XIII. THE ACTION OF INSULIN IN NORMAL YOUNG RABBITS.

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THE early publications by the discoverers of insulin gave evidence that under the influence of insulin the glycogen content of the liver increased. Later, however, contradictory results were obtained. Dudley and Marrian [1923] state that, using normal mice, they found that the sugar disappearing from the blood under the action of insulin is not stored in the liver or the muscles as glycogen. Further they found that a dose of insulin big enough to produce convulsions depletes the liver and muscles of glycogen. Collazo, Händel and Rubino [1924], however, investigating the glycogen of liver and muscles in normal guinea-pigs 4 hours after the injection of 7 units of insulin, found a marked increase in both situations. Their animals were starved for 18 hours and received 3 g. of glucose by stomach tube before the injection of the insulin. The degree of rise in glycogen in these experiments both in liver and muscle was about 50 to 70 % above the controls. Cori [1925] found that starving rabbits showed no appreciable change in their liver-glycogen in the first hour of insulin action and that this was true whether the initial glycogen content was high or low and whether the fall in blood-sugar was large or small. In the second hour after insulin the liver-glycogen was found to remain constant or decrease. Similar results were obtained with mice, the average liver-glycogen of 16 injected animals being 39 % lower than the average of 16 controls. On the other hand completely phloridzinised and starving cats, dogs and rabbits were found to deposit liver-glycogen under the influence of insulin. Cori [1924] found an increased glycogen formation as a result of insulin action. Grevenstuk and Laqueur [1925], however, questioned this result. Frank and Hartmann [1926] criticised the findings of Grevenstuk and Laqueur, and Frank, Nothmann and Hartmann [1925] claim to have found an increase in liver-glycogen in fasting rabbits using small doses of insulin. Barbour *et al.* [1927], using a large number of standard rats, examined the changes in blood-sugar and muscle- and liver-glycogen induced by insulin. The effect produced depended on the dose of insulin. Thus, with 1 or 2 units per kg. body weight the liver-glycogen fell during the first 1½ hours and rose slightly after 2 hours: with 3 units per kg. there occurred a steadily progressive fall in the liver-glycogen. The muscle-glycogen of starving animals fell in all cases after insulin. In fed animals,

however, the total fall in liver-glycogen was almost balanced by a rise in the muscles. Cori and Cori [1928], after studying sugar utilisation in normal fasting rats, concluded that, "after insulin, the utilisation of absorbed sugar in the peripheral tissues (mainly muscles) is increased to such an extent that there is almost no sugar available for glycogen deposition in the liver." On this view, therefore, the main action of insulin is to bring about increased oxidation of glycogen in the peripheral tissues. Other experimenters found definite decreases in muscle-glycogen after insulin, for example, Dudley and Marrian [1923], Andova and Wagner [1924], and Kay and Robison [1924]. According to Hetenyi [1925] the "total sugar" of muscles falls after insulin. The work of Dale and co-workers [1926] has demonstrated that deposition of glycogen can be brought about in the eviscerated spinal cat by means of insulin, whereas without insulin perfusion with glucose does not lead to glycogen formation. Best, Hoet and Marks [1926] regard the immediate action of insulin as being a production of a more rapid combustion of sugar with a coincident further synthesis of glycogen.

It is apparent that as far, at any rate, as the liver-glycogen is concerned no final decision has been reached. The interpretation of any given series of results will naturally depend on the view taken of the glycogenic function. The two main views of the glycogenic function are the so-called storage theory and the secretory theory. The distinction between these views is fundamental: the former states that glycogen is deposited in the liver as a passive store which represents the excess of absorbed over utilised carbohydrate, and that the function of the liver is to keep the blood-sugar below certain levels. The latter regards glycogen as a true internal secretion and considers that the liver is constantly manufacturing this substance and liberating it as glucose in order to keep the blood-sugar above hypoglycaemic levels. The evidence for the secretory view is accumulating rapidly and has been fully discussed by Cramer [1928] and experimentally supported by Markowitz [1925].

A further matter which must be constantly kept in mind is the irreversibility of muscle-glycogen. Once glycogen is formed in the muscles it can no longer contribute to the upkeep of the blood-sugar. The experiments of Mann and Magath [1925] show that in hepatectomised dogs adrenaline can no longer bring about a rise in blood-sugar, in spite of the presence of glycogen in the muscles. From the point of view of available carbohydrate which could conceivably be of value in the relief of insulin convulsions it seems clear that muscle-glycogen is valueless. Strangely enough Chaikoff [1925] suggests that the condition for the recovery from convulsions is the presence of muscle-glycogen.

Dudley and Marrian [1923] concluded that muscle-glycogen was almost completely absent when convulsions occurred, but Chaikoff [1925] showed that this was not the case, a finding with which we entirely agree.

EXPERIMENTAL.

The first point which had to be considered was the choice of animals which could provide reliable controls. Barbour *et al.* [1927] state that rabbits are unsuitable because of the marked irregularity in the glycogen content of the tissues, even when every precaution is taken to have the animals fed and otherwise treated alike. This is certainly true of adult rabbits but it is not for very young ones, particularly if they are from the same litter. The values for muscle-glycogen are not as uniform as those for liver-glycogen. It was therefore decided to breed our own rabbits and carry out comparative experiments on members of the same litter. The animals were allowed to grow for periods of about 6 weeks, their diets being bran, oats and abundant green stuff as soon as they became independent of the mother. Occasionally there is some difficulty in keeping them healthy, as they are very susceptible to infections. Our best litters were obtained from primiparae, particularly if the young were all dominants.

The young animals were placed in a cage with no food for 24 hours before experiment. They were then divided into two groups, one being taken for controls. Only the most definitely healthy animals were taken.

Blood-sugar was determined by MacLean's method, heart-blood being generally taken.

Glycogen was determined in liver and muscle in the following manner. The animal was killed by a sharp blow on the back of the head, extended and the thorax and abdomen were rapidly opened. 0.2 cc. blood was taken immediately from the heart and transferred to the sodium sulphate used in the blood-sugar method. The thorax was packed with swabs and the liver quickly removed, the gall bladder detached and the liver weighed. Boiling 60 % potassium hydroxide (1 cc. to 1 g. tissue) was added. This process, from the time of killing the animal until the addition of the potassium hydroxide, did not occupy more than 90 seconds. In general the whole liver was worked up, but in a few cases part of it was taken for the estimation of fat. The liver was never minced but cut into small pieces with sharp scissors.

Meanwhile the posterior extremities were skinned and the gastrocnemii muscles removed, weighed and worked up for glycogen in the usual way. The separation of the muscles is easy in young rabbits because there is very little fat and one obtains what is almost all muscle tissue. The precipitate of glycogen was allowed to settle during 24 hours, filtered, washed twice with 66 % alcohol, twice with absolute alcohol and with absolute ether. The glycogen was dissolved in boiling water which was allowed to filter through into a volumetric flask. An aliquot part was hydrolysed in 1.8 % HCl, until no more glucose was produced. The period was usually 2 hours when the total glucose was not greater than 60 mg. After cooling and neutralising with KOH, the solution was made up to a known volume and filtered through a starch-free filter-paper.

The glucose was estimated by the method of von Issekutz and von Both

[1927] which depends on the reduction of potassium ferricyanide as in the Hagedorn and Jensen blood-sugar method, and the quantities have been so adjusted that amounts of glucose only up to 15 mg. can be estimated. The technique is particularly suitable for accurate glycogen estimations. In general, in this work, the quantities were adjusted so that no estimation involved more than 10 mg. of glucose. Many duplicates were carried out, always with the most satisfactory results.

The insulin used in these experiments was that prepared by Messrs Burroughs and Wellcome. Dilutions were made up in distilled water so that 1 cc. contained 1 clinical unit, and were used only on the day of preparation.

Exp. 1. Six young rabbits, 10 weeks old, from the same litter. Starvation for 24 hours before experiment. Three were used as controls and three were injected subcutaneously with 0.5 clinical unit of insulin. The first of the insulinised animals was killed 1 hour after the injection and the remaining two at the first sign of convulsions. The onset of convulsions in these young animals is always introduced by a period of incoordination followed by flaccidity. These are followed generally by a certain period of recovery, then a typical convulsion. In this experiment the animals were not allowed to go into convulsions but killed at the onset of flaccidity.

Blood for sugar estimation was taken from the heart. The fat content of the liver was also determined in this experiment.

<i>Controls.</i>							
No.	Wt. (g.)	Blood-sugar (g. per 100 cc.)	Glycogen (%)		Liver- glycogen (mg.)	Liver- fatty acid (%)	Time after insulin (hrs.)
			Liver	Muscle			
1	338	0.077	0.33	0.12	31	3.91	—
2	397	0.162	0.32	0.16	38	8.59	—
3	373	0.120	0.31	0.21	30	7.75	—
<i>Insulinised animals (0.5 unit in each case).</i>							
4	340	0.062	0.79	0.17	72	3.80	1
5	415	"0"	1.63	0.25	204	9.60	2½
6	414	"0"	1.76	0.18	248	6.68	3

It will be seen that the blood-sugars of Nos. 5 and 6 are given as "0."

This symbol indicates that the thiosulphate equivalent of the blood-sugar by MacLean's method was less than 0.2 cc. It was frequently noticed in these experiments that a blood-sugar of these vanishing proportions was not necessarily immediately associated with convulsions, and in the above animals there was merely loss of coordination and flaccidity.

In the insulinised animals the liver-glycogen displayed a rise, compared with that of the normal animals, of over 100 % in 1 hour and over 700 % in 3 hours. The muscle-glycogen did not show any significant variations. The values for the liver-fat gave no information at all. The iodine values were 83, 92 and 101 respectively for the controls, and 105, 90 and 118 for the insulinised animals, thus showing no definite change in the degree of saturation of the fatty acids.

Exp. 2. In this experiment the dose of insulin administered was 0.2 unit per animal. Four young rabbits from the same litter, 6 weeks old, were taken. Starvation for 24 hours before experiment.

Controls.

No.	Wt. (g.)	Blood-sugar (g. per 100 cc.)	Glycogen (%)		Liver-glycogen (mg.)	Remarks
			Liver	Muscle		
1	565	0.090	0.60	0.06	124	—
2	427	0.110	0.15	0.19	21	—

Insulinised animals (0.2 unit per animal).

3	523	0.065	1.40	0.08	272	1 hr. 25 mins. after insulin
4	473	"0"	0.95	0.23	160	2 hrs. after insulin

In this experiment the same effect is seen as far as the liver-glycogen is concerned. The liver-glycogen in No. 1 was unusually high, but in spite of this there could be no doubt as to the very large increase. The results with the muscles were difficult to interpret.

In the next experiment the glycogen deposit at the beginning of convulsions was compared with that in animals after death following convulsions.

Exp. 3. Three young rabbits from the same litter, 6 weeks old. The dose of insulin was 0.5 unit per animal.

No.	Wt. (g.)	Blood-sugar (g. per 100 cc.)	Glycogen (%)		Liver-glycogen (mg.)	Remarks
			Liver	Muscle		
1	384	0.11	0.27	0.43	41	Control
2	479	"0"	0.92	0.20	160	Slight convulsion 1½ hrs. after insulin. Killed 1 hr. 40 mins. after insulin
3	427	"0"	2.03	0.15	292	Violent convulsions 1½ hrs. after insulin. Died in convulsions 3 hrs. after injection

Again the very large rise in glycogen in the liver is seen, the animal actually dying in convulsions having about seven times as much in its liver as the control. This quantity of glycogen converted into glucose and discharged into the blood-stream would have been ample to avert convulsions and death for a considerable time. Whatever theory be accepted as to the action of insulin, it is quite clear that the glycogen is "locked" in the liver and not even the violent convulsions, with, probably, the stimulation of the adrenals, could in these animals bring about an effective glycogenolysis. The values for muscle-glycogen showed that, at any rate, death was not due to the absence of glycogen, as was suggested by Chaikoff [1925]. There was, however, definitely less glycogen per cent. in the insulinised animals than in the controls. Attention must be drawn to the fact that there was nearly twice as much glycogen in the liver of the animal allowed to die of convulsions as in that of the one killed at the onset of the hypoglycaemic attack. This, we consider, is clear proof that the action of insulin, in so far as it influences the deposition of glycogen, is independent of the convulsions. In fact we formed the impression that the

animal, as far as its glycogenic mechanism is concerned, was divided into two separate systems: one amassing glycogen in the liver and preventing its escape as glucose, and the other depleting such glycogen as was already present in the peripheral tissues. In these young animals the convulsions are never so violent as they are in adult animals and so the fall in muscle-glycogen is not so marked.

In the next experiment it was decided to find how far the very marked effect of insulin on the liver-glycogen could be influenced by the injection of adrenaline. It is well known that adrenaline exerts an antagonistic action to insulin by stimulating glycogenolysis and so preventing hypoglycaemia. The old observation of Gatin-Gruzewska [1906] that adrenaline depletes the glycogen of the body is well known, but a considerable time of action and repeated injections are necessary to produce this effect. Pollak [1909] found that repeated injections of adrenaline into glycogen-free rabbits brought about a deposition of glycogen of the same order as that found in carbohydrate-fed animals.

Drummond and Paton [1904] obtained similar results. Kuriyama [1918] found an increase of glycogen in the livers of fasting rabbits after injections of adrenaline, and Cori [1928] found a marked increase in rats. Muscle-glycogen is found to fall and it has been suggested that adrenaline brings about an increased breakdown of muscle-glycogen to lactic acid, which then can be built up again to glycogen in the liver. In a recent communication on the action of adrenaline in depancreatized dogs Chaikoff and Weber [1928] claim to have shown that it can bring about a marked gluconeogenesis from fat. There is no doubt that adrenaline can produce a very marked hyperglycaemia in animals that have been starved for long periods or otherwise treated until the liver contains only very small amounts of glycogen. If, as seems very probable, these experiments indicate that adrenaline stimulates gluconeogenesis and at the same time glycogenolysis, then its effect on insulin action will depend upon the difference between these two processes.

What we wished to determine was whether any evidence could be obtained that adrenaline influenced the action of insulin directly.

Exp. 4. Four young rabbits from the same litter, 6 weeks old. Starvation 24 hours.

No.	Wt. (g.)	Blood-sugar (g. per 100 cc.)	Glycogen (%)		Liver-glycogen (mg.)	Remarks
			Liver	Muscle		
1	384	0.110	0.27	0.43	41	Control
2	363	0.256	0.38	0.28	49	0.25 mg. adrenaline subcutaneously. Killed 1½ hrs. later
3	366	"0"	1.56	0.36	190	0.5 unit insulin subcutaneously. Killed at onset of convulsions 1 hr. 35 mins. later
4	364	0.146	1.34	0.20	182	0.25 mg. adrenaline, 0.5 unit insulin at same time. Killed 2 hrs. later

Exp. 5. Two rabbits from the same litter, 6 weeks old. Starved 24 hours.

No.	Wt. (g.)	Blood-sugar (g. per 100 cc.)	Glycogen (%)		Liver- glycogen (mg.)	Remarks
			Liver	Muscle		
1	328	0.040	0.34	—	38	Control: blood-sugar un- usually low
2	402	0.062	3.86	—	465	2.12 p.m. 0.5 unit 5.38 p.m. 0.5 unit 6.45 p.m. Convulsion 6.55 p.m. Convulsion. 1 mg. adrenaline Killed 2 hrs. later, after complete absence of con- vulsions for 2 hrs.

It is clear from these experiments that 0.25 mg. adrenaline acting for $1\frac{3}{4}$ hours had no effect on the total liver-glycogen of animal No. 2, experiment 4, although there was hyperglycaemia: but there was a suggestively lower muscle-glycogen. No. 3 showed the usual large increase in liver-glycogen. Animal No. 4 showed that 0.25 mg. adrenaline could not prevent the accumulation of glycogen under the action of insulin, although sufficient sugar was liberated to prevent all hypoglycaemic manifestations. When we consider that, throughout the period of 2 hours after the injections in animal 4, there was a high discharge of sugar from the liver and probably an increased peripheral utilisation, and that in spite of this the accumulation of glycogen was of the same order as in animal 3, it seems not unreasonable to suppose that the adrenaline had contributed to the process of glycogenesis as well as given rise to an increased glycogenolysis.

In experiment 5, 1 mg. of adrenaline, whilst bringing about complete recovery from the insulin convulsions, did not do so by inhibiting the effect of insulin on the liver-glycogen. In fact the increase in liver-glycogen was to about 12 times the control value, in spite of a continuous discharge of sugar for 2 hours.

Up to this point, then, our results indicate that, in the starving young rabbit, insulin brings about a large increase in liver-glycogen and possibly a decrease in muscle-glycogen: further, that the increase in the liver can proceed in spite of the fact that the animal may be approaching death, which could be averted if this glycogen were liberated. It has also been seen that in these young animals death may occur in insulin convulsions from exhaustion, without the glycogen of the muscles having been used up. In addition it has been found that adrenaline in doses sufficient to produce a powerful hyperglycaemia does not necessarily deplete the glycogen in the liver, nor does it inhibit the glycogen increase following the injection of insulin.

Our next series of experiments was carried out with the object of finding what agents could bring about an inhibition of the glycogenesis induced by insulin. From several previous experiments we had satisfied ourselves that anaesthesia with ether or chloroform produces in rabbits a very high and lasting

hyperglycaemia, which involves the whole cardio-vascular system. The question as to whether this effect is due to a stimulus to glycogenolysis or to an inhibition of insulin action presented itself.

It was decided to use ether because of the less severe strain on the vascular system, chloroform being definitely dangerous to such young animals as we were using.

In examining the results obtained it must be understood that in no case in which we have injected insulin into young rabbits has there been any question of the immense rise in liver-glycogen.

Exp. 6. The animals used were 6 weeks old and from the same litter as those in experiment 5. The two rabbits of 5 are included in the following table for comparison. The plan of experiment was to anaesthetise an animal when it developed insulin convulsions and to find the effect of insulin in another animal when it was fully under ether. Muscle-glycogen was not determined.

No.	Wt. (g.)	Blood-sugar (g. per 100 cc.)	Liver-glycogen		Remarks
			%	mg.	
1	328	0.040	0.34	38	Control
2	402	0.062	3.86	465	Insulin and adrenaline, <i>vide</i> Exp. 5
3	342	"0"	0.69	84	2.13 p.m. 0.5 unit insulin
					4.30 p.m. Convulsion
					Open ether—complete recovery
					5.42 p.m. 0.5 unit insulin
					6.45 p.m. Convulsion
					Open ether—complete recovery
					7.17 p.m. Convulsion
					Open ether—complete recovery
					7.40 p.m. Killed
4	518	0.104	0.49	84	11.30 a.m. Blood-sugar 0.131 g. per 100 cc.
					11.45 a.m. Open ether and kept under
					11.46 a.m. 0.5 unit insulin
					11.55 a.m. Blood-sugar 0.122 g. per 100 cc.
					12.45 p.m. Blood-sugar 0.117 g. per 100 cc.
					1.30 p.m. 0.5 unit insulin
					1.35 p.m. Blood-sugar 0.077 g. per 100 cc.
					3.15 p.m. Killed after 3½ hrs. anaesthesia

The first point which emerges from this experiment is that the insulin convulsions were completely stopped by the ether anaesthesia. In the case of animal 4 the onset of convulsions was entirely prevented. With animal 3 the anaesthesia was light, so that convulsions occurred when it wore off somewhat: but in the case of 4 the continuous and deep anaesthesia almost entirely inhibited the action of the insulin, thus, in 3½ hours after the two injections of 0.5 unit each, the blood-sugar had only fallen from 0.131 to 0.104 g. per 100 cc. Further, in neither case did there occur the very large rise in liver-glycogen which occurs invariably after insulin alone. There was, it is true, over twice as much glycogen in the livers of 3 and 4 as in the control, but this is a very small effect compared to that, for example, in No. 2.

We conceive that in animal 3 the glycogen content of the liver was rising and would have continued to do so had the animal been allowed to go on living

for as long as it could under the circumstances. The action of the insulin would come in as the effect of the ether wore off.

In animal 4, on the other hand, the continuous anaesthesia tends to promote a continuous flow of sugar from the liver whilst the insulin ineffectually antagonises this by exerting a "locking" action on the liver. This effect is different from that of adrenaline, for, as we have seen in experiments 4 and 5, an injection of this substance sufficient to prevent convulsions produces hyperglycaemia and does not prevent the insulin from promoting a large deposition of glycogen in the liver. It seems to us that the effect of the anaesthetic is due to a toxic action on the liver cells, making it difficult for them to elaborate glycogen from exogenous or endogenous carbohydrate.

In animal 3 there must have been at 4.30 p.m. a large increase in liver-glycogen over the value at the outset, so that the anaesthesia must have liberated it again, this happening each time the convulsed rabbit was given ether. In animal 4 there did not occur any considerable hyper- or hypoglycaemia throughout the $3\frac{1}{2}$ hours of the experiment, hence the inability to lay down any largely increased amount of glycogen must have been due to some effect on the liver itself.

The very powerful liberation of sugar which takes place in rabbits under ether is shown in the following experiment on an adult animal.

Time after ether anaesthesia (mins.)	Blood-sugar, g. per 100 cc.				
	0	10	40	48	58
Peripheral vein	0.129	0.193	0.327	0.383	0.383
Inferior Vena Cava	—	—	0.330	0.340	0.378
Portal vein	—	—	0.293	0.327	0.362

The fact that this effect was absent in animal 4, experiment 6, shows that glycogenolysis was at its normal level and hence the failure to lay down any greatly increased quantity of glycogen localises the inhibition in the liver itself.

The failure of insulin to produce its typical effects in certain cases of very severe diabetes may be analogous to this effect of ether. The intense acidosis and poisoning with the toxic products of abnormal metabolism may produce this antagonism to a successful action of insulin by rendering the cell incapable of forming glycogen. Thus Sevringhaus [1926] found that injections of sodium acetoacetate into fasting rabbits retarded the blood-sugar-lowering action of insulin. Koehler [1926] found that in diabetics with well-marked acidosis (blood- p_H 6.8 to 7.2) doses of 100 to 300 units in 24 hours had no marked effect. The partial loss of tolerance to carbohydrate, induced by the ingestion of NH_4Cl , $CaCl_2$ or $NaHCO_3$ in big doses, is probably due to a similar toxic condition of the hepatic cell. The same is probably true of the intolerance to carbohydrate arising in starvation.

If this view of the action of ether is correct, its effect in preventing insulin convulsions and the accumulation of glycogen in the liver becomes understandable.

Adrenaline acts by liberating hepatic carbohydrate, at the same time probably increasing gluconeogenesis, and so does not, within the period of our experiments, interfere with the action of insulin in promoting an accumulation of liver-glycogen, although the blood-sugar may be maintained at high levels. Ether, on the other hand, prevents insulin from exerting its typical effect, by making it difficult or impossible for the liver cells to transform sugar to glycogen.

The next matter which was examined was the question as to how far the action of insulin could be influenced by convulsions. There seems to be no doubt that convulsions in a muscle deplete its glycogen, although there is some evidence that a muscle can respond to induction shocks even when its glycogen content has been reduced to vanishing proportions by injection of convulsants [Olmsted and Harvey, 1927]. Now in our young animals the convulsions induced by insulin are not powerful enough to deplete either muscle- or liver-glycogen. What we wished to determine was whether convulsions produced by, say, strychnine sulphate could in the presence of insulin prevent the accumulation of glycogen in the liver. Two types of experiment were possible: (1) to induce strychnine convulsions during the action of insulin, and (2) to inject insulin during and between strychnine convulsions.

Exp. 7. Seven young rabbits from the same litter, 6 weeks old. Starvation 24 hours before experiment in each case.

No.	Wt. (g.)	Blood-sugar (g. per 100 cc.)	Glycogen (%)		Liver- glycogen (mg.)	Remarks
			Liver	Muscle		
1	451	0.113	0.59	0.30	78	Control
2	448	0.090	0.32	0.14	56	2.48 p.m. 0.2 mg. strychnine 2.52 p.m. Convulsion—typical 3.20 p.m. Died after 28 mins. of convulsions
3	457	0.109	0.26	0.19	50	1.35 p.m. 0.05 mg. strychnine 2.06 p.m. 0.10 mg. strychnine 2.36 p.m. 0.10 mg. strychnine 2.54 p.m. 0.20 mg. strychnine 2.57 p.m. Convulsion 3.34 p.m. Died after 37 mins. of convulsions
4	461	0.058	1.37	0.16	230	2.50 p.m. 0.5 unit insulin 5.02 p.m. 0.2 mg. strychnine 5.12 p.m. Strychnine convulsions 5.13 p.m. Died—11 mins. after injection of strychnine
5	442	0.113	1.90	0.15	374	2.00 p.m. 0.5 unit insulin 3.05 p.m. Hypoglycaemic reac- tion 3.21 p.m. Insulin convulsion with recovery 3.22 p.m. 0.2 mg. strychnine 3.27 p.m. Insulin convulsion with recovery 3.34 p.m. Strychnine convulsions —very violent 3.39 p.m. Died—17 mins. after injection of strychnine

No.	Wt. (g.)	Blood-sugar (g. per 100 cc.)	Glycogen (%)		Liver- glycogen (mg.)	Remarks
			Liver	Muscle		
6	454	0.110	0.46	0.15	79	5.28 p.m. 0.2 mg. strychnine 5.50 p.m. Violent convulsions 5.51 p.m. 0.5 unit insulin 6.14 p.m. 0.2 mg. strychnine 6.30 p.m. Died—62 mins. after first dose of strychnine and 39 mins. after injection of insulin
7	453	0.056	0.26	0.21	46	6.59 p.m. 0.2 mg. strychnine 7.32 p.m. Convulsion with re- covery 7.53 p.m. 0.15 mg. strychnine 8.04 p.m. Strychnine convulsion. 0.5 unit insulin 8.40 p.m. Strychnine convulsion ceased 8.56 p.m. 0.2 mg. strychnine 9.06 p.m. Intermittent convul- sion 9.21 p.m. Killed—after 3 hrs. 22 mins. strychnine action and 1 hr. 17 mins. insulin action

Several facts emerge from this experiment. First, it is clear that these young animals can die after convulsions lasting over 30 minutes without losing all the glycogen from the liver or muscles, or, indeed, depleting it to any considerable extent. This is probably due to the fact, already mentioned, that the convulsions are not as violent as in an adult animal and that the young animals die of exhaustion. Nos. 2 and 3 illustrate this point. It is also to be observed that the period of convulsion was in both animals about half an hour.

In the cases of Nos. 4 and 5 the strychnine was injected when they were in a definitely hypoglycaemic condition and it was found that both animals died extremely rapidly. The effect was very striking, in No. 4 death followed in 1 minute after the onset of convulsions and in No. 5 in 5 minutes. In Nos. 4 and 5 the hypoglycaemia had been remedied by the strychnine convulsions, the values being 0.058 % and 0.113 % respectively. Increases were observed, moreover, in the glycogen in the liver of the same order as that when insulin acts alone, and the muscles contained amounts not significantly different from those of other young animals. Our attitude to the muscle figures is governed by the principle that we are in search of big effects and are loath to base hypotheses on small absolute changes. One thing, however, is clear, namely, that these animals did not die from hypoglycaemia or from depletion of muscle- and liver-glycogen. Of course in both cases the strychnine convulsions only lasted from 1 to 5 minutes, so that one could hardly expect much effect, but the fact that death followed so quickly seems to point to some hypersensitivity to the alkaloid in the insulinised animal.

When, however, we examine the results obtained from animals 6 and 7 the picture is quite different in several respects. In animal 6, 0.2 mg. strychnine brought about violent convulsions in 22 minutes. 0.5 unit insulin was at once injected and the animal had no further convulsion during the succeeding 24 minutes. An additional 0.2 mg. strychnine was then injected and the

animal died in 16 minutes, that is, 62 minutes after the injection of the first dose of alkaloid and after the insulin had been acting for 40 minutes.

In animal 7, 0.2 mg. strychnine induced convulsions in 33 minutes, but recovery soon occurred. Another dose of 0.15 mg. induced violent convulsions in 11 minutes, when 0.5 unit insulin was injected. Convulsions of the strychnine type continued for 36 minutes and then ceased. After 16 minutes without any convulsion a further 0.2 mg. strychnine was injected and in 10 minutes violent intermittent convulsions set in. The animal was killed 15 minutes later, that is, 3 hours, 22 minutes after the first injection of strychnine and 1 hour, 17 minutes after the injection of insulin. In neither of these animals did a decided hypoglycaemia occur, the heart blood-sugars being 0.110 % and 0.056 %. As seen from animals 2 and 3, strychnine does not bring about a hyperglycaemia, so there is no analogy to the actions of adrenaline and ether, although the convulsions may conceivably stimulate the adrenals to some extent. Examining the results further, it is readily seen that the glycogen contents of both liver and muscle showed no significant departure from those of untreated animals, thus contrasting strikingly with the results in the cases of animals 4 and 5.

It appears then that during the violent convulsions following strychnine, insulin does not bring about its typical effect. Death, when it occurs in these conditions, does not result from the action of insulin but from that of the alkaloid.

It is tempting to imagine some interference arising from the action of the strychnine on the spinal cord, but much further evidence is necessary. There is, indeed, some evidence that insulin exerts its effect *via* the cord, but it is still only in the presumptive stage.

DISCUSSION.

We take the provisional view that the action of insulin is to prevent the discharge of glycogen from the liver. We conceive that the process of absorption into the liver of glycogen-producing substances goes on from the gut and the blood as freely in the starving insulinised animal as in the control, but for some reason yet to be discovered the process of glycogenolysis is prevented. Peripheral utilisation continues and the blood-sugar reaches levels at which symptoms appear. This "locking" effect on liver-glycogen can be readily and strikingly demonstrated in young fed rabbits as well as starving animals.

Exp. 8. Two young rabbits from the same litter, 6 weeks old. Taken off food during the period of experiment only.

No.	Wt. (g.)	Blood-sugar (g. per 100 cc.)	Glycogen (%)		Liver- glycogen (g.)	Remarks
			Liver	Muscle		
1	297	0.156	9.94	0.66	1.39	Control
2	373	"0"	9.26	0.31	2.04	1.05 p.m. 0.5 unit insulin
						3.00 p.m. 0.5 unit insulin
						5.25 p.m. 0.5 unit insulin
						7.05 p.m. Convulsion with re- covery
						7.15 p.m. Killed

Exp. 9. Two young rabbits from the same litter, 6 weeks old. Taken off food during the period of experiment only.

No.	Wt. (g.)	Blood-sugar (g. per 100 cc.)	Liver- glycogen (g.)	Remarks
1	542	0.143	1.56	Control
2	517	"0"	1.03	1.10 p.m. 0.5 unit insulin
				4.30 p.m. 0.5 unit insulin
				5.15 p.m. Convulsion
				5.30 p.m. Convulsion
				5.40 p.m. Convulsion
				5.43 p.m. Killed

Here we have animals, No. 2 in each experiment, which are *in extremis* for the want of blood-sugar and which possess between 1 and 2 g. of glycogen in the liver. Experiment 8 shows also the very much lower muscle-glycogen of the insulin-treated animal. The blood contained practically no copper-reducing substance and the liver was incapable of yielding any to it.

That in the fed animal it takes much longer and much larger doses of insulin to produce hypoglycaemia than in the starved animal we conceive as due to the difficulty of increasing the glycogen in a liver already highly charged with it. Thus the removal of blood-sugar would depend largely on peripheral utilisation. In the last two experiments there did not occur any significant change in the liver-glycogen, the sum of the two controls being 2.95 g. and in the insulinised animals 3.07 g.; so that if we accept the idea that insulin "blocks" the release of glycogen we must also be prepared to consider, in the fed animal, the probability of a factor relating to the regulation of absorption, because the gut in the fed animals was full of carbohydrate and other food in process of digestion. It seems reasonable to suppose that when the liver-glycogen reaches a certain level it is impossible to raise it further, so that the glycogenic function would become a self-limiting process. Insulin acting in these circumstances would block the outward delivery of sugar and the liver itself would inhibit any further uptake of carbohydrate from other sources. The probable existence of a limiting glycogen content in the liver, characteristic for each animal, can be demonstrated by an experiment using rabbits fed on a diet containing an excess of carbohydrate for varying periods. The following was such an experiment.

Exp. 10. Seven adult rabbits were used. Two were kept as controls on their usual diet of bran, oats and green vegetables. The remaining five were given carrots and greens for the periods indicated. They were then killed and the livers and muscles analysed for glycogen and fat. The fat was estimated to see if the excessive carbohydrate was perhaps stored as fat.

No.	Glycogen (%)		Fatty acid (%)		No. of days on carrots Control
	Liver	Muscle	Liver	Muscle	
1	2.60	0.40	—	1.65	
2	0.40	0.26	4.03	2.02	
3	6.30	0.41	2.75	1.49	" 2
4	11.29	0.05	2.07	1.29	7
5	7.54	0.21	2.74	1.31	12
6	4.90	0.11	2.62	1.68	15
7	8.60	0.06	2.68	1.20	31

We take these figures to show that on such a diet the liver-glycogen cannot be made to exceed about 11 %, and although we have not yet tried this in adult rabbits we think that the injection of insulin would not bring about any higher level than this. The view, then, put forward is that the absorption of sugar from the gut or the formation of sugar by gluconeogenesis is governed by the amount of glycogen already present in the liver. Insulin, by "locking" the liver-glycogen, tends to prevent both the processes of absorption and of gluconeogenesis. It is not, we suggest, the insulin as such which does this, but it is the result of the accumulation of glycogen. We conceive that it is the actual presence of the colloid glycogen which imposes a self-limiting action on the liver.

On this view the action of insulin might be explained as due to two factors: (a) the blockage of carbohydrate in the liver, and (b) the self-limiting mechanism of the liver.

Let us consider briefly the application of this view to the cases of (1) the starving animal, (2) the fed animal, and (3) the diabetic.

(1) The starving animal, with its small amount of glycogen in the liver, keeps up its blood-sugar by absorption from the gut and later by gluconeogenesis from protein and fat (the evidence for the latter source of carbohydrate is doubtful and will be the subject of a further communication). The endogenous internal secretions of the pancreas, thyroid and adrenal control this process so that an optimum balance is struck, conserving the body tissues as far as possible. Insulin given to such an animal blocks the release of carbohydrate from the liver, but offers no obstruction to absorption and gluconeogenesis, because the liver-glycogen is still far from the limiting value. The result is a large rise in liver-glycogen. And, as we have seen, this occurs even when adrenaline is acting strongly. The necessary and sufficient condition for this action of insulin is a healthy liver.

(2) In the animal which already possesses a high liver-glycogen the discharge of sugar into the blood is as steady as and not at a much higher level than in the starving animal. This discharge is obviously not accompanied by an equal replenishment, otherwise the liver-glycogen would not fall as we know it does. This, in our view, is due to a limitation of absorption and stoppage of gluconeogenesis. In such an animal insulin, although blocking the discharge from the liver, does not secondarily bring about much change in the liver-glycogen, because the high glycogen content has imposed the self-limiting process on the liver. Thus the animal goes into hypoglycaemia much more slowly, since the disappearance of peripheral sugar will depend mostly on peripheral utilisation. This continues until the animal may die in convulsions without liberation of sugar from the liver.

(3) In the diabetic subject absorption of carbohydrate goes on rapidly from the gut because of the low glycogen values in the liver and the loss of blockage power, due to the deficiency of insulin. Gluconeogenesis proceeds at a rate which is much greater than in the starving animal because of the loss

in conserving power. The R.Q. is low because of the high degree of gluconeogenesis counterbalancing the high R.Q. of carbohydrate utilisation. The height of the R.Q. under any circumstances must be the algebraic sum of these processes. Insulin, provided the liver cells are not damaged and are capable of elaborating glycogen, blocks the discharge of carbohydrate from the liver, the liver-glycogen increases until as a consequence excessive absorption is stopped and gluconeogenesis is inhibited. The R.Q. now rises because the latter process is controlled. The condition for effective action is again the condition of the liver cells.

The whole problem, therefore, resolves itself into how insulin "blocks" the liver-glycogen.

There does not appear to be any evidence that insulin increases metabolic rate more than can be brought about by normal effective metabolism. It has always seemed anomalous that the diabetic should have a more or less normal metabolic rate, and that even when his metabolic processes are controlled by means of insulin the total metabolism shows no significant increase. This, it seems to us, can only be explained by a readjustment of the partition of energy among fat, protein and carbohydrate, which comes about by prevention of uncontrolled release of carbohydrate from the liver and the consequent depression of gluconeogenesis.

SUMMARY.

1. Young rabbits, 6 to 10 weeks old, of the same litter, are well adapted to comparative experiments with insulin. When starved for 24 hours they show a very satisfactory uniformity in their liver-glycogen. The average of twelve such animals from various litters was 50 mg. glycogen per liver.

2. Comparing the glycogen content of such rabbits, starved for 24 hours and treated with insulin, with that of controls from the same litter, there invariably occurred a very large rise in liver-glycogen. The average for twelve such animals was 237 mg.

3. Young rabbits treated with insulin may die in hypoglycaemic convulsions and still possess a very much increased liver-glycogen and a very considerable muscle-glycogen.

4. Adrenaline, whilst relieving hypoglycaemia, does not interfere with the essential action of insulin in increasing liver-glycogen.

5. Light ether anaesthesia relieves the convulsions due to hypoglycaemia. During continuous deep anaesthesia insulin cannot bring about the usual very large increase in liver-glycogen. This is considered to be due to a toxic action on the liver cell.

6. The hypoglycaemic animal is very susceptible to strychnine and dies in a very much shorter time than the normal animal. Continuous strychnine convulsions can prevent the typical action of insulin in promoting an increased glycogen content of the liver.

7. In the fed animal insulin does not bring about an increase in liver-glycogen and the animal goes into hypoglycaemic convulsions with large quantities of glycogen in the liver. Muscle-glycogen is definitely lowered.

8. These results are discussed and a theory of insulin action is suggested, *viz.* that the immediate effect of insulin is to inhibit the release of liver-glycogen. All other effects are regarded as secondary to this. The application of this view to certain cases is discussed.

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