81%		- 30%		- 57%	
Significance	P > .1		P < .01		P > .02		P < .01	

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
× Time after hypophysectomy

content of livers of hypophysectomized rats which was observed in the first series, since in this case, the livers were approximately half those of normals. To put it in other words, part of this decrease was due to the low liver weights of the hypophysectomized rats. Likewise, the apparent beneficial effect of hormonal treatment on the liver noradrenaline content may be explained by an indirect effect of the hormones on the weights of livers. Figure X shows a more prominent dependency of the concentration of noradrenaline on the liver weight of normal rats. This effect of liver weight is almost quantitatively comparable to that found for heart; however, this relationship does not present a problem since no significant differences or consistent trend were observed between the liver noradrenaline concentration of any experimental group and that of the normal group. Thus, it does not appear that hypophysectomy significantly modified the noradrenaline content of livers.

In the case of adrenaline a different situation is present. In the first series it may be observed in Table 44 that hypophysectomy caused a marked decrease in the total adrenaline content of livers. Moreover, this decrease was such that it could be reflected by a corresponding decrease in the concentration of adrenaline despite the low weight of livers of hypophysectomized rats. This decrease in the concentration of liver adrenaline was smaller and not

Table 45

ADRENALINE CONTENT OF LIVERS OBTAINED FROM VARIOUS GROUPS OF RATS †

Number of rat	Normal Fasted † µg/g	Normal Fasted † µg/liver	Hypophysectomized Thyroxine • µg/g	Hypophysectomized Thyroxine • µg/liver	Hypophysectomized Growth Hormone ○ µg/g	Hypophysectomized Growth Hormone ○ µg/liver	Hypophysectomized Thyroxine and Growth Hormone ⊙ µg/g	Hypophysectomized Thyroxine and Growth Hormone ⊙ µg/liver
1	.0020	.0170	.0040	.0239	.0026	.0253	.0032	.0227
2	.0029	.0212	.0026	.0172	.0020	.0134	.0017	.0111
3	.0034	.0250	.0042	.0226	.0036	.0280	.0029	.0215
4	.0034	.0302	.0039	.0220	.0045	.0318	.0050	.0314
5	.0041	.0255	.0044	.0299	.0029	.0203	.0034	.0226
6	.0022	.0164	.0031	.0193	.0047	.0316	.0019	.0171
7	.0026	.0194	.0014	.0085			.0042	.0412
8	.0052	.0429	.0028	.0194				
9	.0044	.0384	.0040	.0238				
10	.0018	.0165	.0022	.0116				
11	.0055	.0565	.0058	.0388				
12	.0019	.0158						
Mean	.0033 ± .001 *	.0270 ± .013	.0035 ± .001	.0215 ± .008	.0031 ± .001	.0250 ± .007	.0032 ± .001	.0239 ± .010
% Change			+ 6%	- 20%	+ 3%	- 7%	- 3%	- 11%
Significance			P > .5	P > .5	P > .5	P > .5	P > .5	P > .5

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
‡ Normal rats were fasted for 28 days

• 1.237mg injected over a period of 26 days
○ 1mg injected daily for 26 days
⊙ Same dose of each hormone injected concurrently for 26 days.

Table 46

ADRENALINE CONTENT OF LIVERS OBTAINED FROM NORMAL AND HYPOPHYSECTOMIZED RATS[†Δ]

Number of rat	Three weeks[×]				Eight weeks			
	µg/g		µg/liver		µg/g		µg/liver	
	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized
1	.0039	.0046	.0386	.0274	.0099	.0023	.0589	.0114
2	.0037	.0038	.0337	.0254	.0062	.0035	.0456	.0197
3	.0057	.0016	.0469	.0117	.0040	.0052	.0233	.0279
4	.0037	.0020	.0316	.0114	.0050	.0059	.0274	.0280
5	.0051	.0023	.0436	.0143	.0048	.0026	.0282	.0137
6	.0026	.0026	.0259	.0185		.0043		.0222
7						.0035		.0167
Mean	.0041±.0011[*]	.0028±.0011	.0367±.0078	.0181±.0070	.0059±.0023	.0039±.0011	.0366±.0150	.0220±.0073
% Change	−31%		−50%		−34%		−40%	
Significance	P > .05		P < .01		P > .05		P > .02	

* Standard deviation
† All rats were given ordinary water as drinking fluid
× Time after hypophysectomy
Δ Normal rats with which eight hypophysectomized rats are compared were fasted for 20 days

Table 47

ADRENALINE CONTENT OF LIVERS OBTAINED FROM VARIOUS GROUPS OF RATS †

Number of rat	Normal Fasted ‡		Hypophysectomized					
			Thyroxine •		Growth Hormone ○		Thyroxine and Growth Hormone ⊙	
	µg/g	µg/liver	µg/g	µg/liver	µg/g	µg/liver	µg/g	µg/liver
1	.0021	.0122	.0043	.0240	.0044	.0255	.0028	.0222
2	.0042	.0204	.0033	.0173	.0027	.0158	.0026	.0189
3	.0044	.0253	.0045	.0260	.0044	.0275	.0054	.0447
4	.0049	.0272	.0026	.0173	.0042	.0273	.0032	.0258
5	.0042	.0228	.0044	.0241	.0043	.0235	.0023	.0230
6	.0051	.0268	.0041	.0248	.0041	.0250	.0051	.0398
7	.0037	.0220	.0044	.0244	.0014	.0085	.0030	.0228
8	.0043	.0247	.0045	.0260	.0025	.0158	.0042	.0349
9	.0078	.0453	.0044	.0251	.0028	.0163	.0040	.0235
10	.0035	.0206	.0052	.0336	.0053	.0311	.0025	.0196
11	.0071	.0383	.0031	.0188	.0026	.0168	.0035	.0277
12	.0063	.0287	.0053	.0321				
13	.0061	.0264	.0037	.0223				
14	.0023	.0143	.0043	.0240				
Mean	.0047± .002 *	.0253±.008	.0041±.001	.0242±.005	.0035±.001	.0211±.007	.0035±.001	.0275±.008
% Change			−12%	− 4%	−25%	−16%	−25%	+8%
Significance			P > .2	P > .5	P > .1	P = .2	P > .05	P > .5

* Standard deviation
† All rats were given ordinary water as drinking fluid
‡ Normal rats were fasted for 30 days

• 1.0375mg injected over a period of 25 days
○ 1mg injected daily for 16 days
⊙ .0625mg of thyroxine and 16mg of growth hormone injected concurrently for 16 days.

statistically significant but was a consistent finding, as will be seen in the second series. It may be seen in Table 45 that hormonal treatment of hypophysectomized rats reduced these differences to insignificant changes. In the second series, Table 46, it can be seen that as found previously there is a decrease in the total adrenaline content of livers of hypophysectomized rats accompanied by a lower decrease in the concentration of adrenaline. Again, Table 47 shows that hormonal treatment reduced these changes to insignificant differences. It is difficult to decide which treatment was more effective since these differences are so small. The amount of adrenaline present in the liver, being so small, does not permit accurate discrimination between these differences.

It could be objected that these effects of the hormones on the total adrenaline content of livers, in general, would probably not be significant if compared to the values obtained for hypophysectomized rats. Reasons for this could entail such factors as slight variation in preparations of reagents which do bore on the sensitivity of analysis, and other factors which could not be controlled. On the other hand, it is felt that evaluation is more accurately possible when the experimental values are compared with normal values obtained under the same conditions of analysis as well as experimental conditions identical or

Table 48

CATECHOLAMINE CONTENT AND WEIGHTS OF LIVERS OBTAINED FROM VARIOUS GROUPS OF RATS†

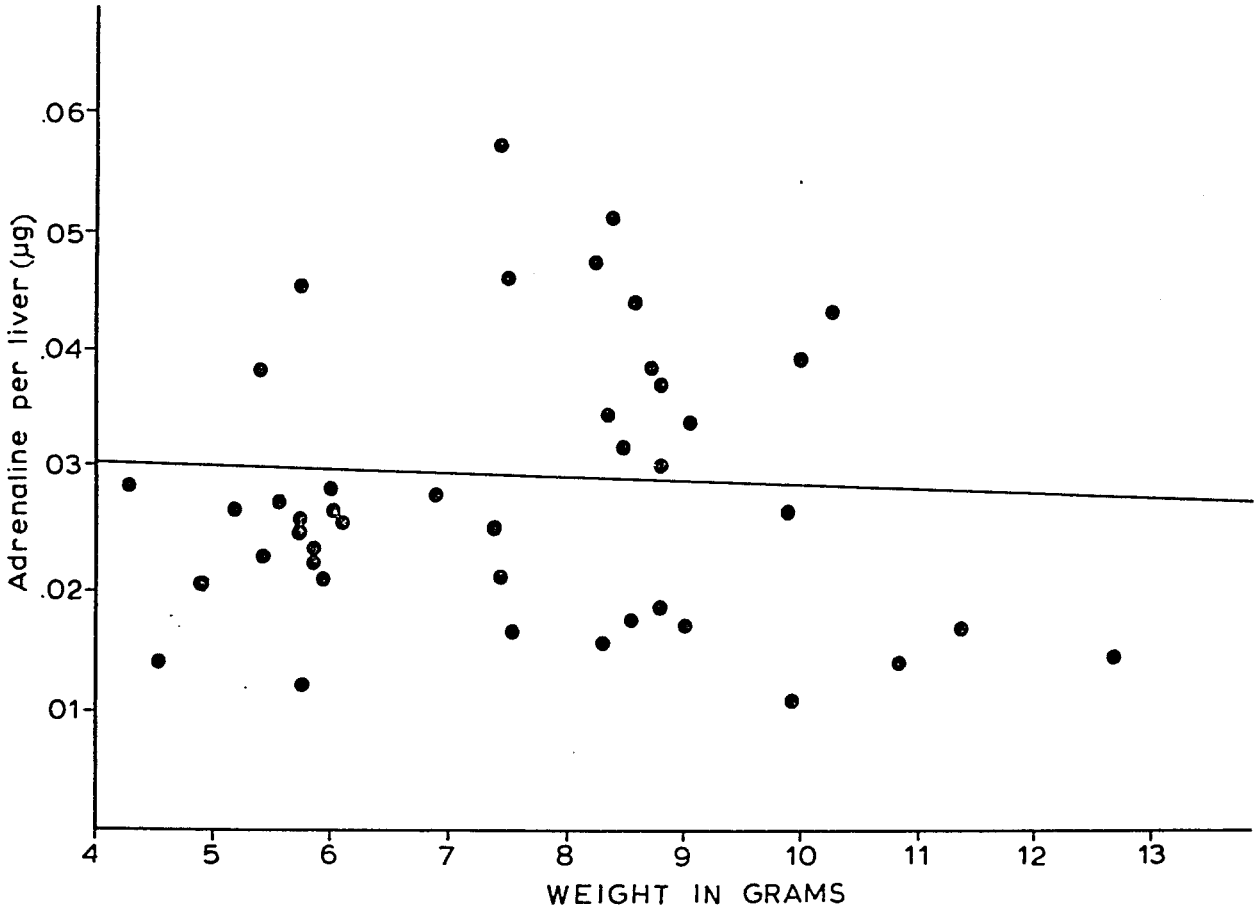
	Time after Hypophysectomy Weeks	Group	Noradrenaline µg/g	Noradrenaline µg/liver	Adrenaline µg/g	Adrenaline µg/liver	Liver Weight g
A*	-	Normal	.0508 ± .120	.5572 ± .084	.0011 ± .0007	.0123 ± .0065	10.62 ± 1.35
	3	Hypophysectomized	.0735 ± .020	.3638 ± .096	.0005 ± .0007	.0023 ± .0029	4.99 ± .72
	-	Normal	.0635 ± .013	.5185 ± .097	.0052 ± .0009	.0406 ± .0089	8.26 ± 1.3
	8	Hypophysectomized	.0665 ± .009	.3329 ± .054	.0036 ± .0004	.0171 ± .0035	5.05 ± .88
	-	Normal Δ	.0803 ± .016	.6481 ± .125	.0033 ± .0013	.0271 ± .013	8.18 ± .87
	11	Thyroxine 1	.0775 ± .011	.4721 ± .075	.0035 ± .0012	.0215 ± .0082	6.25 ± .55
	11	Growth Hormone 1	.0713 ± .124	.5256 ± .081	.0034 ± .0011	.0250 ± .0072	7.75 ± .96
	11	Thyroxine + Growth Hormone 1	.0780 ± .165	.5739 ± .142	.0032 ± .0012	.0239 ± .0097	8.03 ± .13
B×	-	Normal	.0543 ± .016	.4847 ± .130	.0041 ± .0011	.0367 ± .0078	9.06 ± .23
	3	Hypophysectomized	.0733 ± .0085	.4730 ± .065	.0028 ± .0011	.0181 ± .007	6.45 ± .18
	-	Normal ▲	.0735 ± .024	.4822 ± .190	.0059 ± .0023	.0366 ± .015	6.30 ± .70
	8	Hypophysectomized	.0614 ± .014	.3194 ± .080	.0039 ± .0011	.0220 ± .0073	5.04 ± .52
	-	Normal ▽	.0781 ± .037	.4292 ± .200	.0047 ± .0017	.0253 ± .0085	5.50 ± .83
	10	Thyroxine 2	.0561 ± .024	.3082 ± .140	.0041 ± .0008	.0242 ± .0047	5.90 ± .38
	10	Growth Hormone 2	.0691 ± .014	.4183 ± .085	.0035 ± .0012	.024 ± .0069	6.01 ± .32
	10	Thyroxine + Growth Hormone 2	.0552 ± .020	.4392 .170	.0035 ± .001	.0275 ± .009	7.94 ± .88

† Mean values are given with standard deviation
* All rats were given 5% sucrose as drinking fluid
× All rats were given ordinary water as drinking fluid
Δ Fasted for 28 days
▲ Fasted for 20 days
▽ Fasted for 30 days

1 See footnotes Table 32
2 See footnotes Table 34

Figure XI

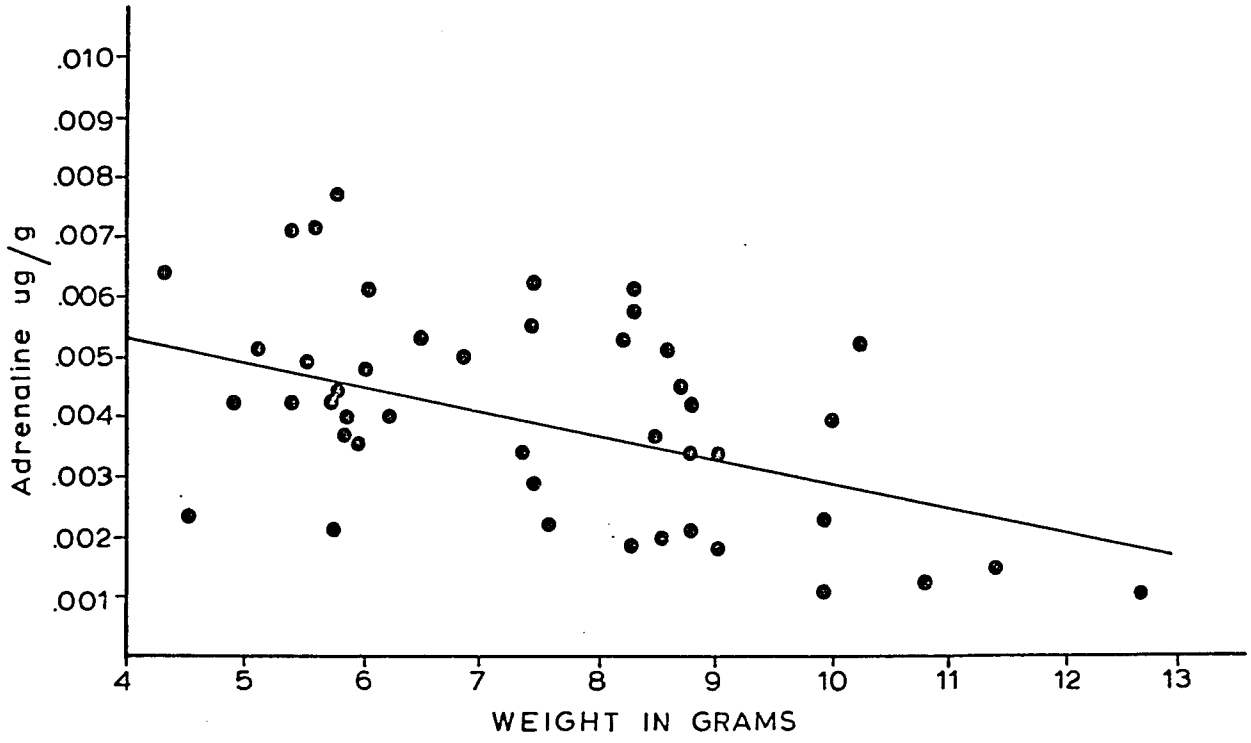
INDEPENDANT VARIATION BETWEEN TOTAL ADRENALINE CONTENT AND WEIGHTS OF LIVERS OBTAINED FROM NORMAL RATS



Line plotted by the method of least squares

Figure XII

RELATIONSHIP BETWEEN ADRENALINE CONCENTRATION AND WEIGHTS OF LIVERS OBTAINED FROM NORMAL RATS



Line plotted by the method of least squares

more similar to hormone-treated groups. Figure XI shows that the total adrenaline present in the liver is independent of the weight of this organ. Thus it appears that the results obtained in considering the total adrenaline content gives a true picture of the effects obtained. On the other hand, the adrenaline concentration seems to be dependent on the size of liver as can be noted in Figure XII. However, as already mentioned the decrease in total liver adrenaline of hypophysectomized rats was great enough to overcome this effect and decreases in adrenaline content of livers from hypophysectomized rats were consistently obtained with both means of expression. The data are summarized in Table 49.

In conclusion, it may be said that hypophysectomy does not appear to modify significantly the noradrenaline content of livers and likewise hormonal treatment does not bring any change. But the hepatic adrenaline content of hypophysectomized rats was decreased by about 50%. Administration of thyroxine, growth hormone or both reduced this difference to a non-significant change.

In the brain

The fact that very little variation was found in the brain weight of the various groups of rats greatly

Table 49

NORADRENALINE CONTENT OF BRAINS OBTAINED FROM NORMAL AND HYPOPHYSECTOMIZED RATS†

Number of rat	Three weeks×				Eight weeks			
	µg/g		µg/brain		µg/g		µg/brain	
	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized
1	.4528	.4271	.7199	.6577	.4104	.4293	.6896	.6697
2	.3586	.4389	.6239	.5616	.3953	.4892	.6975	.7177
3	.3712	.5033	.5976	.7146	.4247	.4102	.7687	.6975
4	.3758	.4359	.6463	.6960	.5069	.4859	.7047	.8310
5	.2926	.4462	.4798	.7094	.4389	.4669	.7242	.7425
6		.3886		.6178				
7		.4209		.6944				
Mean	.3702±.057*	.4371±.034	.6135±.087	.6645±.057	.4332±.045	.4561±.035	.7169±.032	.7316±.061
% Change		+18%		+8%		+5.2%		+2.5%
Significence		P > .02		P>.2		P>.2		P>.3

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
× Time after hypophysectomy

Table 50

NORADRENALINE CONTENT OF BRAINS OBTAINED FROM VARIOUS GROUPS OF RATS†

Number of rat	Normal		Hypophysectomized					
	Fasted‡		Thyroxine•		Growth Hormone○		Thyroxine and Growth Hormone○	
	µg/g	µg/brain	µg/g	µg/brain	µg/g	µg/brain	µg/g	µg/brain
1	.3877	.6982	.3796	.6075	.4098	.7132	.4019	.7597
2	.4164	.6997	.3966	.7020	.4286	.7672	.3222	.6090
3	.4060	.7147	.4631	.8152	.4495	.7192	.3557	.6225
4	.4240	.7845	.4093	.7410	.5345	.8820	.3022	.6045
5	.4241	.7380	.2511	.4245	.4168	.6877	.3608	.7252
6	.4769	.8490	.3964	.7057	.4836	.8512	.3668	.6090
7	.4542	.8040	.4075	.7327	.5027	.8100	.3915	.6930
8	.4321	.7432	.4192	.6641			.3395	.6612
9	.4339	.7117	.2468	.4147			.3508	.6525
10	.4273	.7777						
11	.4259	.7710						
12	.3710	.7172						
13	.4096	.7620						
14	.3710	.6567						
15	.3948	.7462						
16	.3724	.6480						
Mean	.4142±.030*	.7368±.052	.3744±.074	.6452±.140	.4607±.047	.7757±.074	.3546±.031	.6767±.059
% Change			−9%	−12%	+11%	+4%	−14%	−8%
Significance			P > .05	P ≥ .02	P < .01	P > .3	P < .01	P < .02

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
‡ Normal rats were fasted for 28 days

• 1.237mg injected over a period of 26 days
○ 1mg injected daily for 26 days
⊙ Same dose of each hormone injected concurrently for 26 days.

Table 51

NORADRENALINE CONTENT OF BRAINS OBTAINED FROM NORMAL AND HYPOPHYSECTOMIZED RATS †Δ

Number of rat	Three Weeks×				Eight Weeks			
	µg/g		µg/brain		µg/g		µg/brain	
	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized
1	.3353	.3977	.5466	.6285	.5030	.4475	.6892	.6400
2	.3661	.3258	.5707	.4920	.4044	.4208	.5622	.7029
3	.3422	.4450	.5715	.6765	.3893	.5264	.6386	.8055
4	.4581	.5284	.6808	.8032	.4300	.5436	.6210	.8535
5	.4647	.6185	.6042	.7590	.4109	.4820	.6486	.7665
6	.4151	.4632	.6387	.7319		.4846		.7620
7						.4190		.7201
8						.5265		.7740
Mean	.3966±.057*	.4631±.100	.6095±.050	.6818±.011	.4275±.044	.4780±.049	.6363±.045	.7530±.065
% Change	+14%		+11%		+12%		+18%	
Significance	P > .1		P > .1		P > .02		P < .01	

* Standard deviation
† All rats were given ordinary water as drinking fluid
× Time after hypophysectomy
Δ Normal rats with which eight weeks hypophysectomized rats are compared were fasted for 20 days

facilitates evaluation of data; the results then are at least qualitatively the same with both means of expression.

In the first series Table 49 shows the noradrenaline content of brains obtained from hypophysectomized rats compared to normal rats. No statistical differences can be observed between the noradrenaline content of the experimental and normal groups but small increases may be noted in the brain of hypophysectomized rats. The effect of hormonal treatment on the brain noradrenaline of hypophysectomized rats is shown in Table 50. Administration of growth hormone to hypophysectomized rats did not bring much change, the brain noradrenaline content in this group being still higher than normal. However, treatment of hypophysectomized rats with thyroxine alone or in combination with growth hormone decreased the noradrenaline content to levels which are slightly below normal. This effect is statistically significant when both hormones are given and with both means of expression but the differences are not as significant when thyroxine alone is administered. In the second series of experiments, it may be observed (Table 51) that, as found previously, the noradrenaline content of brains from hypophysectomized rats is higher than normal. But still, this effect is not very prominent and is not statistically significant except for eight weeks-hypophysectomized rats.

Table 52

NORADRENALINE CONTENT OF BRAINS OBTAINED FROM VARIOUS GROUPS OF RATS †

Number of rat	Normal		Hypophysectomized					
	Fasted ‡		Thyroxine •		Growth Hormone ○		Thyroxine and Growth Hormone ⊙	
	µg/g	µg/brain	µg/g	µg/brain	µg/g	µg/brain	µg/g	µg/brain
1	.4001	.6442	.2698	.4290	.3023	.4860	.3191	.5745
2	.4191	.6622	.3294	.5205	.3125	.5062	.2575	.4507
3	.4318	.7297	.2393	.4117	.3264	.5092	.2089	.3802
4	.5011	.7867	.2746	.4942	.2817	.5212	.2478	.4387
5	.3629	.5625	.1698	.2752	.3379	.5407	.2373	.4057
6	.3812	.5909	.2089	.3615	.2922	.4762	.2987	.5377
7	.3226	.4968	.2498	.4342	.3670	.6202		
8	.3863	.6645	.2500	.4200	.3925	.6202		
Mean	.4006±.053 *	.6421±.092	.2489±.047	.4182±.070	.3265±.038	.5349±.056	.2615±.041	.485±.076
% Change			-37%	-34%	-18%	-16%	-32%	-27%
Significance			P < .01	P < .01	P < .01	P < .02	P < .01	P < .02

* Standard deviation
† All rats were given ordinary water as drinking fluid
‡ Normal rats were fasted for 30 days
• 1.0375mg injected over a period of 25 days
○ 1mg injected daily for 16 days
⊙ .0625mg of thyroxine and 16mg of growth hormone injected concurrently for 16 days.

Table 53

ALKALINE CONTENT OF BRAINS OBTAINED FROM NORMAL AND HYPOPHYSECTOMIZED RATS†

Number of rat	Three weeks×				Eight weeks			
	µg/g		µg/brain		µg/g		µg/brain	
	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized
1	.0067	.0083	.0116	.0127	.0095	.0093	.0159	.0145
2	.0104	.0101	.0167	.0128	.0169	.0192	.0306	.0282
3	.0115	.0055	.0198	.0078	.0111	.0095	.0198	.0162
4	.0152	.0060	.0249	.0095	.0059	.0139	.0081	.0239
5		.0036		.0058	.0068	.0118	.0111	.0188
6		.0071		.0112				
7		.0013		.0021				
Mean	.0109±.0035*	.0060±.0029	.0182±.0056	.0081±.0039	.0100±.004	.0127±.0040	.0171±.008	.0203±.0057
% Change	- 45%		- 51%		+ 27%		+ 18%	
Significance	P > .02		P < .01		P > .3		P > .5	

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
× Time after hypophysectomy

Table 54

ADRENALINE CONTENT OF BRAINS OBTAINED FROM VARIOUS GROUPS OF RATS†

Number of rat	Normal Fasted‡		Hypophysectomized					
			Thyroxine•		Growth Hormone○		Thyroxine and Growth Hormone⊙	
	µg/g	µg/brain	µg/g	µg/brain	µg/g	µg/brain	µg/g	µg/brain
1	.0049	.0088	.0104	.0166	.0132	.0217	.0070	.0132
2	.0045	.0075	.0018	.0032	.0092	.0152	.0068	.0154
3	.0050	.0089	.0126	.0228	.0105	.0186	.0099	.0198
4	.0044	.0079	.0065	.0111	.0081	.0131	.0050	.0100
5	.0082	.0141	.0054	.0096			.0099	.0164
6	.0052	.0085	.0036	.0064			.0085	.0150
7	.0036	.0065	.0067	.0106			.0069	.0134
8	.0025	.0046	.0028	.0048			.0117	.0220
9	.0041	.0080						
10	.0083	.0155						
11	.0094	.0167						
12	.0021	.0040						
13	.0014	.0024						
Mean	.0049±.002*	.0087 ± .004	.0062±.004	.0106 ± .006	.0102±.002	.0171±.004	.0085±.002	.0156±.003
% Change			+ 26%	+ 21%	+108%	+96%	+73%	+79%
Significance			P = .3	P ≥ .3	P < .01	P < .01	P < .01	P < .01

* Standard deviation
† All rats were given 5% sucrose as drinking fluid
‡ Normal rats were fasted for 28 days
• 1.235mg injected over a period of 26 days
○ 1mg injected daily for 26 days
⊙ Same dose of each hormone injected concurrently for 26 days

Table 55

ADRENALINE CONTENT OF BRAINS OBTAINED FROM NORMAL AND HYPOPHYSECTOMIZED RATS†Δ

Number of rat	Three weeks×				Eight weeks			
	γ/g		γ/brain		γ/g		γ/brain	
	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized	Normal	Hypophysectomized
1	.0069	.0116	.0113	.0194	.0079	.0054	.0103	.0078
2	.0078	.0102	.0122	.0154	.0133	.0130	.0135	.0217
3	.0071	.0050	.0118	.0076	.0066	.0145	.0108	.0222
4	.0143	.0226	.0212	.0277	.0195	.0102	.0282	.0150
5	.0135	.0032	.0176	.0049	.0106	.0071	.0180	.0111
6	.0102	.0047	.0172	.0075		.0083		.0132
7						.0120		.0197
8						.0043		.0074
Mean	.0106±.0037	.0095±.0072	.0152±.0040	.0135±.0086	.0115±.0051	.0093±.0036	.0172±.0071	.0147±.0060
% Change	-10%		-11%		-19%		-14%	
Significance	P > .5		P > .5		P > .3		P > .4	

* Standard deviation
† All rats were given ordinary water as drinking fluid
× Time after hypophysectomy
Δ Normal rats with which eight weeks hypophysectomized rats are compared were fasted for 20 days

Table 56

ADRENALINE CONTENT OF BRAINS OBTAINED FROM VARIOUS GROUPS OF RATS †

Number of rat	Normal		Hypophysectomized					
	Fasted‡		Thyroxine•		Growth Hormone°		Thyroxine and Growth Hormone⊙	
	µg/g	µg/brain	µg/g	µg/brain	µg/g	µg/brain	µg/g	µg/brain
1	.0111	.0176	.0124	.0198	.0125	.0201	.0100	.0180
2	.0104	.0177	.0099	.0156	.0116	.0189	.0092	.0161
3	.0069	.0108	.0108	.0186	.0111	.0174	.0088	.0160
4	.0053	.0082	.0101	.0183	.0092	.0170	.0079	.0140
5	.0115	.0178	.0072	.0117	.0130	.0209	.0069	.0123
6	.0051	.0078	.0089	.0154	.0092	.0150	.0092	.0157
7	.0087	.0150	.0079	.0138	.0086	.0145		
8			.0087	.0147	.0108	.0171		
Mean	.0084±.003*	.0135±.004	.0094±.002	.0159±.003	.0107±.002	.0176±.002	.0086±.001	.0153±.002
% Change			+11%	+17%	+27%	+30%	+2%	+13%
Significance			P > .3	P > .2	P < .01	P > .02	P > .5	P > .3

* Standard deviation
† All rats were given ordinary water as drinking fluid
‡ Normal rats were fasted for 30 days
• 1.0375mg injected over a period of 25 days
o 1mg injected daily for 16 days
⊙ .0625mg of thyroxine and 16mg of growth hormone injected concurrently for 16 days.

It can be seen, from Table 52, that as found before thyroxine administered alone or conjointly with growth hormone reduced the noradrenaline content of hypophysectomized rats to levels which are lower than normal. In this case however this effect was more pronounced being approximately two to three times that found previously. Growth hormone had similar effects although these were less than those found for thyroxine.

In the case of adrenaline (first series, Table 53) there appears to be a significant decrease of 50% in the amount of this catecholamine in the brains of rats which were hypophysectomized for three weeks. But eight weeks after hypophysectomy the adrenaline content of brain is not significantly different from normal. In Table 54 it may be observed that the adrenaline content of hypophysectomized rats treated with the different hormones is above normal but these differences are only statistically significant in the groups of rats injected with growth hormone alone or together with thyroxine. In the second series, Table 55 shows that there is no significant differences between the adrenaline content of brains from hypophysectomized rats compared to normal. Again, Table 56 shows that the adrenaline content of brains from hypophysectomized rats treated with thyroxine and growth hormone is higher than normal. However, these increases are much less than found before and are not as statistically

Table 57

CATECHOLAMINE CONTENT AND WEIGHTS OF BRAIN OBTAINED FROM VARIOUS GROUPS OF RATS †

	Time after hypophysectomy Weeks	Group	Noradrenaline µg/g	Noradrenaline µg/brain	Adrenaline µg/g	Adrenaline µg/brain	Brain Weight g
SERIES A*	-	Normal	.3702 ± .057	.6135 ± .087	.0109 ± .0035	.0182 ± .0056	1.62 ± .100
	3	Hypophysectomized	.4371 ± .034	.6645 ± .057	.0060 ± .0029	.0081 ± .0039	1.53 ± .130
	-	Normal	.4332 ± .045	.7169 ± .032	.0100 ± .0044	.0171 ± .0088	1.66 ± .170
	8	Hypophysectomized	.4561 ± .035	.7316 ± .061	.0127 ± .0039	.0203 ± .0057	1.60 ± .100
	-	Normal△	.4142 ± .030	.7388 ± .052	.0049 ± .0025	.0087 ± .0043	1.78 ± .063
	11	Thyroxine 1/	.3744 ± .074	.6452 ± .140	.0062 ± .0037	.0106 ± .0064	1.72 ± .085
	11	Growth Hormone 1/	.4607 ± .047	.7757 ± .074	.0102 ± .0022	.0171 ± .0038	1.70 ± .087
	11	Thyroxine + Growth Hormone 1/	.3546 ± .031	.6767 ± .059	.0085 ± .0021	.0156 ± .0035	1.83 ± .110
SERIES B×	-	Normal	.3969 ± .057	.6095 ± .050	.0106 ± .0037	.0152 ± .0040	1.55 ± .140
	3	Hypophysectomized	.4631 ± .100	.6818 ± .011	.0095 ± .0072	.0135 ± .0086	1.50 ± .140
	-	Normal▲	.4275 ± .044	.6363 ± .045	.0115 ± .0051	.0172 ± .0071	1.50 ± .140
	8	Hypophysectomized	.4788 ± .049	.7330 ± .065	.0093 ± .0036	.0147 ± .0060	1.57 ± .098
	-	Normal▽	.4006 ± .053	.6421 ± .092	.0084 ± .0027	.0135 ± .0045	1.60 ± .074
	10	Thyroxine 2	.2489 ± .047	.4182 ± .070	.0094 ± .0017	.0159 ± .0027	1.68 ± .075
	10	Growth Hormone 2	.3265 ± .038	.5349 ± .056	.0107 ± .0016	.0176 ± .0022	1.64 ± .091
	10	Thyroxine + Growth Hormone 2	.2615 ± .041	.4645 ± .076	.0086 ± .0011	.0153 ± .0019	1.76 ± .060

† Mean values are given with standard deviation
* All rats given 5% sucrose as drinking fluid
× All rats given ordinary water as drinking fluid
△ Fasted for 28 days
▲ Fasted for 29 days
▽ Fasted for 30 days
1/ See footnotes Table 32
2 See footnotes Table 34

significant.

The above mentioned data are summarized in Table 57 together with absolute brain weights.

In conclusion, the noradrenaline content of hypophysectomized rats tended to be slightly higher than normal and hormonal treatment especially thyroxine not only corrected this trend but decreased the noradrenaline content to levels that were below normal. In the case of adrenaline, no consistent change could be observed as a result of hypophysectomy. On the other hand, the brain adrenaline content of hypophysectomized animals treated with hormones tended to be high as compared to normal.

DISCUSSION

It is evident from the foregoing section that when one deals with changes in catecholamine content of tissue, which are induced by experimental conditions which also modify the size of the organs concerned, one is confronted with a serious problem. It is indeed difficult to decide whether these changes in catecholamine content are due as a consequence of these changes in weights or to a direct effect of the different experimental conditions. These difficulties were resolved by showing that in general, in normal organs the total noradrenaline or adrenaline content is not influenced by the size of the organs. Thus, where the results are expressed per organ, the various differences obtained can not be attributed to changes in size of organs and this mode of expression appears to be more valid. That the catecholamine content per organ is constant over a wide range of tissue weight has important consequences; it follows from this that small-size organs will have a greater concentration of catecholamine than larger-size organs. Thus, neglected weight factors in the evaluation of data can lead to false conclusions or apparently paradoxical results. In the foregoing section there are examples of these apparent contradictions when the

values are considered on a per gram basis and the weights of the normal organs are different from the experimental weights. However, when the weights of normal tissue are comparable to that of the experimental the results are no different than when the values are expressed per organ. It is possible that much of the confusion reported in the literature dealing with concentration of catecholamine is the result of inappropriate consideration of this possible weight variable.

This constancy in the total content of catecholamine in heart is in fact not surprising. There has been considerable evidence that the stores of noradrenaline are influenced by the presence of sympathetic nerves. Cooper et al (238) showed that in dogs denervation of heart causes a decrease in the catecholamine content. In dogs and sheep, sympathectomy lowered the noradrenaline content but apparently had no effect on adrenaline (239). Goodall (240) also demonstrated that degeneration of the adrenergic nerves is accompanied by a diminution of the catecholamine content of the organs supplied by these nerves. Similar findings were reported by Cannon and Lissak (241) in the heart and liver of cats. In vitro it was shown by Goodall and Kirshner (242) that sympathetic nerves are capable of synthesizing noradrenaline from tyrosine, and local synthesis of catecholamine by

heart has been demonstrated (243,244,245). Part of the endogenous catecholamine in heart has also been suggested to be derived from circulating catecholamine (246,245,248) and the uptake of both circulating noradrenaline and adrenaline has been shown to be dependent on the presence of sympathetic nerves (247,248). The catecholamines are found in greatest amount in the atria (249) which receive most of sympathetic innervation. It is evident then, that the amount of catecholamine is dependent on sympathetic nerve supply. It may be expected that nervous supply will not be influenced by the size of the organ it innervates and consequently the amount of catecholamine will logically remain unchanged in different size organs. Liver noradrenaline, in contrast to liver adrenaline or cardiac catecholamines, does not appear to be strictly independent of organ weight. It may be that sources other than of nervous origin may be responsible for the synthesis of noradrenaline in liver and these sources are affected by factors influencing the weight of this organ. However, this effect of weight on the total noradrenaline content of liver was small and may be considered to be negligeable with small differences in the weight of liver.

The decrease in noradrenaline and probably also the adrenaline content in hearts of hypophysectomized rats is of extreme interest regarding the corresponding low heart

rate observed in these animals. The chronotropic effect of both catecholamines on heart is well known, and this effect is elicited equally well with both adrenaline and noradrenaline (250,251). It has been a general finding that proper maintenance of the cardiac stores of catecholamine may be important for the normal functioning of heart. It has already been mentioned in the introduction of this thesis (page 37 to 42) that catecholamine depleting drugs such as reserpine and guanethidine alleviated the tachycardia observed in hyperthyroid animals. Similar results were also found in normal animals. Cooper et al (238) demonstrated that the decrease in catecholamine content of hearts of dogs induced by the denervation of these hearts resulted in an inhibition of the inotropic and chronotropic response to tyramine. Data provided by Macmillan (253) show that when atria were removed from rabbits previously treated with reserpine their rate of beating was 115/min. compared to 130/min. for normal rabbits. Trendelenburg and Gravenstein (254) observed that the heart rate of dogs whose myocardial catecholamine had been depleted by reserpine was slower and was not increased by accelerans nerve stimulation as readily as the hearts from normal animals. More striking are reports in which experimental heart failure in guinea pigs and dogs has been associated and attributed to a decrease in cardiac noradrenaline stores (255,256). Low myocardial catecholamine

content has also been found to occur in patients who have died from heart failure (257).

From the foregoing considerations, it is tempting to establish a relationship of cause to effect between the low catecholamine stores found in hearts of hypophysectomized rats and their low cardiac rate. However, as such, the data does not provide definite evidence that this is so nor is it always compatible with this hypothesis. In our experimental conditions there is a lack of strict correspondence between the stores of catecholamine and heart rate. In general, the percent decrease in both noradrenaline and adrenaline content per heart of hypophysectomized rats was greater than the corresponding percent decrease in heart rate. A similar disproportionality can be observed in the hormone-treated groups. Assuming that the decrease in catecholamine content of hypophysectomized rats is responsible for the low heart rate then, it is logical to expect that thyroxine, which increased the heart rate of hypophysectomized rats, will also increase the catecholamine content of these hearts. Although this was the case for noradrenaline, in the first series, the hearts of hypophysectomized rats treated with thyroxine alone or with growth hormone, still contained about 20% less noradrenaline than normal. The heart rates in these groups however were only 10% lower than normal. Growth hormone which was less

effective in restoring the heart rate of hypophysectomized rats had an effect on the noradrenaline content which was identical to that of thyroxine. In the second series, the heart rate of the thyroxine-treated groups was not different from normal but the catecholamine content per heart was significantly less than normal, at least in the case of noradrenaline, and this despite the small number of determinations made. The situation is somewhat similar to that arrived at by Barker and Nakiuchi (114) who obtained a decrease both in heart rate and myocardial noradrenaline content of rats treated with guanethidine. The decrease in catecholamine content was greater than the corresponding decrease in heart rate. Although thyroxine increased the heart rate to almost normal levels the hormone had no effect on the noradrenaline content. In our case, although thyroxine seemed to have some increasing effect on the noradrenaline content of hearts from hypophysectomized rats, this effect appears to be only partial and is no different than that obtained with growth hormone. Thus it is only reasonable to doubt that the observed decrease in noradrenaline content of heart of hypophysectomized rats is totally responsible for the observed decrease in the heart rate of these animals. It is even less likely that the synergism observed with thyroxine and growth hormone on the maximal cardiac performance of hypophysectomized rats (252) may be

explained by an increase above normal in the heart stores of the catecholamines.

With respect to the heart rate however, a total participation of low stores of catecholamine in the observed bradycardia of hypophysectomized rats cannot be at the present time definitely rejected. This opinion is based on the observation that hyperthyroidism has been classically associated with tachycardia. It is rather intriguing that doses of thyroxine which are sufficient to produce cardiac hypertrophy of hypophysectomized rats above that observed for normal rats have failed to also raise the heart rate above normal. Thus this relative insensitivity of hypophysectomized rats to the action of thyroxine on the cardiac rate may be explained by the lower content of myocardial noradrenaline. It may be surmised then, that a certain level of cardiac catecholamine may be necessary in order that thyroxine may fully exert its action on the heart rate. The absence of normal catecholamine stores may be due to the atrophy of the adrenals. Although adrenalectomy or adrenal demedullation was shown not to affect myocardial catecholamine content (258,259), repletion of myocardial catecholamine after depletion with reserpine treatment was slower in adrenalectomized rats (259).

It is unfortunate that the results obtained with adrenaline were not completely satisfactory. The low

adrenaline content of the growth hormone group, at best, confirmsthat the adrenaline content of hypophysectomized rats is low, assuming of course, that growth hormone has no depleting effect on the adrenaline content of hearts. It is impossible to decide however, whether or not thyroxine has some restoring effect on those low myocardial adrenaline stores. The finding that the adrenaline content of liver was also decreased in hypophysectomized rats does not lend support to the idea that the decrease in myocardial adrenaline may be linked in a special way to some hemodynamic factor such as heart rate or other.

The results partly confirm those obtained by Hokfelt (///) who reported a similar decrease in the noradrenaline content (µg/kg body wt.) of hearts from hypophysectomized rats. This decrease could be observed five days after hypophysectomy and reached about 33% that of normals nineteen days after the operation. On the other hand, contrary to our results, an increase was noted in adrenaline content (µg/kg body wt.) of hearts of hypophysectomized rats. Growth hormone was found to increase the noradrenaline content of hearts from hypophysectomized rats but to levels which were above those of normal rats. Growth hormone had no effect on myocardial adrenaline. The method used by Hokfelt for the determination of catecholamines was biological and this may account for the difference in results

obtained for adrenaline in the hearts of hypophysectomized rats.

In order to assess a true relationship of cause to effect between the cardiac catecholamine stores and heart rate it would be necessary to begin a more detailed and systematic study of the time relationship existing between these two parameters. It would then be possible to find out whether or not the decrease in cardiac catecholamine content precedes the decrease in heart rate, which is obviously a necessary prerequisite. More fruitful information could be gained also with the more sensitive radioactive methods involving studies of uptake or binding of catecholamine by heart in the diverse experimental groups of rats. It has already been mentioned in an earlier part of this thesis and this work confirms the opinion that results obtained with catecholamine contents may not provide a satisfactory answer to physiological problems. Possibly, this may be due to the lack of knowledge regarding the relative proportion of free active catecholamine or the subcellular distribution of catecholamine when this method of approach is used.

It should be mentioned also, that the possibility cannot be dismissed that other factors which have no direct relation to catecholamine may be partially or totally involved in these cardiac changes. Physiological

readaptation and nutritional factors have been mentioned but parasympathetic activity may also be another means by which the diverse effects could be obtained.

In conclusion, it may be said that although the data provide reasonable evidence that the cardiac stores of noradrenaline and most probably adrenaline also are reduced in hypophysectomized rats, it cannot be decided from thesedata alone that these changes are totally responsible for the low heart rate and hypotension observed in such rats.

In the livers, no important change occurred in the noradrenaline content of this organ in hypophysectomized animals. This is in accordance with Hokfelt's results (111). But contrary to our findings this worker found that hypophysectomy increased the adrenaline content of livers. This discrepancy between his results and ours, when the adrenaline is considered is very suggestive that methodology may be a contributing factor. It is felt that perhaps some substances other than adrenaline may have been present in Hokfelt's samples and may have been detected by his biological method used for the determination of catecholamine. At any rate, our results show that the liver adrenaline content of rats was diminished by hypophysectomy.

This low adrenaline content may be of importance with regard to the extreme sensitivity of the hypophysectomized animals to insulin (240, 241, 242, 243). Several

explanations were offered to explain this reaction. Wall et al (260) concluded from their work that the hypophysectomized animals were more sensitive to the action of insulin because A) the uptake of glucose under the influence of insulin was greater in hypophysectomized dogs than normal and B) the livers of hypophysectomized dogs were not as effective as normal livers in discharging glucose in blood to overcome the hypoglycemia resulting from insulin. The latter observation in turn may be the result of decreased gluconeogenesis or decreasedglycogenolysis. Evidence for decreasedgluconeogenesis is provided by reports showing greatly diminished nitrogen excretion in fasting hypophysectomized dogs (264, 265, 266). On the other hand, the involvement of adrenaline in the decreased glycogenolysis has been a debated question. Several workers claimed that the hyperglycemia resulting from subcutaneous injections of adrenaline was less in hypophysectomized rats than in normals (267, 268, 269). Russel and Cori (270) attributed these differences to lack of absorption of subcutaneously administered adrenaline since in their hands intravenous injection did not bring any change in the hyperglycemic response of hypophysectomized animals compared to normals. However, results contrary to those of Russel and Cori were obtained by other workers using perfused liver preparations (272, 273, 274). Furthermore, de Bodo et al (271) were able

to show that the amount of liver glycogen in hypophysectomized animals was not a limiting factor in the low hyperglycemic response to adrenaline observed in these animals. These authors also showed that when the anterior pituitary was kept intact, normal blood sugar responses to adrenaline were obtained. The data obtained with the adrenaline content of livers of hypophysectomized rats give additional evidence that adrenaline may be concerned in the sensitivity of hypophysectomized rats to insulin but in a different way than that proposed by the above mentioned workers (de Bodo et al (271). Although an increase in the release of glucose from the liver, as a result of hypoglycemia, may be attributed to an increase in the release of adrenaline from the adrenals, this may not necessarily be the case and the adrenals need not participate in this process. Using C^{14} glucose de Bodo et al (275) were able to demonstrate that administration of growth hormone to hypophysectomized dogs increased the rate of glucose release from the liver. In another paper (276) it was concluded that growth hormone also enhanced glucose release from the liver of hypophysectomized dogs as a result of hypoglycemia induced by insulin. No explanation is given on the mechanism by which these effects are brought about. Our results show that low adrenaline content occurs in the livers of hypophysectomized

rats and this is not likely to be due, at least totally, to abnormal functioning ofadrenals since when thyroxine or growth hormone is administered the values are not significantly different from normal. It is not impossible that local release of adrenaline could occur in the liver and be responsible for glycogenolysis and increase in blood sugar, when the animals are in great need of glucose such as in hypoglycemia. If this is the case, then the liver of the hypophysectomized rat, with its low adrenaline content, could not cope as efficiently as normal liver in its response to hypoglycemia and hence the great sensitivity of hypophysectomized rats to insulin. Growth hormone and thyroxine, by maintaining the proper amounts of adrenaline in liver, may then be able to correct this defect. It has been mentioned that de Bodo et al (27) reported an increase in the amount of glucose released by the livers of hypophysectomized rats as a result of treatment with growth hormone. It would be interesting to investigate the effect of thyroxine on the blood sugar response of hypophysectomized rats to insulin. Sensitivity to insulin has also been observed in thyroidectomized animals (6) and thyroid feeding could restore normal responses to injections of insulin.

In view of the greatly increased insulin sensitivity of hypophysectomized animals which attained 30-60 times that

observed for normal animals (271), possibly both a low liver adrenaline content and a decreased glycogenolytic effect of adrenaline may contribute to this effect in hypophysectomized rats. However, the finding of de Bodo et al (276) that growth hormone, despite its effect on the release of glucose from the liver, does not alleviate the insensitivity of hypophysectomized dogs to the hyperglycemic effect of adrenaline makes this latter factor less likely to be important.

It seemed that the brain of hypophysectomized rats contained slightly greater amounts of noradrenaline as compared to normal. This effect was too small to be statistically significant but it was consistently observed. This result is different than that obtained with the other tissues and once more places the brain in a different category. It is improbable that this 5-10% increase has some important physiological consequences. Thyroxine appears to be totally responsible for this effect since it was no longer observable after the hypophysectomized rats had been given this hormone. The lowering effect of thyroxine on the noradrenaline level of brain is rather unexpected on the basis that the data presented in the first part of the experimental did not show any difference in the level of noradrenaline in the brain of hyperthyroid rats. On the other hand, the experimental conditions were not the same

and may account for these differences. The greater effect of thyroxine on depletion of noradrenaline which was accompanied by a lesser but similar effect of growth hormone in brain of hypophysectomized rats not given sucrose is difficult to explain. It may be taken as an indication of an indirect unspecific action of thyroxine. Thus, one could speculate that a metabolic stress is induced in hypophysectomized rats by administration of thyroxine and this may result in a greater noradrenaline discharge by the central nervous system. In the absence of sucrose it may be expected that a greater metabolic stress occurs with thyroxine and may also be extended to growth hormone thus resulting in a greater release of the catecholamine by brain of hypophysectomized rats. Of course this is only a tentative explanation which does not rely on a very firm basis. The absence of pertinent work related to this subject in general does not permit a fruitful comparative discussion of these results.

In a general way, it may be concluded from this work that the physiological interpretation or significance of results is limited when one deals with changes in catecholamine content of tissues under various experimental conditions. This is not surprising because of the limited amount of information that can be obtained in using this method of approach. However, it serves the purpose of suggesting possible relationships and thus may encourage further research along these lines.

SUMMARY OF RESULTS

Asterisks mark claims to original work

* 1) To study the effect of hyperthyroidism in rats, catecholamine contents, monoamine oxidase activity and catechol-O-methyl transferase activity were measured in the liver of each experimental and normal rat. Catecholamine determinations were also made in the heart and brain of the same rat.

2) The livers and hearts of hyperthyroid rats were hypertrophied and the hepatic soluble proteins were increased.

3) Hepatic monoamine oxidase activity was decreased by 25% in hyperthyroid rats which had not encountered an appreciable loss of weight.

4) Catechol-O-methyl transferase activity in the liver of hyperthyroid rats was not significantly different from that of controls.

5) No significant changes could be observed in the noradrenaline and adrenaline content of the heart, liver and brain obtained from hyperthyroid rats.

* 6) The other mentioned effects were quantitatively the same whether or not the rats were deprived of vitamin B_{12}.

7) The effect of vitamin B_{12} deficiency was studied on liver monoamine oxidase and catechol-O-methyl transferase activities and also on the catecholamine content of the liver, heart and brain. No effect of vitamin B_{12} deficiency could be noted on the liver catechol-O-methyl transferase activity or on the catecholamine content of the liver, heart and brain.

8) Vitamin B_{12} deficiency increased the activity of monoamine oxidase in the liver of hyperthyroid or normal rats.

9) <u>In vitro</u>, vitamin B_{12} had no effect on monoamine oxidase activity.

10) <u>In vitro</u> also, vitamin B_{12} or methylcobalamin had no effect on catechol-O-methyl transferase activity.

11) In normal animals, the noradrenaline and adrenaline content per heart was constant and did not depend on the size of the heart.

12) There was a slight dependance of the noradrenaline content per liver on the size of liver but the hepatic adrenaline did not seem to vary with the size of this organ.

13) The noradrenaline and adrenaline concentrations of both the liver and heart were greater in small size organs but were lower in heavier organs.

14) The body weight, absolute liver and heart weights were decreased in hypophysectomized rats but only the relative weights of the heart, adrenals and testes were significantly reduced.

15) Thyroxine caused hypertrophy of both the liver and heart and had no effect on the body weight of hypophysectomized rats.

16) Growth hormone had a general effect on growth and stimulated liver and heart weight to the same extent it increased body weight.

17) The heart rate of hypophysectomized rats was reduced by about 30%, eight weeks after the operation.

18) Thyroxine increased the heart rate of hypophysectomized rats to normal in one series of experiments but could not do so in the series where all rats were given 5% sucrose as drinking fluid.

* 19) The noradrenaline content and probably the adrenaline content of hearts of hypophysectomized rats were reduced. In the case of noradrenaline the fall extended to 28%.

* 20) Thyroxine did not restore this decrease in myocardial noradrenaline stores completely to normal and growth hormone appeared to have a similar effect.

* 21) No synergism could be consistently obtained with both hormones in either body weight, organ weight or catecholamine content of any tissue.

* 22) The liver of hypophysectomized rats contained less adrenaline than normal livers and this decrease amounted to an average of 50%.

* 23) With thyroxine or growth hormone administration the liver adrenaline content of hypophysectomized rats is not significantly different from normal.

* 24) There was a tendency for brain noradrenaline to be slightly high in hypophysectomized rats and this increase could be corrected by administration of thyroxine.

* 25) Thyroxine administration to hypophysectomized rats decreased the brain noradrenaline to levels which were below normal and this effect was more prominent in rats not given 5% sucrose as drinking fluid.

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