XXXIII. INSULIN AND GLUCONEOGENESIS.

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THE principal theories of the action of insulin may be shortly classified as follows.

(1) The theory of increased oxidation of carbohydrate which states that insulin brings about its typical effects by stimulating the peripheral tissues to a more rapid and greater catabolism of carbohydrate. The evidence adduced in favour of this is drawn from observations on diabetic subjects and particularly from the changes in the respiratory metabolism. All such experiments have depended on the interpretation put upon the respiratory findings. This theory demands that in the normal organism the effect of insulin be accompanied by a depletion of both liver- and muscle-glycogen, for as the peripheral carbohydrate is oxidised it is conceived that the liver delivers up its glycogen until, when both sources are exhausted, convulsions supervene and eventually the animal dies of carbohydrate deprivation.

(2) The second view is that in addition to increased oxidation there occurs also a polymerisation of carbohydrate to glycogen or to substances as yet unidentified.

In the eviscerated spinal cat, where the whole metabolism takes place in muscle, a very satisfactory balance has been struck between the sugar disappearing from the perfusion fluid on the one hand and the glycogen formation and oxygen utilisation on the other [Best *et al.*, 1926]. Cori and Cori [1928] have also been able to recover about 90 % of the sugar to be accounted for in standard rats. These authors also found that in glucose-fed rats the action of insulin was to bring about a fall in liver-glycogen and an almost equal rise in muscle-glycogen. Barbour *et al.* [1927] corroborate this finding. Cori and Cori state that under the action of insulin the peripheral need for sugar is so great that there is none available for the formation of glycogen in the liver.

(3) The third view is that originally propounded by Laufberger [1924] and more recently supported by Best *et al.* [1926] and Bissinger and Lesser [1926] as a partial explanation of the action of insulin. It states that the essential action of insulin is to inhibit the formation of glycogen from fat and protein. Laufberger strongly opposed the oxidation theory as a result of respiratory experiments on various types of animals. He also, by determining the non-sugar carbon (Restkohlenstoff) in blood, showed that the existence of a new carbohydrate complex in the blood of insulinised animals, as suggested by Macleod, was very improbable and Macleod himself has

recently admitted that the conception is unnecessary. In common with other workers Laufberger found that insulin caused the disappearance of glycogen from the liver of the normal animal, and he conceived that death from excess of insulin occurred as a result of (1) exhaustion of existing glycogen in the liver and tissues and (2) inhibition of replenishment from non-carbohydrate sources. The whole of this theory stands or falls, as Laufberger himself says, on the truth of the observation that insulin brings about an exhaustion of liver- and muscle-glycogen. He emphatically states that in no type of animal has he ever failed to find this glycogen-depleting action of insulin, whether convulsions did or did not occur.

(4) The fourth theory is that insulin produces a change in the nature of sugar so that a more readily utilisable form is produced. Various substances have been suggested but the evidence is very meagre.

It seemed to us that the fundamental matter to decide unequivocally was whether insulin did in fact diminish the liver- and muscle-glycogen in the normal animal. The diversity of the results in the literature made it probable that some underlying factor was preventing a uniformity in the findings of different workers, for there seemed no possibility of explaining the differences merely on criticisms of technique. The question arose as to whether it might not be explained by the variability in development in different species of animal. There can be no doubt that in different species the activity of such glands as the adrenal, pituitary, thyroid and sex glands is a variable factor and that this applies also to different stages in the development of the same animal. It was decided to see if uniform results could be obtained in very young animals. Young rabbits were used and comparisons made between members of the same litter. The findings with these animals have already been published [Goldblatt, 1929]. It was found that in every case after a dose of insulin (0.2–0.5 unit) sufficient to bring about convulsions in the young rabbit starved for 24 hours previously, there occurred a very large rise in liver-glycogen. Further, this increase was associated in all the cases with a blood-sugar of vanishing proportions and a muscle-glycogen not significantly affected. It was observed that the greatest rises in liver-glycogen occurred when the animals were allowed to proceed right to the onset of convulsions. In fact it was thought that with a given dose of insulin the concentration of glycogen might be a roughly linear function of the time of action.

Frank *et al.* [1927], using adult rabbits, state that if the animals be starved for 4 to 6 days their liver-glycogen does not exceed 0.2 % and they are therefore suitable for controls in comparative work with insulin. The muscle-glycogen, they state, shows no uniformity. Using 6 such animals (liver-glycogen varying from 0.05 to 0.297 %) they found that in a series of similarly starved animals treated with insulin in doses of 0.1 clinical unit, the liver-glycogen rose to 3.8 % (variations from 0.6 to 3.8 %) even when the blood-sugar fell relatively little. These authors do not state the weight or age of

their animals. In some experiments which we carried out on adult animals of 4 kg. weight the liver-glycogen after 4 days' starvation was in several cases between 2 and 3 %. In two such animals used for comparative work with insulin the control after 4 days' starvation had a liver-glycogen of 2.1 % and the other animal after injection of 0.5 clinical unit on two occasions gave a value of 2.73 %, the blood-sugar having fallen from 0.113 to 0.062 %, the total time of experiment being 3 hours 11 minutes. The difference between these results and those of Frank *et al.* may be due to the age and activity of the rabbits used, young animals losing their glycogen much more quickly on starvation than old ones. The conclusion drawn by Frank *et al.* from their experiments was that the theory of Laufberger, later supported by Dale and by Bissinger and Lesser, that the action of insulin was essentially to inhibit gluconeogenesis, is no longer tenable, Frank considering that the extra liver-glycogen is derived from body-protein. As there is no analysis of nitrogen excretion given this cannot yet be said to be proven.

There can, however, be no doubt that both in Frank's experiments and our own, using starved animals, a new formation of glycogen was taking place under the influence of insulin, and that this new glycogen was not derived from muscle-glycogen, since it has been abundantly shown that the latter cannot contribute to circulating carbohydrate [Bollman, Mann and Magath, 1925; Markowitz, 1925; Soskin, 1927].

Further consideration of the facts seems to complicate the problem. How is the fact that insulin brings about a large increase in the liver-glycogen of the fasting rabbit to be reconciled with the further fact that animals to which sugar is administered and into which insulin is injected in large doses show a progressively diminishing glycogen content in the liver? This latter fact is emphasised by Barbour *et al.* [1927], who say: "There can be no doubt that very large doses of insulin given to animals which are absorbing glucose from the alimentary canal cause more glycogen to be deposited in the muscles accompanied by an approximately corresponding decrease in the amount deposited in the liver."

This statement was made as a result of work done with standard rats, but we show below that it is also true for the young rabbit. Frank, attempting to explain this apparent anomaly, considers that, since, in order to produce convulsions in the fed animal, it is necessary to use large and unphysiological doses of insulin, there occurs a large utilisation of sugar peripherally so that the liver tends to replenish the muscle-glycogen by delivering up its own glycogen. Thus there occurs a fall in hepatic glycogen. We cannot, however, accept this view because it would necessitate in all cases the association of largely depleted liver- and muscle-glycogen with the onset of convulsions, which is not a fact [Goldblatt, 1929].

If we consider that in our young rabbits, given a dose of insulin sufficient to reduce the blood-sugar to almost zero and bring about the convulsive syndrome, there occurs a large accumulation of glycogen in the liver which

if liberated as glucose could preserve the animal's life for a considerable period, and, further, that if to such animals glucose be administered *per os* or parenterally recovery occurs, then it seems clear that, although there is a definite inhibition of glycogen release, free sugar is in quite a different position and can up to a point pass freely through the liver. This matter will be returned to later in this paper.

EXPERIMENTAL.

Having shown in a previous communication [Goldblatt, 1929] that the injection of insulin into young rabbits is followed invariably by a very large accumulation of glycogen in the liver, it was important to see if this occurred also in other species. For this purpose we first of all chose the rat. The very large amount of work done by Barbour *et al.* [1927] and by Cori and Cori [1928] on the metabolism of the rat made it improbable that our results would be likely to add to our knowledge of the action of insulin. These investigators have in the main obtained similar results, that insulin depletes the liver-glycogen in the starving rat and inhibits glycogen formation in the fed rat. Cori's view as a result of his work is that insulin produces so great an increase in peripheral oxidation that the liver is forced to give up its glycogen or does not form glycogen at all.

These results are so out of harmony with those we obtained with young rabbits that it is felt that there is some fundamental difference between the metabolism of these animals. In order to satisfy ourselves that the large increase in liver-glycogen could not in fact be reproduced in rats, the following experiments were carried out. The methods of analysis were the same as those detailed in our previous work.

Experiments 1 and 2 show that with doses of insulin varying from 0.2 unit to 4 units significant increases in glycogen could not be demonstrated in the liver of the rat starved for 24 hours. It is further clear that rats vary enormously in their susceptibility to insulin. Thus animal 3 in Experiment 2 developed convulsions after 0.75 unit in 1 hr. 7 mins., whilst animal 4 showed no symptoms 2 hrs. 36 mins. after 1.25 units. It is also to be remembered that the metabolism of the rat is very rapid and that during starvation they lose weight very rapidly due to the acceleration of the processes of gluconeogenesis which are necessary to replace the glycogen which they exhaust very quickly. These animals must therefore possess mechanisms, probably regulated by their endocrine system, by means of which they can rapidly mobilise carbohydrate for the needs of their muscular system. This state of affairs is in marked contrast to that found in the rabbit, which has a relatively much lower heat production. Having regard to these considerations it was to be expected that, if insulin produced a very much increased peripheral utilisation of sugar and at the same time inhibited gluconeogenesis, the rat would be a particularly suitable animal for demonstrating a rapid depletion of liver- and even muscle-glycogen.

Exp. 1. Four albino rats were starved for 24 hours. They were then treated as detailed below and determinations made of blood-sugar and liver-glycogen. The injections of insulin were made subcutaneously.

No.	Wt. g.	Blood- sugar g. per 100 cc.	Liver-glycogen		per 100 g. body-wt. mg.	Remarks
			g. %	mg.		
1	169	0.072	0.85	38	22.5	Control—no treatment
2	129	0.069	0.69	26	20.2	0.2 unit—killed 2 hrs. 40 mins. later
3	149	0.081	0.65	28	18.8	0.4 unit—killed 2 hrs. 54 mins. later
4	106	0.051	1.00	29	27.3	0.6 unit—killed 3 hrs. later

Exp. 2. Six albino rats were starved for 24 hours and then treated as below.

No.	Wt. g.	Blood- sugar g. per 100 cc.	Liver-glycogen		per 100 g. body-wt. mg.	Remarks
			g. %	mg.		
1	184	0.100	0.59	34	18.5	Control
2	119	0.018	0.67	27	22.7	2.13 p.m. 0.5 unit 3.10 " Killed
3	110	—	0.82	28	25.6	2.18 " 0.75 unit 3.25 " Convulsions 3.30 " Killed
4	138	0.033	0.95	33	23.9	2.24 " 1.25 units 5.0 " Killed
5	188	—	1.11	52	27.7	2.30 " 1.5 units 5.51 " 2.0 units 7.12 " Killed
6	199	0.020	0.74	45	22.6	Hypoglycaemic during 40 mins. 2.35 " 2 units 5.12 " 2 units 5.35 " Convulsions and killed

Dale and his co-workers have indeed drawn attention to this point. But our results cannot be brought into line with any theory which attributes an inhibition of gluconeogenesis to insulin, for whatever may have occurred in the peripheral tissues there certainly did not occur any exhaustion of liver-glycogen, which leaves no doubt in our mind that formation of carbohydrate from non-carbohydrate sources was proceeding quite adequately in spite of the active effect of the insulin.

Exp. 3. Four young rats were taken without previous starvation. One was kept as a control and the other three received subcutaneous injections of 1, 2, 3 units (clinical) of insulin. The latter animals were allowed to die in convulsions. The control was killed after the others had died. No food was allowed during the experiment.

Carcass signifies what remained after decapitation and removal of the abdominal viscera.

No.	Wt. g.	Blood- sugar g. per 100 cc.	Liver- glycogen		Carcass- glycogen		Total glycogen per 100 g. body-wt.	Remarks
			g. %	mg.	g. %	mg.		
1	57	0.030	0.35	8	0.21	47	96	3 units—convulsions 29 mins. after injection, lasted 37 mins., when animal died
2	53	"0"	0.53	11	0.12	33	83	2 units—convulsions 22 mins. after injection, lasted 50 mins., when animal died
3	49	"0"	0.42	10	0.21	46	114	1 unit—convulsions 24 mins. after injection, lasted 52 mins., when animal died
4	61	0.113	0.49	11	0.24	64	123	Control—killed after the others had died

The first noteworthy point in Exp. 3 is the exceedingly low values for glycogen in these small animals, even unstarved, which gives some idea of the necessity for frequent feeding in order to spare the tissues. The enormous doses of insulin used and the very severe convulsions which supervened were clearly not able to bring about an exhaustion of either liver- or carcass-glycogen. Definitely less glycogen was found in the insulinised animals than in the control, but this did not apply to any extent in the liver.

Exp. 4. Four albino rats were starved for 24 hours and were then given a mixture of 3 g. flour and 5 g. glucose worked up into a soft mass with a little water. They all ate the mass readily and after 3 hrs. had consumed almost all of it. Injection of insulin took place immediately on presenting the food mixture.

No.	Wt. g.	Liver-glycogen			Remarks
		g. %	mg.	per 100 g. body-wt. g.	
1	132	4.63	182	1.38	Control—killed 4 hrs. after presenting food
2	163	2.86	142	0.87	0.2 unit insulin—killed 4 hrs. after presenting food
3	152	4.27	177	1.16	0.4 unit insulin—killed 4 hrs. 10 mins. after presenting food
4	138	4.95	187	1.36	0.6 unit insulin—killed 4 hrs. 30 mins. after presenting food

Unfortunately in this experiment the blood-sugar values could not be determined. The figures are, however, of some interest in view of the findings of Barbour *et al.* that up to 3 hours after the injection of moderate doses of insulin in fed rats there occurs a retardation of glycogen deposition in the liver. Of course, no more can be concluded from this experiment than that it is at least possible to have rats which, when fed with plenty of carbohydrate and treated with doses of insulin varying from 1 to 5 units per kg., can lay down quantities of glycogen of the same order. Although this to some extent opposes the findings of Barbour *et al.* [1927] and of Cori and Cori [1928] we do not deny the principle involved in their findings, for similar results to theirs could be obtained using young rabbits, as shown in the next experiment.

Exp. 5. Six young rabbits from the same litter, 7 weeks old, were starved for 24 hours before the experiment. 20 cc. of 50 % glucose solution were given by mouth by means of a tube and syringe. Insulin was injected immediately afterwards and the animals were killed 3 hrs. later.

No.	Wt. g.	Blood-sugar g. per 100 cc.	Liver-glycogen		Remarks
			g. %	Total g.	
1	667	0.154	0.55	0.10	Control—no treatment
2	774	0.224	5.00	1.54	10 g. glucose <i>per os</i>
3	714	0.197	3.82	0.84	" " " —2 units insulin
4	760	0.159	3.56	0.82	" " " —4 "
5	737	0.090	2.20	0.59	" " " —6 "
6	725	0.058	2.15	0.51	" " " —8 "

None of the animals in this experiment showed any symptoms. The progressively lower glycogen in the liver with increasing doses of insulin was very striking, particularly when one considers that it is possible to obtain

values of liver-glycogen up to 4 % in insulinised animals given no food at all (see below).

Examination of the intestinal contents of all the animals which had been given glucose showed that there were still large quantities of reducing sugar available. In spite of this the animals were approaching hypoglycaemia which, as is well known, could have been averted by giving more sugar. This puzzling circumstance recalls the fact that after the administration of sugar to the normal animal the temporary rise in blood-sugar is followed by a marked fall, even to hypoglycaemic levels, at a time when there are still large quantities in the gut and the respiratory metabolism has returned to normal levels. This phenomenon is usually explained as being the result of a stimulus to storage in the liver arising from the raised blood-sugar and the degree of saturation of the muscles with carbohydrate. This conception clearly cannot be applied to such an experiment as No. 5.

If absorption from the gut is at the same rate in every case then there seems to be no alternative but to regard the lower liver-glycogens as due to the excessive utilisation in the muscles brought about by the very large doses of insulin. But we feel that this question of absorption is precisely the difficulty. This matter is being investigated and no final decision can as yet be given.

The question of controlled absorption arises again if we consider the changes in blood-sugar after successive doses of glucose. The following experiment illustrates this.

Exp. 6. The blood-sugar changes in normal men following 4 successive doses of 50 g. glucose each. The first was given 3 hrs. after the morning meal.

Subject 1:		Blood-sugar g. per 100 cc.	Remarks
	Time		
	12.00	0.102	12.05 p.m. 50 g. glucose
	12.30	0.150	
	1.00	0.125	1.00 " "
	1.30	0.135	
	2.00	0.125	2.00 " "
	2.30	0.109	
	3.00	0.125	3.00 " "
	4.00	0.117	
Subject 2:			
	11.40	0.113	11.45 a.m. 50 g. glucose
	12.15	0.122	
	12.45	0.122	
	1.15	0.104	1.30 p.m. "
	2.00	0.122	
	2.30	0.125	2.50 " "
	3.05	0.113	
	3.20	0.129	
	3.35	0.100	3.50 " "
	4.15	0.117	

The first point of interest in these figures is that one cannot force up the blood-sugar of the normal subject by successive doses of sugar: all that is possible is to prevent the fall to the rather low levels which commonly follow the administration of a single dose. Examination of these tables will show that at three points the administration of the sugar was followed in the first

half-hour by a fall in blood-sugar, to be succeeded by a return to the original or a rather lower level. Such a fall has never been observed by us following a single dose of sugar. It appears, therefore, that there exists a very delicate mechanism by means of which the processes of absorption and discharge of sugar from the liver are regulated to the needs of the peripheral tissues. Thus, in starvation, when the immediate needs of the tissues are being supplied by gluconeogenesis, the administration of sugar is followed by blood-sugar values far in excess of the normal which are sustained at high levels for a very prolonged time. The respiratory metabolism in such cases shows much less rise in R.Q. and what rise does occur takes place during the fall in blood-sugar. The same delay in the rise of R.Q. also occurs in the ordinary post-absorptive state. Accepting the usual interpretation of the R.Q. this would mean that there is a lag in the oxidative mechanism after the presentation of the sugar to the tissues, which would suggest that a certain concentration of carbohydrate must be reached in the tissues before the process of oxidation is commenced or the process of gluconeogenesis is diminished.

Now the height of the blood-sugar at any time is proportional to the ratio $\frac{\text{rate of liberation from the liver}}{\text{rate of peripheral utilisation}}$ and the normal organism seems at all times to tend to readjust this ratio to an equilibrium value which keeps the blood-sugar in the region of 0.100 g. per 100 cc. Permanent disturbance in either or both the terms of this ratio must result in a pathological condition.

The possible number of variations of this ratio is nine, according as we have unchanged, increased and diminished activity of the two factors. Every case of ortho-, hyper-, and hypo-glycaemia must be covered by these variations.

Now after the administration in the post-absorptive state of a dose of glucose *per os* there is at first a rapid delivery of sugar to the muscles and a temporarily almost exclusively carbohydrate oxidation. There then seems to occur a fall in sugar oxidation and then the blood-sugar falls. There must hence be a fall in both terms of the above ratio until, when the mass of available carbohydrate reaches a certain level, gluconeogenesis is brought into play in order to keep the ratio as near the equilibrium point as possible.

Now, since an animal can go into insulin convulsions with quantities of sugar in the gut, it seems unavoidable to consider that the concentration of the intestinal sugar has fallen to a level at which the rate of absorption is inadequate to combat the hypoglycaemic action of the insulin, although the absolute amount may be quite sufficient. The effect of dilution can easily be shown by taking 50 g. glucose dissolved in a very large volume of water, say a litre, when the rise in blood-sugar will be found to be very slight indeed.

But the fact, as seen in Exp. 5, that progressively increasing large doses of insulin acting for the same time are associated with progressively less formation of glycogen from free sugar, indicates that the passage of sugar

through the liver is so rapid that a large quantity of glucose has passed through the liver before the dilution effect comes into play. This is not the same as saying that the animal can deplete its liver-glycogen when under the influence of insulin, which is the opinion of Frank and others. For, as we have seen in young animals, and Chaikoff [1925] found in adults, death from insulin convulsions may occur in the presence of high liver-glycogen values. What is suggested is that free sugar can, under the necessities brought about by large doses of insulin, pass rapidly through the liver, the amount remaining available for formation of glycogen being correspondingly less. But such glycogen as is formed, we conceive, is fixed so that it is not available for the relief of the severe condition which later arises.

In the next experiment further evidence was sought for the view that insulin inhibits glycogen release.

Exp. 7. The writer was the subject of experiment. In order to establish a state in which the process of gluconeogenesis was actively proceeding, a preliminary starvation of 40 hours was undergone. Insulin was then injected subcutaneously and when hypoglycaemia was well established glucose was taken. As evidence of gluconeogenesis we may take the degree of ketosis, for a large body of evidence exists that the ketonic acids arise as products of this process. These substances were estimated by a method described by the writer for small quantities of urine [Goldblatt, 1925].

Time	Urine cc.	Rothera reaction	Acetoacetic acid mg. per hr.	β -Hydroxy- butyric acid mg. per hr.	Blood-sugar g. per 100 cc.
11.00 a.m.	33.0	++	6.8	7.4	—
11.30 "	13.0	++	—	—	—
11.52 "	—	—	—	—	0.077
11.54 "	8.0	++	9.3	4.8	—
11.57 "	10 units insulin hypodermically				0.062
12.00 "	—	—	—	—	—
12.30 p.m.	19.0	++	17.4	39.6	0.065
12.45 "	50 g. glucose in 200 cc. water taken				0.054
12.47 "	10.0	++	—	—	—
1.00 "	—	—	—	—	—
1.15 "	10.0	++	11.6	13.6	0.082
1.30 "	8.5	++	—	—	—
1.45 "	9.5	+	6.8	7.6	0.109
2.00 "	10.0	—	0	0	—
2.15 "	15.0	—	0	0	0.122
2.30 "	9.5	—	—	—	—
2.45 "	14.0	—	—	—	0.122
3.00 "	10.0	—	0	0	—
3.15 "	—	—	—	—	0.069
3.30 "	17.0	—	0	0	—
3.45 "	—	—	—	—	0.045
4.00 "	15.5	—	0	0	—
4.15 "	—	—	—	—	0.027

At this stage a strong hypoglycaemic reaction was experienced. Glucose, bread and tea were administered and recovery took place in about an hour. At 5.30 there was glycosuria and a positive Rothera reaction and at 5.45 the blood-sugar was 0.137 g. per 100 cc. In interpreting these findings the following points must be noted.

(a) After this period of starvation there is ample glycogen in the liver and tissues but gluconeogenesis is proceeding as indicated by the ketosis.

Since the latter can be completely abolished by giving glucose or any other good glycogen former, we consider that it is the concentration of glycogen in the liver which determines the degree of gluconeogenesis.

(b) When the excretion of the ketonic acids falls to zero we consider that the glycogen in the liver is at an optimum for the normal supply of carbohydrate to the tissues.

The first point we note in this experiment is the rapidity of action of the insulin, pre-hypoglycaemia being definitely experienced in 33 mins., when the blood-sugar had fallen to 0.054 g. per 100 cc. 50 g. glucose in 200 cc. water having been taken, a severe hypoglycaemic attack occurred 3½ hours later. Examination of the blood-sugar values shows that although the maximum value was only 0.122, the absolute rise above the level attained when the sugar was taken was 0.068 g. per 100 cc., which is not significantly different from the increase usually obtained after such a dose of sugar without insulin. Thus the rate of passage of sugar through the liver was up to this point adequate to counteract the effect of the peripheral utilisation. This maximum point was associated with the disappearance of the ketosis and, as we believe, with a concentration of glycogen in the liver sufficient to inhibit gluconeogenesis.

The phenomena now become different, for the blood-sugar values fall steadily until very low levels are attained (0.027 g. per 100 cc.) and still the ketosis remains absent. No urine could be collected between 4.15 and 5.30 p.m. as the subject was prostrate; but the nitroprusside reaction was distinctly positive at the latter time and at 5.45 p.m., *i.e.* when the action of the insulin had worn off. Examination of the values for the excretion of acetoacetic acid and β -hydroxybutyric acid shows that there occurred a large rise in the amounts of these substances after the injection of the insulin. Similar results have been recorded by Collip [1923], who observed the production of ketone bodies in hypoglycaemic rabbits, and by Burn and Ling [1928], who report very large increases in the ketosis of rats adapted to a fatty diet, following the injection of insulin. We have not been able to confirm these latter findings but the results in the above experiment are suggestive.

Now, although it is probable from Exp. 5 that there was less glycogen in the liver than there would have been had no insulin been injected, there could be no doubt that even when a dangerous hypoglycaemia had been reached there was an amount present high enough to prevent gluconeogenesis. This, we consider, corroborates our view that the outstanding effect of insulin in the intact animal is to inhibit the release of such glycogen as is already present in the liver.

In the following experiments a relationship was sought between the formation of new liver-glycogen, the dose of insulin and its time of action in the starving young rabbit.

Exp. 8. In this experiment the time of action was kept constant and the dose varied. Five young rabbits, 6 weeks old, from the same litter were starved for 24 hrs. and were killed in all cases about 2 hrs. after the injection of the insulin, whether convulsions had or had not supervened.

No.	Wt. g.	Blood- sugar g. per 100 cc.	Liver-glycogen		Increase over control mg.	Remarks
			g. %	Total mg.		
1	493	0.141	0.82	139	—	Control
2	399	0.058	1.71	297	158	0.25 unit—slight convulsive attack. Killed 2 hrs. 10 mins. after insulin
3	472	0.062	1.03	167	28	0.50 unit—no symptoms. Killed after 2 hrs. 9 mins.
4	590	0.058	0.99	185	46	0.75 unit—no symptoms. Killed after 2 hrs. 7 mins.
5	682	0.058	0.69	167	28	1.0 unit—no symptoms. Killed after 2 hrs. 5 mins.

It has been found in these young animals that the glycogen content of the liver after 24 hours' starvation is higher in the winter (when the experiments recorded in this paper were carried out) than in the summer. Further, they seem to be more resistant to insulin in the winter than in the summer, as judged by the rapidity of onset of convulsions after a given dose. The results of the above experiment show that the degree of gluconeogenesis in the liver is not so much a function of the dose of insulin as of the rapidity of approach to the convulsive state. The only animal which showed a rise in the liver-glycogen which we can regard as considerable was No. 2, which was the only one that exhibited a convulsive attack, and it had received the smallest dose of insulin. We must, therefore, consider that the large new formation of glycogen is a response to some urgent necessity arising from the intensity of action of the insulin. In the next experiment the dose of insulin was kept constant and the time of action varied. Estimations of muscle (gastrocnemii)-glycogen were also carried out in each case. Determinations of so-called "free sugar" were also made in liver and muscle. This was done as follows. About 5 g. of tissue were dropped into a weighed stoppered centrifuge tube of about 100 cc. capacity containing 20 cc. absolute alcohol, which had been kept in ice. After weighing, the tissue was minced under the alcohol as finely as possible with sharp scissors, which were washed clean with 20 cc. 70 % alcohol. The contents were now shaken periodically and kept in ice for 2 hours and then centrifuged. The clear supernatant fluid was then pipetted off and run into a graduated 100 cc. flask. The entire operation was then repeated and the supernatant fluid again added to the flask. The alcohol was now removed on the water-bath and the residual fluid, which was of a yellowish colour, worked up for estimation of sugar as follows. 20 cc. of 15 % sodium sulphate and 3 drops of glacial acetic acid were added to the flask and, after warming on the water-bath, 1 cc. of colloidal ferric hydroxide was added. The flask was cooled and the liquid made up to 100 cc. and filtered. Reducing power was estimated in 20 cc. of the filtrate by the method of von Issekutz and von Both [1927]. In regard to this method

it must be mentioned that when dealing with extracts of tissue allowance must be made for iodine-binding substances by a preliminary titration in the cold. Although there can be no question of the figures thus obtained being entirely due to sugar, it was thought that any considerable change in tissue-sugar would show itself by this method.

Exp. 9. Seven young female rabbits from the same litter, 6 weeks old, were starved for 24 hrs. Two were taken as controls, four were given 0.5 unit of insulin subcutaneously and killed at different times afterwards. One was given sufficient insulin to put it into convulsions and then killed.

No.	Wt. g.	Blood- sugar g. per 100 cc.	Liver			Muscle		Dose of insulin and time of action
			Glycogen		"Free sugar" Total mg.	Glycogen g. %	"Free sugar" g. %	
			g. %	Total mg.				
1	546	0	0.81	142	127	0.28	—	Control
2	563	0.122	0.62	155	—	0.29	0.27	"
3	535	0.035	1.28	225	—	0.28	0.25	0.5 unit — 30 mins.
4	538	0.056	0.78	152	66	0.32	—	0.5 " — 60 "
5	535	"0"	1.19	195	—	0.32	0.31	0.5 " — 97 "
6	492	0.053	3.62	761	90	0.30	—	0.5 " —145 "
7	587	0.035	4.43	1159	—	0.23	0.28	0.5 " —3.30 p.m.
								0.5 " —6.25 "
								0.5 " —8.00 "
								2.0 units—8.15 "
								Hypoglycaemia and killed—9.00 p.m.

It was again seen that the control values for liver-glycogen were much higher than those found in similar animals in summer. The muscle-glycogen was, however, of the same order as always found in these animals.

The first point elicited from this experiment was that, although there occurred marked rises in liver-glycogen in animals 3 and 5, animal 4 showed no change, which we think shows that the amassing of liver-glycogen is not merely a function of the time of action of the same dose. The time of action is, however, clearly of great importance as is seen from the findings with animal 6, which, without any manifest symptoms, was found to have a liver-glycogen of 3.62 % and a total of 761 mg., *i.e.* 5 times the control value. It seems as if the process of gluconeogenesis were suddenly stimulated to a very much increased activity by an emergency created by the insulin at its point of maximum effectivity. No. 7 illustrates very strikingly the progressive nature of this glycogen deposition in the liver and demonstrates beyond doubt that insulin, in these circumstances, so far from "clearing out" the glycogen, is bringing about a very rapid gluconeogenesis.

The values for muscle-glycogen were remarkably uniform and left no doubt that whatever carbohydrate was being used peripherally was being adequately replenished from new sources as the insulin continued its action. The "free sugar" in the liver was very suggestive of an inhibition of glycogenolysis, for the ratio of this to glycogen fell from 0.9 in the first control to 0.43 in No. 4 and then to 0.12 in No. 6. In the muscles, however, no such striking changes were found. These results suggest that whilst insulin may

inhibit the change from glycogen to glucose in the liver, it does not do so in the muscles.

From the last two experiments we consider that insulin can stimulate the process of gluconeogenesis in the liver, and that this effect is most obvious when the animal is at the stage of convulsions.

SUMMARY.

1. Evidence is presented that insulin can stimulate the new formation of glycogen from endogenous sources.

2. It is considered that the theory that insulin inhibits gluconeogenesis is untenable.

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REFERENCES.

- Barbour, Chaikoff, Macleod and Orr (1927). *Amer. J. Physiol.* **80**, 243.
Best, Dale, Hoet and Marks (1926). *Proc. Roy. Soc. Lond. B.* **100**, 32.
Bissinger and Lesser (1926). *Biochem. Z.* **168**, 398.
Bollman, Mann and Magath (1925). *Amer. J. Physiol.* **74**, 238.
Burn and Ling (1928). *J. Physiol.* **65**, 191.
Chaikoff (1925). *Trans. Roy. Soc. Canad.* **19**, Sect. 5, 83.
Collip (1923). *J. Biol. Chem.* **55**. *Proc. Soc. Amer. Biol. Chem.* **38**.
Cori and Cori (1928). *J. Biol. Chem.* **76**, 755.
Frank, Nothmann and Hartmann (1927). *Arch. exp. Path. Pharm.* **127**, 35.
Goldblatt (1925). *Biochem. J.* **19**, 626.
— (1929). *Biochem. J.* **23**, 83.
v. Issekutz and v. Both (1927). *Biochem. Z.* **182**, 298.
Laufberger (1924). *Z. ges. exp. Med.* **42**, 570.
Markowitz (1925). *Amer. J. Physiol.* **74**, 22.
Soskin (1927). *Amer. J. Physiol.* **81**, 382.