THE RELATION OF THE THYROID GLAND TO THE ACTION OF INSULIN.

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An experimental investigation of the connection between the thyroid gland and carbohydrate metabolism was first attempted by Eppinger, Falta and Rudinger(1) in 1905, who claimed that in the absence of the gland, glycosuria was not produced by the injection of adrenaline into dogs. Controversy on the truth of this, and of the allied claim that adrenaline hyperglycæmia is less in the thyroidectomised than in the normal animal, still continues, the latest paper being that of Geiger(2) who denies that there is evidence for either. An important observation was made by Cramer and Krause(3), who found that the addition of thyroid gland to the diet of cats and rats resulted in the total disappearance of glycogen from the liver; their work has since been confirmed by Kuriyama(4). Recently, while the investigation described in this paper was in progress, Bodansky(5) has recorded that the hypoglycæmic reaction to insulin is greater in sheep after thyroidectomy, and Ducheneau(6) has found that the lethal dose of insulin is smaller for thyroidectomised than for normal rabbits. Macleod(7) originally put forward the hypothesis that during the hypoglycæmia produced by insulin the liver discharges glycogen in an effort to raise the blood sugar to the normal level. Lately this has been disputed by Cramer(8), who interprets the diminution of liver glycogen observed by Dudley and Marrian(9) as due to diminished glycogen formation. Macleod's conception is supported, on the other hand, by Burn's observation(10) that ergotamine, which paralyses the mobilisation of sugar from the liver by sympathetic impulses and adrenaline, also intensifies the hypoglycæmia produced by insulin. In the following paper we present the results of experiments made to test this hypothesis further, by studying (1) the effect of cutting the splanchnic nerves on the effect of insulin, (2) the relation between the hyperglycæmia produced by adrenaline and the hypoglycæmia produced by insulin in different rabbits, and (3) the effects on both these reactions of thyroidectomy and thyroid feeding.

Section of both splanchnic nerves. The operation of dividing both splanchnic nerves below the diaphragm is one which is better tolerated by the cat than by the rabbit. The observations were therefore made on cats, and owing to the difficulty of making repeated blood sugar determinations, the degree of hypoglycæmia produced by an injection of insulin was judged by the symptoms, which appear with greater regularity, and less abruptly, than in the rabbit. The stages observed were (1) drowsiness and disinclination to walk; (2) increasing ataxia; (3) inability to walk, the cat lying on its side apparently unconscious; (4) convulsions of a violent character.

Each animal was kept on an ample diet of meat with bread and milk. Before an observation it received no food for 16 hours. It was then weighed, and a known amount of insulin was injected under the skin. Starting from doses which produced no symptoms, the effect of increasing amounts was observed. From three to ten observations were made on each animal, both before and after the section of the splanchnic nerves. These were divided in two operations, at an interval of 8 to 10 days, the operations being performed under deep ether anæsthesia, with full aseptic precautions, by the lumbar route. A period of 10–14 days was allowed to elapse after the second operation before the second series of observations with insulin was begun, the immediate effects of operation having disappeared completely by this time. The results obtained are shown in Table I.

TABLE I.

	Weight kgm.	No. of observations	Severe symptoms produced by insulin dosage of mgms. per kgm.	No symptoms produced by dose of mgms. per kgm.
Cat *A* when normal	2·75	10	·2	·14
after operation	2·80	6	·13	·10
Cat *B* when normal	2·2	5	·30	·20
after operation	2·45	3	·10	·05
Cat *C* when normal	2·1	3	·20	·15
after operation	2·8	3	·10	·05

The table gives the weights of each cat before and after the operations, and the number of observations made in order to arrive at the dosage necessary to produce on the one hand, severe symptoms of hypoglycæmia and, on the other, no definite effect. "Severe symptoms" indicates the condition in which the cat is apparently unconscious and lies on its side. In the case of each animal, a dose less than that which was without effect before, produced severe symptoms after the operations. It is evident that the removal of the central control of the liver and

suprarenals increased the hypoglycæmia produced by a given amount of insulin, a result consistent with the view that the compensatory discharge of glycogen from the liver normally attending the insulin effect is in part produced by impulses passing down the splanchnic nerves.

The relation of adrenaline hyperglycæmia to insulin hypoglycæmia. The extent of hyperglycæmia produced by a given amount of adrenaline varies widely in different rabbits, although these receive the same diet for many weeks before the tests are made. Similarly, the hypoglycæmia produced by a given amount of insulin varies in different rabbits. The variation in these two reactions may be connected, for if the second be limited by glycogenolysis prompted by stimulation of sympathetic nerve endings, then it should be smaller in those animals in which the hyperglycæmia produced by adrenaline is relatively large. The following examples illustrate that this is the case.

Exp. 1. Two rabbits, each weighing 3 kgm. received injections (s.c.) of ·5 mgm. adrenaline. Blood sugar determinations were made by the Shaffer-Hartmann method at hourly intervals after injection.

	Initial B.S.	1st hr.	2nd hr.	3rd hr.	4th hr.	5th hr.	Average
Rabbit 1	·12 p.c.	·33	·38	·35	·25	·16	·29
Rabbit 2	·10 „	·20	·22	·24	·18	·13	·19

The hyperglycæmic reaction was much greater in Rabbit 1.

The injection of 1·6 mgm. per kgm. of a sample of insulin hydrochloride into Rabbit 1 produced no symptoms, and the lowest blood sugar observed was ·06 p.c. The injection of less than one-tenth this amount, namely ·15 mgm. per kgm. into Rabbit 2, produced convulsions, and the injection of ·1 mgm. per kgm. reduced the blood sugar to ·04 p.c. The hypoglycæmic reaction was much smaller in Rabbit 1.

Exp. 2. Five rabbits were taken, all of about 2 kgm. weight. The hyperglycæmic reaction to an injection of ·4 mgm. adrenaline was determined for each by taking five samples of blood at hourly intervals after the injection. From the average of these the initial blood sugar was deducted, and the difference used as a measure of the reaction. The hypoglycæmic reaction to the injection of ·2 mgm. insulin hydrochloride was determined by a similar procedure. The results were as follows:

	Hyperglycæmic reaction	Hypoglycæmic reaction
Rabbit 3	·072 p.c.	·063 p.c.
„ 4	·135 „	·023 „
„ 5	·126 „	·022 „
„ 6	·149 „	·036 „
„ 7	·113 „	·018 „

The hyperglycæmic and the hypoglycæmic reactions of Rabbits 4, 5, 6 and 7 were respectively alike. The hyperglycæmic reaction of Rabbit 3

was much less than that of the others, while the hypoglycæmic reaction was greater; at the second hour the blood sugar was ·026 p.c., whereas in the other four animals it was ·06 p.c. or more; at the third hour Rabbit 3 had convulsions when the blood sugar of the others was ·072 p.c. or more.

The relation of the thyroid hormone to the reactions.

(*a*) *The effect of thyroidectomy.* Several observers have recorded (see Geiger(2)) that after thyroidectomy the hyperglycæmia produced by a given amount of adrenaline is less. We have published(11) in a preliminary note figures showing that the hypoglycæmia due to a given amount of insulin is greater, confirming the records of Bodansky(5) and Ducheneau(6). We have now carried out in three rabbits observations both on the insulin hypoglycæmia and the adrenaline hyperglycæmia, before and after thyroidectomy.

Exp. 3. The rabbits were maintained under constant conditions for several weeks before and during the experiments.

A. *Insulin hypoglycæmia.* In each case an attempt was made to determine the minimal convulsive dose of a given preparation of insulin for each rabbit. The level of the blood sugar was also followed at hourly intervals during the experiments. Each figure in the following table represents the results of from three to six separate tests.

	Minimal convulsive dose before thyroidectomy mgm. per kgm.	Minimal convulsive dose after thyroidectomy mgm. per kgm.	Increase in sensitiveness
Rabbit 1	Greater than 1·6	·25	More than 6 times (Probably 9 times)
„ 8	„ 1·2	·25	About 5 times
„ 2	0·15	·05	3 times

The lowest blood sugar recorded following the injection of 1·6 mgm. per kgm. of insulin in Rabbit 1 before thyroidectomy was ·061 p.c., a figure considerably above the convulsive level; probably a dose of 2·4 mgm. would have produced convulsions. Had this been the case the increase in sensitiveness following thyroidectomy would have been nine to ten times. The injection of ·25 mgm. after the thyroidectomy reduced the blood sugar to ·029 p.c. in this rabbit.

The lowest blood sugar observed after the injection of 1·2 mgm. per kgm. insulin in Rabbit 8 before thyroidectomy, was ·042 p.c.; consequently, although this dose did not produce convulsions it was near the convulsive level.

B. *Adrenaline hyperglycæmia.* In each case ·5 mgm. adrenaline was

injected. In the following table the *average* blood sugar during the five hours following the injection is recorded:

	Average hyperglycæmia		
	Before thyroidectomy	After thyroidectomy	Difference
Rabbit 1	·295 p.c.	·235 p.c.	·060
„ 8	·232 „	·207 „	·025
„ 2	·192 „	·183 „	·009

The difference in the adrenaline hyperglycæmia before and after thyroidectomy is small; the figures represent the average of five determinations, however, and therefore the difference, even in the case of Rabbit 2, is probably real. Comparing the two tables, the greatest increase in sensitiveness to insulin occurred in Rabbit 1, in which there is the greatest reduction in the hyperglycæmic reaction; similarly, the least increase in the hypoglycæmic reaction was in Rabbit 2, which showed the least reduction of the hyperglycæmic reaction.

(*b*) *The effect of thyroid feeding.* Experiments have been carried out in which a dried preparation of thyroid gland (Burroughs, Wellcome and Co.) was added to a diet consisting of a mixture of boiled potatoes and crushed oats. Of this a rabbit, of weight 2 kgm. received 100 gm. a day, containing the powdered substance of four tablets, representing 1·2 gm. of fresh gland, according to the manufacturer's indication; occasionally this amount was increased to 1·8 gm. of fresh gland. The effect of this feeding was to cause the animals to lose weight. Thus, in 20 days, a rabbit starting at 2·2 kgm. fell to 1·35 kgm.

The extent of the hypoglycæmic reaction to insulin rapidly diminishes after a few days' thyroid feeding. Fig. 1 shows the initial reaction of a thyroidectomised rabbit, to an injection of insulin which produced convulsions, and also the much smaller reaction after 8 days' thyroid feeding to five times this dose of insulin. The injection of ten times this dose did not produce any symptoms. Other examples of similar effects are shown in the following records.

Rabbit 9. Initially ·25 mgm. per kgm. insulin produced convulsions. After 8 days' thyroid feeding, the injection of ·75 mgm. per kgm. produced a hypoglycæmia in which the blood sugar did not fall below ·06 p.c. After 12 days the injection of 1 mgm. per kgm. did not produce convulsions.

Rabbit 10. Initial injection of ·25 mgm. per kgm. produced convulsions. After 8 days' thyroid feeding 1 mgm. per kgm. insulin produced a fall of blood sugar in which the lowest value was ·053 p.c.

These results indicate that the presence of large amounts of the thyroid hormone in the circulation enables the organism to prevent the

occurrence of severe hypoglycæmia, in spite of the injection of relatively large doses of insulin.

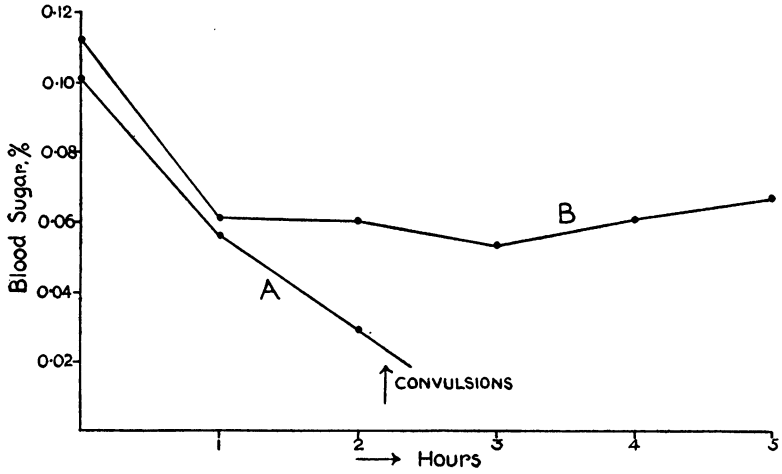

Fig. 1. Curve *A* shows the hypoglycæmic reaction of a thyroidectomised rabbit to ·05 mgm. per kgm. of insulin. Curve *B* shows the reaction of this rabbit to five times the dose after 8 days' thyroid feeding.

After thyroid feeding there is an increase in adrenaline hyperglycæmia, which is more uniform than the decrease produced by thyroidectomy. We have observed this increase with intravenous as well as with subcutaneous administration of adrenaline. The following are examples:

		Reaction when normal	Reaction after thyroid feeding
Rabbit 11	·05 mgm. adrenaline i.v.	·02 p.c.	·04 p.c.
„ 12	·2 „ s.c.	·03 „	·09 „

The reaction in Rabbit 11 is the difference between the average blood sugar during 1 hour after the injection (three samples of blood) and the initial blood sugar; the change observed occurred after 7 days' thyroid feeding. The reaction in Rabbit 12 is the difference between the average blood sugar during 5 hours after the injection (five samples of blood) and the initial blood sugar; the change occurred after 14 days' thyroid feeding.

By prolonging thyroid feeding beyond 10–14 days we have found that the hypoglycæmic reaction to insulin after the initial great reduction, once more increases. Fig. 2 *a* shows the changes in the hypoglycæmic reaction of a rabbit to a given amount of insulin during 18 days' thyroid feeding. The reaction was measured by taking the average figure of five blood sugar determinations at hourly intervals and sub-

tracting it from the initial blood sugar figure. After becoming progressively less in intensity and duration, the hypoglycæmia afterwards

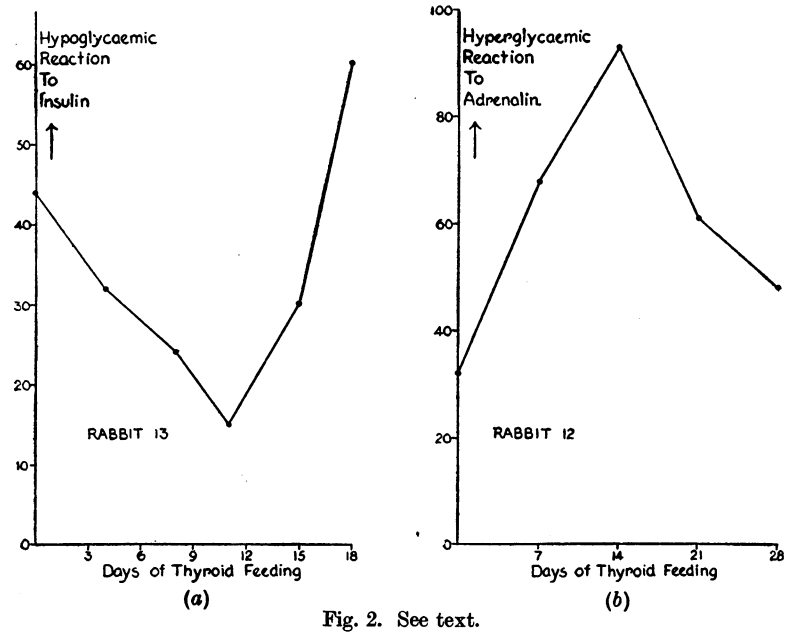

Fig. 2. See text.

increased so that on the eighteenth day the rabbit had severe convulsions following the injection, and died, in spite of subcutaneous injection of sugar. Fig. 2 *b* shows, again, that the adrenaline hyperglycæmia undergoes changes inversely corresponding to the changes in the insulin hypoglycæmia, as thyroid feeding progresses; after having increased during the earlier period it decreases during the later period of thyroid feeding. The hyperglycæmic reaction in the figure is expressed as the difference between the average of the five determinations of the blood sugar percentage, determined at hourly intervals after the injection, and the initial blood sugar.

Changes in liver glycogen during thyroid feeding.

Since Cramer and Krause(3) have shown that thyroid feeding leads to the disappearance of liver glycogen, and since we suppose that the output of sugar from the liver is the most important factor limiting the hypoglycæmia produced by insulin, it was important to determine the course of the disappearance of the glycogen store during the changes in the hypoglycæmic reaction.

The livers of normal rabbits, which have been kept on the diet described above, have been found to contain from 3 to 6 p.c. of glycogen. A rabbit of 2 kgm. weight which was fed with thyroid gland for 8 days, receiving 1·2 gm. fresh gland daily, showed at the end of that time a considerable diminution of the hypoglycæmic reaction. Four times the amount of insulin which initially produced convulsions did not lower the blood sugar below ·053 p.c. It was then killed, and a determination in duplicate of the liver glycogen showed 3·5 p.c. A second rabbit of similar weight fed for 35 days, receiving 1·2 gm. fresh gland daily showed a diminution of the hypoglycæmic reaction during the first 8 days, and subsequently a small but progressive increase; by the thirty-fifth day this was considerable; nevertheless, when killed, the liver glycogen amounted to 2·8 p.c. On the other hand, Rabbit 13, of which the changes are shown in Fig. 2 *a*, at the end of 18 days' thyroid feeding, when the hypoglycæmic reaction was greater than before the feeding began, had no detectable liver glycogen. A similar absence of glycogen under these conditions has been observed in four other rabbits. It seems clear, at least, that the stage of diminished reaction to insulin in the early days of thyroid feeding is not attended by any serious fall in liver glycogen.

There is no indication on the other hand of an increased deposit. The diminished sensitiveness to insulin may reasonably be attributed to increased responsiveness of the liver, while the glycogen reserve is still adequate. During the subsequent period of increasing sensitiveness to insulin this reserve is being depleted, and is finally exhausted so that the compensatory action of the liver disappears.

Spontaneous hypoglycæmia. When all glycogen has thus disappeared from the liver, rabbits die without warning in hypoglycæmic convulsions, although they may never have received, throughout the whole course of the feeding, any dose of insulin. In two such cases a sample of blood taken at death contained only ·03 p.c. sugar, and rigor set in immediately after death, as it does in animals dying in hypoglycæmic convulsions produced by insulin.

The convulsive level in the thyroid-fed animal.

During the late stage of thyroid feeding the blood sugar may persist for several hours without symptoms appearing in the rabbit at a value lower than that at which normal animals have convulsions. The following are examples:

Rabbit 14. Hypoglycæmic reaction following injection (s.c.) of 1 mgm. per kgm. insulin.

	1st hr.	2nd hr.	3rd hr.	4th hr.	5th hr.
15th day of thyroid feeding	·029	·040	·027	·045	·066
22nd „ „	·034	·033	·024	·040	·053
34th „ „	·028	·020	·030	·028	·039

On none of these occasions did symptoms appear. Three days after the last test, two and a half times the dose of insulin was given; this produced constantly recurring convulsions and the animal died.

Rabbit 15. Injection of 1 mgm. per kgm. insulin.

	1st hr.	2nd hr.	3rd hr.	4th hr.	5th hr.
15th day of thyroid feeding	·040	·034	·043	·040	·048
22nd „ „	·026	·029	·034	·040	·047
34th „ „	·033	·039	·032	·030	·036

It is well known that a rabbit may temporarily have a blood sugar lower than ·045 p.c. without suffering from symptoms, but we consider the cases quoted, in which the blood remained below ·040 p.c. for several hours, to be exceptional and worthy of record. Since thyroid gland preparations generally contain a certain amount of parathyroid substance, it seemed possible that the absence of convulsions might be due to a specific effect of the contained parathyroid in preventing convulsions; this point was therefore tested by feeding two rabbits with parathyroid tablets (Parke, Davis and Co.). We were unable to observe any increase in the amount of insulin necessary to produce convulsions during 14 days' feeding of two rabbits weighing 2 kgm. each with four tablets a day.

Discussion.

The effect of all the evidence which we have presented is to confirm the view that the liver by liberating sugar from its glycogen store antagonises the hypoglycæmia produced by excess of insulin in the circulation. This compensating action is weakened by section of the splanchnic nerves, or by thyroidectomy; increased by thyroid feeding so long as the reserve of glycogen persists; annulled when the thyroid feeding is pushed to the length of exhausting the reserve.

To the much debated question whether the sensitiveness of the liver function to sympathetic stimulation is affected by the amount of thyroid hormone in circulation, our experiments seem also to give a positive answer. The changes in hyperglycæmic response to a single large dose of adrenaline, produced by thyroidectomy on the one hand, and by thyroid feeding on the other, though definite, are not impressive in degree. The results gain significance, however, when it is recognised

that every tested condition, natural or artificial, which has been shown to enhance the hyperglycæmic response to adrenaline has been observed to depress, in a more striking manner, the hypoglycæmic response to insulin, while conditions which impair the response to adrenaline, accentuate that to insulin.

It seems reasonable to suppose that the prolonged stimulation of the liver cells by sympathetic impulses or adrenaline when evoked as a reaction to the effect of insulin, is a more truly physiological process than the more evanescent and intense effect produced by sudden injection of adrenaline itself, and consequently more easily modified by changing the amount of thyroid hormone in circulation. As to the precise mechanism of this compensatory action of the liver, we have little evidence to offer. That it is in part produced through the central nervous system appears to be demonstrated by the increased effect of insulin when the splanchnic nerves are divided. It cannot be supposed, however, that this operation annuls the reaction completely. More peripheral sites of stimulation must exist. But our results afford no evidence as to the nature of the stimulus. Whether on nerve centres, suprarenal glands, or the liver cells themselves, the stimulus, so far as our evidence goes, may be due either to lack of dextrose or to excess of insulin itself in the blood.

The view that the liver thus modifies the insulin hypoglycæmia, by accelerated output of dextrose, has been opposed by Cramer(8), who advances two principal arguments against it. He states, in the first place, that hyperthyroidism empties the liver of glycogen, while diminishing sensitiveness to insulin. In the second place, he suggests that in hyperthyroidism, though the liver is depleted, the blood sugar is abnormally high. It appears to us that our experiments dispose of these difficulties. The effect of thyroid feeding on carbohydrate metabolism in the rabbit is relatively slow in development. It is easy to distinguish, in this species, the earlier stage, in which the accelerated glycogenolysis diminishes the response to insulin, as suggested by Cramer; but at this stage the liver has still an adequate glycogen store. Similarly, it is true that hyperthyroidism may be accompanied by hyperglycæmia; but our evidence does not support Cramer's suggestion that under these conditions the liver glycogen is already exhausted. On the contrary, when that stage of the effect has been reached, we find that the animal passes into a spontaneous, acute and fatal hypoglycæmia.

SUMMARY.

1. Section of both splanchnic nerves in the cat increases the hypoglycæmic reaction to insulin.

2. The hyperglycæmic reaction to adrenaline varies in different rabbits as does the hypoglycæmic reaction to insulin; in those animals in which the former is large, the latter is small; and *vice versa*.

3. Thyroidectomy diminishes the hyperglycæmic reaction to adrenaline and increases the hypoglycæmic reaction to insulin.

4. Thyroid feeding, so long as the glycogen store in the liver is not diminished, increases the hyperglycæmic reaction to adrenaline and decreases the hypoglycæmic reaction to insulin.

5. When, as a result of prolonged thyroid feeding, the liver glycogen disappears, the hyperglycæmic reaction to adrenaline diminishes and the hypoglycæmic reaction to insulin increases.

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