612.445:615.361.37

INSULIN AND THE THYROIDECTOMIZED RABBIT.

BY M. W. GOLDBLATT.

(*From the Sherrington School of Physiology, St Thomas's Hospital, London.*)

(*Received August* 9, 1935.)

THE blood-sugar response of a normal animal to insulin depends upon the balance of two factors: the increased oxidation of carbohydrate by the tissues and the liberation of glycogen from the liver. The nature of the stimulus to carbohydrate metabolism is very difficult to picture, and no good evidence exists as to the precise position of the point of attack. Whereas insulin appears to be the principal humoral agent in the control of the peripheral utilization of carbohydrate, several mechanisms exist for the mobilization of liver glycogen to overcome the fall in blood sugar due to insulin. Any temporary or permanent inadequacy of the mechanisms for glycogen mobilization will result in a hypersensitivity to insulin, which does not imply increased speed of oxidation of glucose but that the sugar in the blood is not replenished as its level falls.

The replenishment of the blood sugar in hypoglycæmia appears to be mainly due to a stimulation of the sympathetic system by secretion of adrenaline, but it is not established that this is the only factor involved.

The explanation of the hypersensitivity to insulin after adrenalectomy is obvious. The recent work of Cope and Marks [1934] offers an explanation of the hypersensitivity to insulin which follows hypophysectomy. These workers put forward the view that the anterior pituitary lobe possesses a substance which is responsible for the mobilization of liver glycogen, and that in its absence the usual hyperglycæmic effect of adrenaline is impaired. They showed, by means of the denervated pupil and the surviving rabbit's intestine, that the hypophysectomized animal secretes adrenaline in hypoglycæmia. The hypersensitivity to insulin of the hypophysectomized animal is thus to be regarded as due to the resistant state of the liver glycogen. Whether this is due to changes in the effectiveness of the enzyme systems in the liver or to a lowered sensitivity of the sympathetic is not yet clear.

Now the increased sensitivity to insulin of the thyroidectomized animal is of much smaller degree than that of the adrenalectomized or hypophysectomized animal. Doses of insulin sufficient to bring about severe convulsions in the latter may only produce a mild hypoglycæmia in the former. In the following experiments an attempt was made to elucidate the hypersensitivity of the thyroidectomized rabbit to insulin.

Experimental.

Thyroidectomy was performed when the animals were young. It was not necessary to ligate any of the thyroid vessels. Great care was taken to remove all the connective tissue in front of the trachea in the region of the thyroid isthmus, as hypertrophy of gland fragments in this region is very rapid after removal of the main lobes. Injury to the recurrent laryngeal nerves invariably led to the death of the animals from respiratory complications. Meticulous asepsis is unnecessary. The diet was a mixture of bran and oats with a sufficiency of green stuff: it was allowed *ad lib.*

Effect of thyroidectomy on the growth of young rabbits. The operation was performed on a litter of rabbits about 5 or 6 weeks after birth. As the experiments were to be carried out when the animals had grown to adult size the opportunity was taken to follow the growth curves of the thyroidectomized and normal littér mates. Table I gives the course of

Table I.

	Days after operation								
	0	10	21	37	44	60	71	81	101
	Controls—weight in g.								
1	950	1025	1325	1550	1650	1725	—	—	—
2	700	700	850	1200	1350	1650	1950	2050	2250
	Thyroidectomized.								
1	800	850	1050	1300	1400	1700	1750	1800	1850
2	650	575	750	1100	1150	1525	1750	1850	2000
3	700	875	1050	1250	1425	1700	1725	1750	2150
4	550	575	825	875	1050	1250	1325	1400	1750

the weight changes in such a litter. It shows that the disturbance in metabolism resulting from thyroidectomy in rabbits of the above age cannot be a very serious one. By no external symptom or sign could these animals be distinguished from the controls. In spite of this the increased sensitivity to insulin could easily be demonstrated.

Effect of insulin on the blood sugar of thyroidectomized rabbits. In each case a comparison was made under the same conditions and at the same time between thyroidectomized and control rabbits from the same litter. Blood sugar was determined by the method of Hagedorn and Jensen.

The figures in Table II show: (1) That the initial rate of fall of blood sugar in the thyroidectomized animal is no more rapid than in the normal. (2) That in both the normal and thyroidectomized animals, when not starved, this fall to hypoglycæmic levels is followed by a

TABLE II.

Exp. 1. Insulin, 1 unit/kg. subcut. Blood sugar, mg./100 c.c. *c* = convulsions.

		Time after injection (hours)						
Thyroid	Wt. g.	0	½	1	1½	2	2½	3
		(*a*) Not starved.						
Normal	1875	154	81	65	54	63	90	90
Removed	1825	154	90	74	50	66	57	66
		(*b*) Starved 24 hours before injection.						
Normal	1800	141	70	54	50	68	—	—
Removed	1700	125	75	57	48*c*	47*c*	—	—
		(*c*) Starved 24 hours before injection.						
Normal	1950	141	—	75	—	84	—	—
Removed	1700	139	—	61	—*c*	54	—	—

secondary rise in blood sugar which is of smaller proportions in the latter, but in both is sufficient to obviate convulsions. In the presence, therefore, of considerable reserves of glycogen in the liver, the thyroidectomized animal is able to compensate the fall in blood sugar. (3) That after 24 hours' starvation the rate of fall of blood sugar is still the same in both control and operated animals, but whereas the former show a marked secondary rise in blood sugar the latter fail to do so and develop violent intermittent convulsions.

In seeking some hypothesis to explain this hypersensitivity it was decided first to see if it could be increased by ergotamine. This substance increases the sensitivity to insulin by inhibiting the hyperglycæmic action of adrenaline. In Table III three experiments are recorded showing that normal and thyroidectomized rabbits treated with ergotamine reach the convulsive level of blood sugar in the same time.

It appears, therefore, that with the paralysis of the motor sympathetic the normal animal becomes indistinguishable from the thyroidectomized as regards sensitivity to insulin. Further, ergotamine does not increase the sensitivity of the starved thyroidectomized rabbit to insulin. It seems probable, therefore, that in the thyroidectomized animal the adequate secretion of adrenaline or its action is in some way involved in the increased sensitivity to insulin. The next matters to be made clear were (1) does the thyroidectomized rabbit secrete adrenaline adequately in hypoglycæmia, and (2) if so does it react normally to adrenaline?

TABLE III. Effect of ergotamine on the hypersensitivity to insulin.

Exp. 2. Comparisons were always made between litter mates, starved 24 hours before injection; ergotamine tartrate 2 mg./kg. subcut.; insulin 1 unit/kg. subcut. Blood sugar, mg./100 c.c. *c* = convulsions.

Thyroid	Wt. g.	Time after insulin (hours) 0	½	1	1½	2	2½	3
		(*a*) Ergotamine alone.						
Normal	1650	129	136	132	138	149	155	—
Removed	1375	134	146	141	139	141	139	125
		(*b*) Ergotamine followed after a few minutes by insulin.						
Normal	2100	143	129	88	54	61*c*	56*c*	—
Removed	1700	148	99	92	70	65*c*	47*c*	—
		(*c*) Insulin followed by ergotamine.						
Normal	2050	143	—	65	—	66*c*	—	—
Removed	1700	136	—	79	—	72	—*c*	—

Effect of hypoglycæmia on the adrenalinæmia of thyroidectomized rabbits. Direct evidence of the presence of adrenaline in the blood during hypoglycæmia was looked for by drawing 1 c.c. of blood from the right heart into a syringe containing a small volume (0·05 c.c.) of a solution of heparin and tested directly on a preparation of surviving small intestine of a rabbit in 35 c.c. of oxygenated Tyrode solution at 37° C. The heparin itself has no effect on the gut. The removal of 1 c.c. of blood from an adult rabbit can be repeated six or seven times at hourly intervals without any harmful effect on the animal or significant change of effect of the blood on the surviving intestinal strip. It is well known that rabbit's blood frequently contains a substance with stimulant action on intestinal muscle, but the amount of this action varies greatly in different rabbits and even in the same rabbit at different times. It is most marked in our experience if the blood is taken from a peripheral vessel: when the blood is taken direct from the heart we do not find the effect at all marked and frequently it is entirely absent.

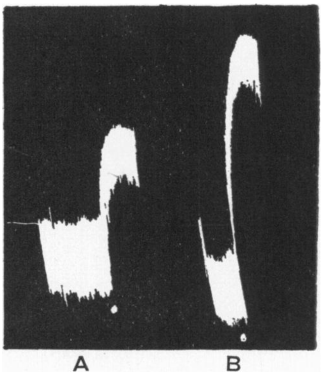
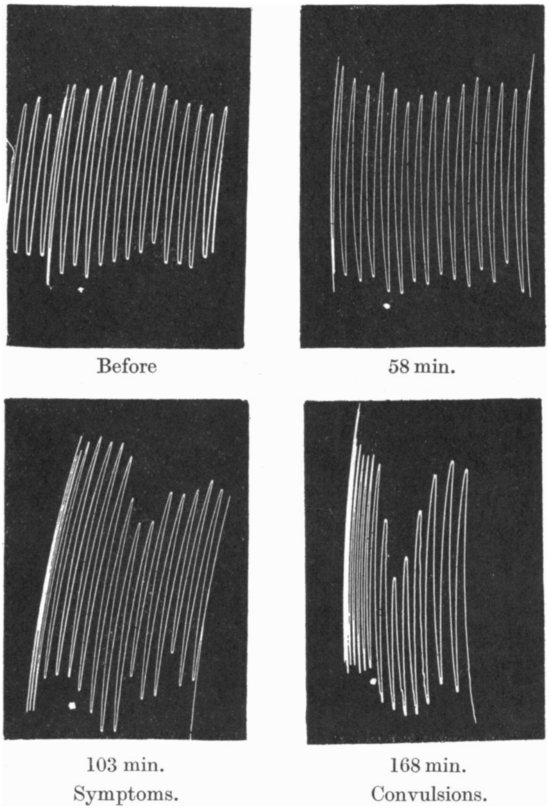


Fig. 1. Rabbit's small intestine. 35 c.c. O_2 Tyrode. Slow speed. Effect of 1 c.c. ear-vein blood (heparinized). A, normal adult rabbit; B, thyroidectomized adult rabbit.

In Fig. 1 are given tracings (very slow kymograph) of the effect of two specimens of blood taken from the ear vein of a normal and of a thyroidectomized animal. It is clear that in the presence of so powerful a tonic action any moderate increases in adrenaline content of the blood would be masked. (See, however, tracings in paper by Cope and Marks [1934].)

Having regard to these observations heart blood was always used and striking results could readily be obtained.



Before 58 min.

103 min. Symptoms. 168 min. Convulsions.

Fig. 2. Normal rabbit starved 24 hours.

Figs. 2, 3, 4. Rabbit's small intestine. 35 c.c. O_2 Tyrode. Medium speed. Effect of 1 c.c. heart blood (heparinized) at various times (given below tracings) after injection of 1 unit insulin per kg. subcutaneously. Same strip of intestine throughout.

In Figs. 2, 3 and 4 are shown the results of an experiment carried out as follows. A normal and thyroidectomized animal, from the same litter and grown to adult size, were starved for 24 hours. In order to make the onset of convulsions certain in both animals they were injected with 2 mg. ergotamine tartrate 15 min. after having received 1 unit

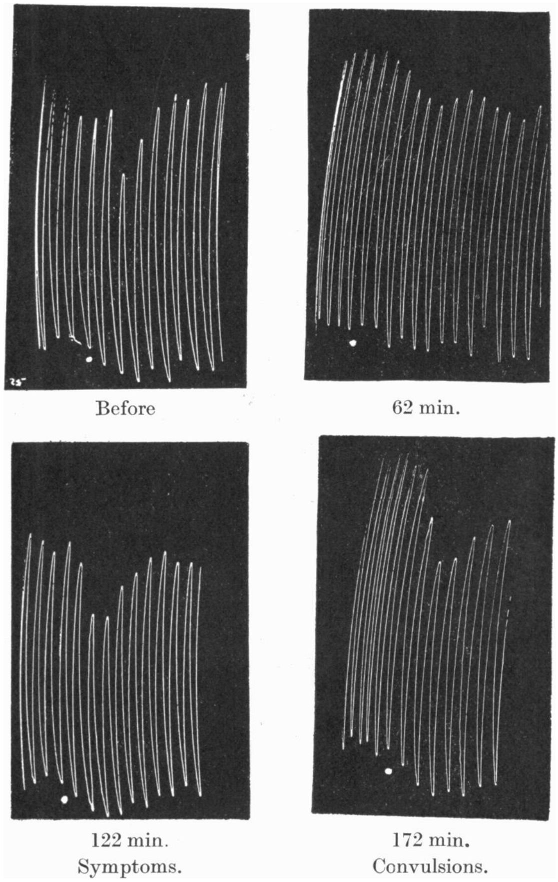


Fig. 3. Thyroidectomized litter mate starved 24 hours.

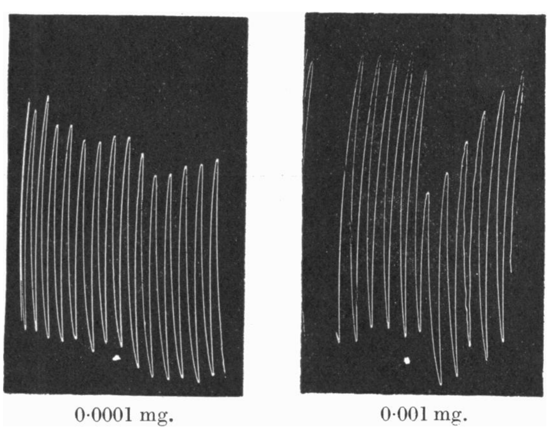


Fig. 4. Adrenaline for comparison.

insulin per kg. subcutaneously. Blood was drawn from the right heart and added to an intestine preparation within 10 sec. The animals were quiescent and the heart puncture could be made without any excitement or disturbance of the animals and without any manipulation other than deflection of the thorax somewhat to the side into which the needle is entered.

The points to which we draw attention in these tracings are the following:

(1) The heart blood (1 c.c.) exerted no tonic action on the muscle.

(2) Definite relaxation was produced by the blood of both animals at a little over 100 min. after the injection of insulin. This effect became very marked during hypoglycæmic convulsions which appeared in both animals at almost the same time.

(3) Comparison with Fig. 4 shows that the secretion of adrenaline was very considerable in both animals.

It may, therefore, be considered as certain that the thyroidectomized animal secretes adrenaline in hypoglycæmia in quantities adequate to produce readjustment of the blood sugar, provided it can react to adrenaline normally.

Effect of adrenaline on blood sugar of thyroidectomized rabbits. Cope and Marks [1934] give blood-sugar curves in normal and thyroidectomized animals after injection of 0·2 mg. adrenaline, which indicate that in the latter the rise in blood sugar is less and that the return to initial levels is rather more rapid. They do not state how long the animals were thyroidectomized. In Exp. 3 (Table IV) the animals were all from the same litter and the operation had been carried out 100 days previously.

TABLE IV.

Exp. 3. Average increases of blood sugar, mg./100 c.c., following the intravenous injection of 0·05 mg. adrenaline. 24 hours' starvation.

	Time after injection (hours)					
	0	½	1	1½	2	3
Controls (2)	0	75	67	34	8	4
Thyroidectomized (3)	0	43	50	52	54	46

The difference in these responses to adrenaline can be summed up as follows:

(1) The controls reach a maximum much earlier than do the thyroidectomized animals, and this maximum is of considerably greater proportions than in the latter.

(2) The controls re-establish a normal level of blood sugar in about 2 hours, whilst the thyroidectomized animals have a greatly raised blood sugar 3 hours or more after the adrenaline injection.

Now although these results are out of harmony with those of Cope and Marks [1934] as represented in the curves given on p. 167 of their paper, the actual figures given on p. 168 indicate that the normal animal reaches a blood-sugar maximum sooner and returns to normal levels sooner than the thyroidectomized animal. If, as we believe, this is the true response of the thyroidectomized animal to adrenaline it can be explained on the hypothesis that both the glycogenolytic response to adrenaline (sympathetic response) and the insulin secretory mechanism are more sluggish than normal.

There is considerable evidence in favour of this. Clinically the myxœdema patient gives an abnormally high and prolonged blood-sugar curve after a test dose of glucose.

Conversely, abnormalities of carbohydrate metabolism have frequently been described in cases of hyperthyroidism as a manifestation of the general hyperactivity or hyperexcitability of the sympathetic. Prolonged administration of thyroid to animals, it is well known, leads to an inability to retain glycogen in the liver and also to an excessive sensitivity of the insulin secretory mechanism with low blood-sugar curve. Further, animals fed for several days with large amounts of thyroid, as shown by Burn and Marks [1925], become almost insensitive to doses of insulin which previously produced convulsions, an observation which means that either the sympathetic has become hypersensitive or that the adrenal glands are in a state of much readier response to slight changes in blood sugar. From these facts it would follow that the chronic absence of thyroid secretion would tend to depress both the sympathetic glycogenolytic response and the response of the mechanism of insulin secretion to raised blood-sugar values.

The hypersensitivity to insulin of the thyroidectomized animal is thus to be explained not on any failure to secrete adrenaline in response to a low blood sugar nor to any immobilization of liver glycogen but to a time factor in the glycogenolytic response to sympathetic stimulation. This is also borne out by the fact that ergotamine renders the normal and thyroidectomized animal equally hypersensitive to insulin. That this sluggishness is not to be supposed to apply indiscriminately to all sympathetic phenomena in the thyroidectomized animal is shown by the following experiment.

Effect of adrenaline on the blood-pressure of the normal and thyroidectomized rabbit. A normal and a thyroidectomized rabbit (105 days after the operation) were taken from the same litter and blood-pressure records obtained from the left carotid artery. After the various cannulæ were inserted the anæsthetic (ether) was discontinued. When the excess ether had been expired the animal was quiescent and gave a slight

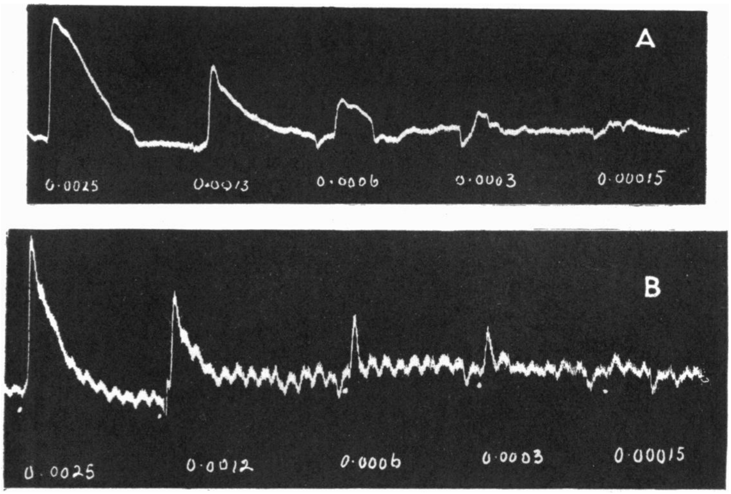


Fig. 5. Rabbit. Ether. Vagi cut. Carotid blood-pressure following adrenaline intravenously. Doses in mg. given on tracing. Ether off throughout period of injection. A, normal rabbit; B, thyroidectomized litter mate—110 days after the operation.

TABLE V. Effect of adrenaline on the blood-pressure of the normal and thyroidectomized rabbit (vagi cut).

Dose of adrenaline mg. per kg.	Rise in blood-pressure mm. Hg: Control normal 2·3 kg.	Rise in blood-pressure mm. Hg: Thyroidectomized 1·9 kg.
0·010	66	74
0·005	50	58
0·0025	44	57
0·0012	28	42
0·0006	15	20
0·0003	6	16
0·00015	0	0

corneal reflex. Fig. 5 shows the results of a series of intravenous injections of adrenaline after the vagi were both cut. In Table V are recorded the actual increases in blood-pressure.

So far from finding any diminution of response in the thyroidectomized animal the actual rise in blood-pressure was consistently greater in this animal until the ineffective dose was reached at about 0·15 γ/kg.

It is not suggested that thyroidectomy in all species does not lead to a general depression of the sympathetic for this is manifestly not so. But in the rabbit, which shows so little sign of disturbance after thyroidectomy, there seems to be an unequal degree of disturbance of different sympathetic mechanisms, that responsible for glycogenolysis being sluggish in response when the vaso-constrictor system appears to be as sensitive and to react as normally.

Another process which seems to be sluggish in the thyroidectomized rabbit is that responsible for the liberation of lactate from muscle glycogen.

Effect of adrenaline on blood lactate in thyroidectomized rabbits. The injection of adrenaline is normally followed by an increase in blood lactate. This increase occurs even when for any reason there is no rise in blood sugar after the injection [Goldblatt and Elkington, 1933]. The rise in blood lactate is much slower than that of blood sugar, but in general a marked rise is found in a little over an hour after the injection of the adrenaline (Table VI).

TABLE VI.

Exp. 4. Control and thyroidectomized rabbits from the same litter. Starved 24 hours. Blood lactate by the method of Friedeman, Cotonio and Shaffer carried out on heart blood. Adrenaline 0·05 mg./kg. Changes from initial blood lactate, mg./100 c.c.

	Time after injection (hours)			
	0	1	2	3
Control	0	- 9	+24	+40
Thyroidectomized	0	+14	- 5	+44

The rise of 14 mg. after 1 hour in the thyroidectomized animals cannot be regarded as a true adrenaline effect, for variations of almost this magnitude are frequently met with in rabbits under normal conditions. This is borne out by the value after 2 hours. The absolute rises in blood lactate are the same in both groups after 3 hours, but it appears that the initiation of the effect takes a considerable time longer in the thyroidectomized animals.

It appears, therefore, that the processes of glycogenolysis, both in the liver and the muscles, are less readily brought about in the thyroidectomized rabbit than in the normal.

Insulin and the thyroidectomized young rabbit. The peculiar effect of insulin on the starving young rabbit in bringing about a very great increase in liver glycogen has engaged the attention of several workers. Cope and Corkill [1934] give strong evidence in support of the view that this effect "requires the cooperation of adrenaline from the suprarenal medulla" and Corkill, Marks and White [1933] show that the effect is not obtainable in hypophysectomized young rabbits. The results of the latter workers are the more surprising in that Cope and Marks [1934] showed that hypophysectomy tended to immobilize liver glycogen, but that adrenaline was secreted as usual during hypoglycæmia. If in the hypophysectomized animal adrenaline can liberate lactate from muscle glycogen and the liver can resynthesize glycogen from lactate, it will be difficult to interpret the insulin effect on the adrenaline hypothesis. One great difficulty, it seems to the present writer, is that it fails to explain why the increase in liver glycogen is not detected in other normal animals. Cori [1925] found that insulin produces no definite change in blood lactate of rabbits, but other workers and the writer have found that at low blood-sugar values in adult rabbits there is a definite and often considerable rise in blood lactate before convulsions occur. Cori [1931] confirmed the deposition of liver glycogen in young starving rabbits under insulin, and inclined to the view that it was due to the secondary secretion of adrenaline as suggested by the above workers.

There is no doubt that adrenaline can produce a redistribution of glycogen in the young starving rabbit qualitatively similar to that produced by insulin [Goldblatt, 1933], but whereas this effect of adrenaline is also demonstrable in other animals and also in adult rabbits, the effect of insulin is not.

In view of the delay of adrenaline effects in the thyroidectomized animal, it seemed of interest to see if insulin produces the paradoxical effect on liver glycogen in the thyroidectomized young rabbit. Young rabbits from the same litters were taken and the thyroid glands removed. After some days they were treated as detailed in Table VII. The methods

TABLE VII.

Exp. 5.

No.	Wt. g.	Period of thyroidectomy days	Blood: Sugar mg./100 c.c.	Blood: Lactate mg./100 c.c.	Glycogen g. p.c.: Liver	Glycogen g. p.c.: Muscle	Dose of insulin and time of action
				Litter 1.			
1	850	0	115	60	0·22	0·32	—
2	700	9	97	44	0·32	—	—
3	675	13	106	48	0·42	0·33	—
4	750	15	45	60	0·38	0·24	2 units 2¼ hr.
5	700	15	52	94	0·81	0·26	1 unit 2 hr.
				Litter 2.			
1	500	14	—	—	4·12	—	—
2	500	14	—	—	1·52	—	0·5 unit 3 hr.

All the animals that received insulin were killed at the onset of convulsions. In all cases an examination post-mortem verified the complete absence of thyroid tissue.

of analysis were those given in previous papers [Goldblatt, 1929, 1933]. The animals were killed by bleeding from a clean cut across the carotids. The animals were starved for 24 hours.

Reference to the work already quoted will show that the figures given by litter 1 represent practically an abolition of the usual effect of insulin in young rabbits. Litter 2 happened to belong to the type of animal which possesses a high liver glycogen after 24 hours' starvation, and hence the injection of insulin was followed by the usual depletion of the glycogen [Goldblatt, 1929; Rathery and Kourilsky, 1930].

It appears, therefore, that the thyroid gland is necessary for the glycogen accumulation in the liver of the starving young rabbit under insulin.

Having regard to the fact that the secondary secretion of adrenaline in the adult thyroidectomized rabbit is adequate but the responses of both hepatic and muscle glycogenolysis are sluggish, it might be supposed that the lactic acid cycle is set into motion too late to make any great contribution to liver glycogen in the case of the young thyroidectomized rabbit. It is almost certain that the effect of insulin under discussion is not an uncomplicated one, and it seems that several parts of the endocrine system are involved in it.

We have seen above that the secondary secretion of adrenaline can be readily demonstrated in the adult rabbit. Attempts were therefore made to show an increased adrenalinæmia in young rabbits as a result of insulin using the same technique. It is surprising how often samples of 1 c.c. of heart blood can be removed from the young rabbit without producing any apparent disturbance in the animal. During a period of 2 hours we have withdrawn seven specimens of heart blood (1 c.c. each) from a rabbit weighing 600 g. without any ill-effects. When tested, as described above, on the surviving intestinal strip these successive specimens of blood do not produce any increase in inhibition of rhythm of the muscle, showing that the blood-pressure is very rapidly adjusted or, at any rate, does not fall to a level sufficiently low to call forth an increased secretion of adrenaline. In Fig. 6 are shown the results of addition to a preparation of intestinal muscle of 1 c.c. heart blood taken from a normal and a thyroidectomized young rabbit at intervals after the injection of 2 units insulin per kg. and until violent convulsions were established. The strip used in this experiment showed marked relaxation when treated with 0·5γ adrenaline. It will be seen that no trace of relaxation or inhibition of rhythm was produced by the blood even during convulsions. Repetition of this experiment with two similar animals treated with ergotamine and insulin gave exactly similar results.

We do not wish to draw any comprehensive conclusion from a negative finding, nor to infer that there is no secondary secretion of adrenaline in these animals, but we consider it justifiable to state that the normal young rabbit does not during hypoglycæmia secrete relatively as much adrenaline as the adult, and also that it does not secrete more adrenaline in these circumstances than does the thyroidectomized young rabbit. In spite of this latter fact the thyroidectomized young rabbit fails to give the typical liver glycogen response to insulin.

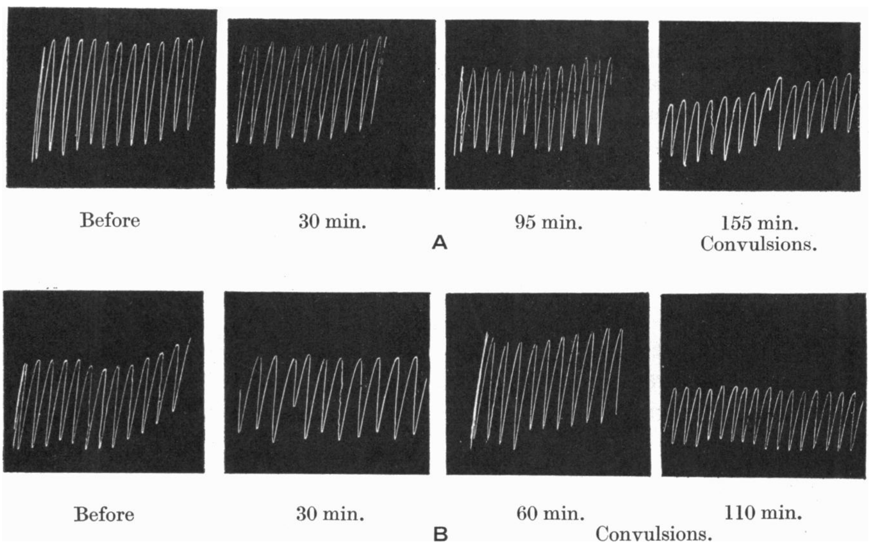


Fig. 6. Rabbit's small intestine. 35 c.c. O_2 Tyrode. Medium speed. Effect of 1 c.c. heart blood (heparinized) at various times after injection of 1 unit insulin per kg. subcutaneously. A, normal control, 750 g.; B, thyroidectomized litter mate, 550 g., 23 days after operation. Intestinal strip readily relaxed by 0·5γ adrenaline.

Whether the administration of thyroxine over a considerable period would re-establish the usual insulin effect in these animals remains to be seen; probably it would, but it seems clear that if it did so it would not be due to any modification of adrenal response.

That a part of the effect of insulin is due to the secondary secretion of adrenaline cannot be doubted in view of the known effects of this hormone and of the results of Corkill, but the findings of Cope and Marks and the data given above strongly suggest that the whole system thyroid-adrenal-pituitary-insulin is necessary. The nervous system does not appear to be involved in the effect, for as we have previously shown

[Goldblatt, 1933] ergotamine in large doses does not inhibit it, and in more recent experiments we have found that atropine in large doses also has no apparent influence. No comprehensive explanation of the phenomenon is yet possible, nor is likely to be until much more detailed knowledge is available on the mechanism of glyconeogenesis in the liver.

SUMMARY.

1. The hypersensitivity of the thyroidectomized rabbit to insulin is due to the failure to initiate adequate glycogenolysis at hypoglycæmic blood-sugar levels. Whilst this is the case in rabbits which have not been starved and those which have, in the former sufficient glucose is liberated into the circulation to prevent convulsions.

2. Ergotamine, whilst increasing the sensitivity of the normal rabbit to insulin, does not increase the already existing hypersensitivity of the thyroidectomized rabbit.

3. In both normal and thyroidectomized adult rabbits an increase in circulating adrenaline can readily be demonstrated during hypoglycæmia.

4. The blood-sugar response to adrenaline is both slower in onset and smaller in degree in the thyroidectomized rabbit than in the normal. The increase in blood lactate following the injection of adrenaline is slower in onset in the thyroidectomized rabbit than in the normal but finally not less in degree.

From these results it is concluded that the hypersensitivity of thyroidectomized rabbits to insulin is due to the sluggishness of response of the sympathetic mechanism responsible for glycogenolysis in the liver. It is shown that this sluggishness does not affect the vaso-constrictor response to adrenaline, which may be as good or better in thyroidectomized than in normal rabbits.

5. Insulin does not produce the usual deposition of glycogen in the liver of starving thyroidectomized young rabbits that is constantly observed in normal young rabbits.

6. Attempts to demonstrate an increased adrenalinæmia in young hypoglycæmic rabbits (normal and thyroidectomized) by methods which are successful in adult rabbits were without positive result.

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