

LXXXVII. THE INFLUENCE OF INSULIN ON THE DISTRIBUTION OF GLYCOGEN IN NORMAL ANIMALS.

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SINCE the action of insulin began to be investigated, a puzzling discrepancy between its action on the diabetic and on the normal animal has attracted attention. In the diabetic animal it readily renews the store of glycogen in the depleted liver; on the other hand, the predominant result of the earlier and of much of the later work was to show that, when injected into the normal animal, if it measurably affected the glycogen in the liver or the muscles, it caused disappearance instead of additional accumulation [cf. Dudley and Marrian, 1923; Macleod and McCormick, 1923; Babkin, 1923; Nitzescu and Popescu-Inotesti, 1923; Brugsch, 1924; Gigon and Staub, 1923]. There was evidence, indeed, that insulin even in the normal animal might initially promote an increase of glycogen in the liver of the rabbit [Cori, Cori and Pucher, 1923], or of the whole mouse [Bissinger, Lesser and Zipf, 1923], though at a later stage of developed hypoglycaemia the glycogen became reduced below the original amount. Cori later found that insulin caused diminution of liver-glycogen in rabbits, guinea-pigs and mice when the proportion was initially high, but not if it was low. There was no room for doubt, however, that the glycogen stores of liver and muscles might in some species be seriously depleted by insulin with a concurrent great reduction of oxidative metabolism; and the problem remained of accounting for the disappearance of circulating glucose under such conditions.

Macleod [1926] and his school have interpreted such results as pointing to the conversion of glucose into some substance unrecognisable by the ordinary methods of estimating carbohydrates, when insulin acts on the normal animal. It was found in this laboratory, however, that when insulin acted under uncomplicated conditions on muscle, the glucose disappearing could be fully accounted for by glycogen storage and oxidation [Best, Dale, Hoet and Marks, 1926]. It was accordingly urged that the phenomena seen in the whole animal were due to complication by another action of insulin, namely, a depression of the new formation of carbohydrate, so that oxidative metabolism was concentrated on that pre-existing, with consequent depletion of reserves.

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Substantially similar views as to this effective transfer of oxidation to carbohydrate had been put forward by Laufberger and by Cori and Cori.

More recent evidence has suggested yet another factor in the total effect. Cori and Cori [1928] have shown that in the rat a suitable dose of insulin diminishes the store of glycogen in the liver and increases that in the muscles, while a small dose of adrenaline affects the distribution in the other direction, causing glycogen to leave the muscles as lactic acid, which the liver rebuilds into glycogen. Experiments by Marks and myself, which are being published elsewhere, have confirmed this effect of adrenaline on muscle-glycogen and brought to light a new discrepancy, since we failed to find enough lactic acid to account for the glycogen thus discharged. Since a hypoglycaemic dose of insulin provokes accelerated secretion of adrenaline, the bearing of this complication on the total effect of an insulin injection had to be considered, and gave promise of reconciliation between the rival conceptions above mentioned.

Meanwhile, however, Goldblatt [1929] had published the results of experiments, made chiefly on young, fasting, normal rabbits. He found that injection into such animals of a dose of insulin sufficient to cause a profound hypoglycaemia, without convulsions, produced a substantial accumulation of glycogen in the liver. The change shown in his experimental records was striking, especially in view of the fact that so many workers, under conditions but slightly different, had looked for such an effect and failed to find it, or had even observed its direct opposite. It proved to be easy, as I shall show, under the conditions of Goldblatt's experiments, to confirm his observation with regularity. He has based on it a theory that the action of insulin is simply to retard the conversion of liver-glycogen into glucose, with resulting accumulation of glycogen and hypoglycaemia.

Such a theory, attractive in its simplicity, might have been easy to accept at a very early stage of the investigations on insulin. Its acceptance to-day would appear to involve the rejection of a large body of evidence obtained by other workers, on a number of species and by various methods. It seemed desirable to examine further the relation of the fact observed by Goldblatt to many which appeared to conflict with it. With this object in view I have studied the effects of nonconvulsant doses of insulin in normal animals of several species.

EXPERIMENTAL.

Methods.

In all experiments convulsions were avoided, the animals being killed when preconvulsive flaccidity and inco-ordination had developed. The animals were killed either by a blow on the head or, in the case of mice and fowls, by decapitation. The thorax and abdomen were quickly opened and blood for sugar estimation was taken from the heart. Glucose was determined by the method

of Hagedorn and Jensen. The liver was quickly removed, weighed and cut up into boiling 60 % potassium hydroxide, allowing 1 cc. per g. tissue. In the case of mice the livers were not weighed but directly dropped into tubes containing boiling potash and the weights determined by difference.

In young rabbits it is possible to remove quickly and cleanly the whole of the posterior thigh muscles, and such samples were invariably taken for muscle-glycogen determinations. The muscle was dropped immediately into boiling 60 % KOH. The anterior thigh and posterior leg muscles were removed for free glucose estimation, being immediately disintegrated in ice-cold absolute alcohol. In fowls a portion of the left pectoralis muscle was removed for glycogen estimation and in ferrets the procedure was to strip the leg and thigh for such samples.

Glycogen was determined by Pflüger's method, the glucose in the acid hydrolysate being estimated by the modified Bertrand method as described by Best, Hoet and Marks [1926]. Muscle-sugar was also determined according to the method used by these workers.

RESULTS.

1. *Experiments on young rabbits.*

The experimental conditions were closely similar to those of Goldblatt's experiments on insulin alone. In each experiment a batch of healthy young rabbits from one litter was taken and kept without food for 24 hours. In each case approximately equal numbers were taken for injection with insulin and as normal controls respectively. The ages of the litters varied from 6½ to 8 weeks, and the weights of individual rabbits in the whole series from 400 to 900 g. The average weight of 11 controls was 654 g. and of 11 rabbits receiving insulin 620 g. In a certain number of his experiments Goldblatt estimated the glycogen in the gastrocnemii of each rabbit as well as that of the liver. The number of such estimates on rabbits receiving insulin alone was hardly large enough, however, to justify the attribution of much significance to the relatively small deficit of muscle-glycogen observed, as compared with that in the few controls; and Goldblatt did not, in fact, lay stress upon it. I have estimated the glycogen in muscles as well as liver in all experiments on rabbits, and, to increase the chance of obtaining representative samples, have taken muscles from the whole of the posterior thigh in each case, as described under Methods. Table I shows the results on the 22 rabbits, estimates of liver-glycogen being available in all cases, and of muscle-glycogen in all except one of the controls, in which it was lost by accident.

There is clear confirmation of the increase of liver-glycogen observed by Goldblatt as the effect of insulin; the lowest figure in the insulin group is nearly 2½ times as great as the highest in the normal group, and the average insulin value more than 7 times as great as the normal. The effect on the muscle-glycogen is not so uniform. Of the 10 normal values, 2 are so low

as to be within the general insulin range, and of the 11 insulin values 4 are high enough to be within general range of the normals. If we compare the simple averages of the two groups, that of the normal is practically twice as high as that of the insulin series. The figure in either case, however, is obviously subject to a rather wide range of error. It seemed worth while, nevertheless, to calculate for the glycogen from the liver and muscles the average total quantities per rabbit in the two groups, even though the figures could be regarded as only rough approximations.

Table I.

A. Normal controls.					
Rabbit wt. g.	Liver wt. g.	Liver-glycogen %	Muscle-glycogen %	Muscle-sugar %	Blood-sugar %
476	13.96	0.24	0.19	0.100	0.131
480	16.08	0.25	0.18	0.099	0.163
462	14.62	0.60	0.21	0.117	0.150
464	17.28	0.20	0.30	0.127	0.141
852	19.33	0.28	(Lost)	0.115	0.152
582	15.18	0.08	0.66	0.103	0.110
800	20.30	0.12	0.42	0.127	0.106
690	20.06	0.57	0.08	0.097	0.132
904	23.97	0.42	0.12	0.103	0.122
804	24.41	0.33	0.30	0.124	0.156
676	17.55	0.22	0.27	0.112	0.138
Av. 654	18.43	0.30	0.27	0.111	0.136
B. After insulin (0.5 unit).					
553	21.73	3.72	0.15	0.094	0.052
581	21.10	3.66	0.13	0.098	0.015
407	16.14	2.10	Trace	0.113	0.041
520	14.53	3.20	0.07	0.098	0.051
645	18.73	1.20	0.27	0.124	0.061
715	20.84	2.73	0.24	0.117	0.051
860	23.25	2.03	0.19	0.112	0.056
672	21.53	1.90	0.04	0.095	0.072
521	15.83	1.47	0.08	0.074	0.050
748	19.98	1.33	0.20	0.115	0.043
598	18.47	1.00	0.12	0.092	0.057
Av. 620	19.28	2.22	0.14	0.103	0.050

The livers were weighed in all cases, but a figure was required for estimating the weight of muscle from the total weight of a rabbit. Best, Hoet and Marks found that the muscles of a cat contributed approximately one-half of the total weight of the intact animal. Estimates were made on two young rabbits by the methods which they used.

Exp. Two young rabbits were kept fasting for 24 hours, weighed and then killed. The skin, the viscera of the abdomen and thorax and the brain were removed and weighed. The difference represented roughly the weight of muscles and bones. The stripped carcass was then boiled, and the bones were cleaned and weighed. The difference again gave the weight of the muscles. Table II shows the results.

Table II.

Rabbit wt. (g.)	809	797
Muscles and bones (g.)	504	482
Bones (g.)	106	79
Muscles (g.)	398	403
Wt. of muscles %	49	50

The muscle weight of the young rabbits, like that of the cat, can accordingly be taken to be about one-half the total body weight. Applying this factor, and the known average weight of the livers, we obtain figures for the average totals of liver- and muscle-glycogen in the two series, as shown in Table III.

Table III.

Averages	Normal	After insulin	Difference
Wt. of animal (g.)	654	620	—
„ muscles (g.)	327	310	—
„ liver (g.)	18.43	19.28	—
Liver-glycogen %	0.30	2.22	—
Muscle-glycogen %	0.27	0.14	—
Total liver-glycogen (g.)	0.055	0.428	+0.373
„ muscle-glycogen (g.)	0.882	0.434	-0.448
„ glycogen (g.)	0.937	0.862	-0.075

It will be seen that, on this calculation, the total loss of muscle-glycogen under insulin is actually slightly greater than the total gain of liver-glycogen. As already indicated, the figures for muscle-glycogen are too irregular and the method of calculation, involving a large and only approximate factor for weight of muscle, too rough to warrant the attribution of any significance to this difference. It is, however, justifiable to conclude that the gain of liver-glycogen, though much easier to detect with certainty, is not a more important item in the total change produced than the loss from the muscles.

Taken by itself, such a result would still be quite compatible with Goldblatt's suggestion as to the mechanism of insulin action. The normal level of the blood-glucose being maintained by liberation at an appropriate rate from the liver-glycogen, a stoppage of this process would lead the blood-sugar to fall, and the hypoglycaemia might in its turn prevent the muscle from replenishing the glycogen used in contractile metabolism. On the other hand, this combination of effects—the disappearance of glycogen from the muscles and its accumulation in the liver—is the direct reverse of that described by Cori and Cori as produced by insulin in the rat. Such a contrast clearly suggests the desirability of examining the effects in some other species.

2. *Experiments on chickens.*

All the chickens used were from a strain of the Plymouth Rock variety bred at the Institute's Farm Laboratories, Mill Hill, and were of a known and uniform age of about 3 months from hatching. The normal well-fed chicken, as others have recorded, has an apparent percentage of blood-glucose, as determined by reduction, about twice as great as that of the average mammal. It is stated that the true percentage of glucose is some 30 mg. lower than

this reduction-value, which is partly due to uric acid, ergothioneine and other non-carbohydrate reducing substances. I have made no attempt to differentiate, recording the total reduction-values in all cases as glucose. It should be noted, however, that if an absolute correction of the amount above indicated were applicable to the figures for "blood-sugar" after insulin, a condition of very severe hypoglycaemia would in many cases be indicated, though no pronounced symptoms, and certainly nothing suggesting convulsions, occurred as the result.

The figures in Table IV, obtained from 3 chickens not deprived of food up to the time of killing, give an idea of the range of the "blood-sugar" and liver-glycogen in such normal birds.

Table IV. *Normally fed chickens.*

"Blood-sugar" %	Liver-glycogen %
0.199	3.64
0.200	2.77
0.188	2.4

It was found, however, that fasting for 24 hours sufficed to reduce the liver-glycogen to a very low proportion, so that in most cases it was below the range of trustworthy estimate by Pfüger's method, and had to be recorded as an unmeasured "trace." Chickens so prepared should accordingly offer an experimental material closely comparable to the young rabbits used in Goldblatt's experiments and my own. Birds, as others have observed, being very resistant to insulin, it was necessary to give it in relatively enormous doses to produce definite hypoglycaemic symptoms, 80 units per bird were injected in hourly doses of 20 units each, and the birds were killed when distinct symptoms of inco-ordination had appeared after the last dose. Table V shows the results on the glycogen content of the liver and muscles of these birds.

Table V. *Chickens after 24 hours' fasting.*

Controls Percentages			Insulin Percentages		
Blood-sugar	Liver-glycogen	Muscle-glycogen	Blood-sugar	Liver-glycogen	Muscle-glycogen
0.199	Trace	0.50	0.052	Trace	0.51
0.175	"	0.26	0.068	"	0.32
0.200	"	(Lost)	0.084	"	0.61
0.188	"	0.31	0.052	"	0.85
0.199	0.26	0.43	0.052	"	0.60
0.199	0.18	0.40	0.068	0.32	0.50
	Av.	0.38		Av.	0.56

It will be seen that, in complete contrast to the effect on young rabbits, there is here no sign of accumulation of glycogen in the liver as the result of the action of insulin. The glycogen of the muscles, on the other hand, shows a small, but probably significant increase under the action of insulin.

The conditions of absorption from the alimentary canal, as also of new formation of carbohydrate in the liver, might of course be different in the chicken and the rabbit, when both were subjected to fasting for 24 hours. It was, therefore, of interest to study the effects on the glycogen of the liver and of the muscles of giving known amounts of glucose by the mouth, with and without insulin, to chickens which had previously fasted for 24 hours. Several experiments of this kind were carried out, and, since the conditions in all were identical, and the results uniform in direction, the latter may conveniently be presented together, as in Table VI. It will be seen that 5 birds were kept fasting as controls, 9 were given glucose alone, in each case 3 g. as 20 cc. of a 15 % solution by tube into the crop, and another 9 the same treatment with glucose and 40 units of insulin hypodermically, 20 units being given at the same time as the glucose, and another 20 units an hour later. Two hours after the administration of the glucose the birds were killed for analysis.

Table VI.

Fasting controls Percentages			3 g. glucose by mouth Percentages			3 g. glucose by mouth 40 units insulin hypodermically Percentages		
Blood- glucose	Liver- glycogen	Muscle- glycogen	Blood- glucose	Liver- glycogen	Muscle- glycogen	Blood- glucose	Liver- glycogen	Muscle- glycogen
0.184	0.18	0.73	0.226	1.95	0.38	0.251	0.08	0.81
0.199	Trace	0.90	0.234	2.45	0.52	0.343	0.58	0.78
0.190	0.22	0.49	0.213	2.28	0.73	0.164	0.29	1.08
0.206	Trace	0.75	0.232	2.12	1.00	0.163	0.55	1.03
0.206	"	0.22	0.232	2.28	0.41	0.152	Trace	(Lost)
Av. 0.197		0.62	0.240	1.81	0.40	0.141	2.02	1.01
			0.243	1.90	0.62	0.102	Trace	0.62
			0.228	2.70	0.85	0.286	"	0.48
			0.282	1.70	0.94	0.127	0.15	0.85
			Av. 0.237	2.02	0.65	0.192		0.83

The results, as regards the liver-glycogen are perfectly clear. Glucose alone by mouth, without extraneous insulin, causes a rapid replenishment of the glycogen store of the liver, depleted by fasting. This is accompanied by a moderate rise in the average level of the blood-sugar. When insulin is given in addition to the glucose, no definite hypoglycaemia occurs, some values being above, others below, the normal and the average being practically the same as in the controls. Nevertheless, except in one aberrant case, the insulin can be said to have prevented, or restricted to very small dimensions, the effect which the same dose of glucose by itself produced on the liver-glycogen. As usual, the figures for muscle-glycogen are more irregular, and the comparison of averages has a consequently less certain validity. They appear to indicate that, whereas glucose alone causes no perceptible change in the glycogen content of the muscles, insulin in addition to glucose causes some increase in the muscle-glycogen, whereas it prevents, as a rule, the accumulation in the liver which glucose alone causes.

Taking these results as they stand, and deferring for the moment any discussion of the processes concerned, we may note that the effects of insulin in the fowl, whether glucose is also given or not, resemble those described by Cori and Cori in the rat, and are in sharp contrast to those observed by Goldblatt and by myself in the rabbit.

3. *Experiments on young ferrets.*

These were chosen as being another type of animal, intermediate in size between the rabbit and the rat, purely carnivorous, and easily obtainable in litters of sufficient size. A few preliminary experiments showed that the liver of the ferret retains its glycogen store with great tenacity, from 2 to 4 % being present after 24 hours' fast, and that the species has a relatively high resistance to insulin, from 12 to 15 units being necessary to produce a recognisable hypoglycaemia in a young ferret. Even with such doses the only symptoms were muscular weakness and inco-ordination; no convulsions were seen.

Six young ferrets of the same litter, 7½ weeks old, were taken for an experiment. All were kept fasting for 24 hours. Three were then set aside as controls, and each of the other 3 received 12 units of insulin hypodermically. Three hours later all the treated animals were visibly flaccid and their movements inco-ordinate. All 6 were killed, and determinations made of blood-sugar, liver-glycogen and muscle-glycogen. The results are shown in Table VII.

Table VII.

Controls Percentages			After insulin Percentages		
Blood-sugar	Liver-glycogen	Muscle-glycogen	Blood-sugar	Liver-glycogen	Muscle-glycogen
0.102	2.4	0.23	0.052	2.4	0.42
0.092	3.8	0.31	0.052	1.9	0.45
0.092	2.8	0.25	0.058	3.9	0.48
Av. 0.095	3.0	0.26	0.054	2.7	0.45

It will be seen that the insulin has produced a definite, though not extreme, hypoglycaemia. The liver-glycogen shows no significant change. The increase of muscle-glycogen, on the other hand, is consistent, and of significant dimensions.

4. *Experiments on mice.*

The effect of insulin on the distribution of glycogen between the liver and the muscles of the rat had been very thoroughly studied by Cori and Cori, and it seemed unnecessary to make further experiments on this animal. In mice, on the other hand, the early experiments of Dudley and Marrian, which first drew attention to the unexpected disappearance of glycogen under insulin action, were few in number. Lesser and his colleagues used a method which did not differentiate between liver and muscles. Further experiments on

mice seemed, therefore, desirable and, in particular, it seemed important to discover whether insulin in any dose, in any relation to food, and at any stage of its action, could be shown to promote such storage of glycogen in the liver of the mouse as it does in that of the young rabbit. Under no conditions, however, has it been possible to demonstrate such an effect, even as a preliminary phase of the action. In some cases the muscle-glycogen was estimated by removing the viscera, the head and the skin, and working up the muscles and bones of the stripped carcass together.

Exp. Ten mice weighing 16 to 20 g. were kept without food for 12 hours. Five were used as controls and each of the other five received 0.05 unit of insulin subcutaneously. The mice were kept at the laboratory temperature, so that the effect of insulin was protracted. The first of the insulin mice showed symptoms of weakness after 70 minutes and was killed, with one of the controls. Thereafter as each injected mouse showed such symptoms, and before any sign of convulsions appeared, it was killed, together with a control mouse. In each case the liver was removed, and dropped into hot potash, and the carcass similarly treated. The 5 control livers were worked up together, and similarly the 5 insulin livers. Separate estimates were made of the carcass-glycogen and of the sugar in the blood taken from each animal at killing. The last mouse was killed about 90 minutes after the insulin was given. The results are shown in Table VIII.

Table VIII.

Controls Percentages			After insulin Percentages		
Blood-sugar	Liver-glycogen (Av.)	Carcass-glycogen	Blood-sugar (Lost)	Liver-glycogen (Av.)	Carcass-glycogen
0.168		0.10	0.041		0.12
0.131		0.22	0.026	None	0.08
(Lost)	2.1	0.15	0.024		0.07
0.113		0.08	0.034		0.07
0.140		0.12			
Av. 0.138		0.15	0.031		0.08

Though the minute "glycogen" precipitate from the combined 5 insulin livers was duly collected and hydrolysed, it did not yield a titratable amount of glucose. The carcass-glycogen shows a probably significant reduction, but has not approached exhaustion.

Exp. Twenty mice were kept without food for 12 hours. Ten were kept as controls and each of the other 10 received 0.02 unit of insulin. All the mice were then placed in a thermostat at 37°, at which temperature the smaller dose of insulin caused symptoms of hypoglycaemia in 15 to 25 minutes. The insulin mice were, as before, killed as they reached the stage of preconvulsive symptoms, and the controls alternately with them. The blood samples and the livers of each group were pooled for analysis, so that the figures obtained and shown in Table IX are average percentages.

Table IX.

Controls		After insulin	
Blood-sugar	Liver-glycogen	Blood-sugar	Liver-glycogen
0.139	1.11	0.034	0.003

Again the liver is practically emptied of glycogen. Several larger series of mice were then taken, which had been feeding up to the time of starting the observation or, in one series, until 2 hours before that time. In each series 5 were taken as controls, and the remainder received 0.05 unit of insulin. All were kept at room temperature, the insulin mice being killed in batches of five at intervals varying from 5 minutes up to 1 hour after the injections. The glycogen percentage was estimated on the pooled livers of each such sample of 5 mice. The results are given in Table X.

Table X. *Liver-glycogen percentages.*

No. of mice	Controls	Time after injection of insulin			
		5 mins.	10 mins.	15 mins.	1 hr.
15	1.67	—	—	0.91	Trace
15	2.54	2.34	—	—	0.01
20	2.07	2.03	1.56	—	Trace

In these well-fed mice, also, there is no suggestion of increased storage of liver-glycogen at any stage. Five minutes after the injection, with the dose only partly absorbed, the liver-glycogen is not certainly diminished, but shows no sign of increase; in 10 minutes the decrease is becoming obvious; after an hour there is practically no glycogen left.

A further similar series of measurements was made on 20 mice which had been kept without food for 16 hours. After 15 had each received 0.02 unit of insulin, they were kept in the thermostat at 37° and killed in batches up to 25 minutes from the time of injection. Table XI shows the percentages of liver-glycogen.

Table XI.

Liver-glycogen %	Time after injection of insulin			
	Controls	5 mins.	10 mins.	25 mins.
	0.30	0.13	0.15	0.001

Again, the first perceptible effect of insulin is a diminution of the liver-glycogen, exhaustion being practically complete in 25 minutes under these conditions. These experiments are typical of a number of others in which the effect of insulin was tested on mice fully fed, or fasting for different periods, and killed for examination at varying intervals after the injection, from 5 minutes onwards. On a total of 310 mice, in no case was any increase of liver-glycogen detected, but always a decrease when a significant change had occurred.

Bissinger, Lesser and Zipf made their observations on mice which, after fasting for 18 hours, were given insulin with 50 mg. glucose. Under those conditions they observed an initial increase in the glycogen of the whole mouse, occurring at an earlier period than in similar mice receiving glucose

alone. It seemed desirable to attempt to repeat this observation, and to estimate the glycogen separately in the liver and in the stripped carcass, consisting chiefly of muscle.

Exp. Eight mice were accordingly kept without food for 18 hours, after which 80 mg. of glucose were injected into each mouse, and 0.04 unit of insulin into each of four, the others being kept as controls. After 30 minutes all were killed and worked up in the usual manner, the livers in each group being pooled, and the carcasses examined separately. Table XII shows the result.

Table XII.

Controls Percentages			After insulin Percentages		
Blood-sugar	Liver-glycogen (Av.)	Carcass-glycogen	Blood-sugar	Liver-glycogen (Av.)	Carcass-glycogen
0.104		0.099	0.103		0.11
0.160	0.15	0.091	0.170	0.08	0.13
0.106		0.055	0.160		0.11
—		0.051	0.101		0.11
	Av.	0.074		Av.	0.12

It will be seen that in this series the supply of glucose by injection has been adequate to prevent, in every case, a fall of the blood-sugar in the mice receiving insulin below the range observed in the controls. The muscles under these conditions show a gain of glycogen, small indeed, but with such regularity that it is probably significant. In the pooled livers, on the other hand, in spite of the maintained blood-sugar, the glycogen has fallen to a value corresponding to an average content of about one-half that in the controls, although the latter is itself already remarkably low.

Experiments on young rabbits with adrenaline.

In certain of his experiments on rabbits Goldblatt gave insulin and adrenaline. He evidently assumed that adrenaline by itself would lessen the glycogen in the liver, and that storage under the combined action, such as he observed, furnished evidence of the prepotency of insulin, keeping glycogen in the liver in spite of the stimulus of adrenaline to its discharge.

Earlier evidence had, indeed, given us clear warrant for the assumption that the action of adrenaline with regard to liver-glycogen is simply to promote its discharge as glucose, whether in the rabbit or other species. There was a body of evidence in favour of its promoting, under suitable conditions, a new formation of carbohydrate in the liver, as well as discharge of what was already stored; so that the balance of affect, whether gain or loss of glycogen, might differ according to the depleted or loaded state of the cells when the adrenaline was given.

Goldblatt's results with insulin on rabbits, however, which my own have closely confirmed, were so unlike those obtained on other species, either by

myself or by other observers, as by Cori and Cori on rats, that it seemed desirable to make a few direct observations on the effects of adrenaline on the glycogen distribution in young rabbits, under the conditions of Goldblatt's and my own experiments with insulin.

Exp. Four young rabbits of the same litter, 8½ weeks old, were kept without food for 24 hours. Two were kept as controls, and the other two each received 0.75 mg. of adrenaline, given as three hypodermic injections of 0.25 mg. each at intervals of 20 minutes. Two hours after the first injection the animals were killed, and the liver and samples of the musculature worked up as usual. Table XIII shows that the well-marked hyperglycaemia was accompanied by loss of glycogen from the liver, and also, though in smaller degree, from the muscles.

Table XIII.

Wt. of rabbit g.	Controls Percentages			Wt. of rabbit g.	After adrenaline Percentages		
	Liver-glycogen	Muscle-glycogen	Blood-glucose		Liver-glycogen	Muscle-glycogen	Blood-glucose
1066	0.36	0.21	0.152	1058	0.03	0.11	0.268
1051	0.51	0.13	0.148	1021	0.02	0.06	0.320

Rabbits treated with such a relatively large dose of adrenaline are, of course, losing carbohydrate from the system through glycosuria. In Cori and Cori's experiments on rats, the doses of adrenaline which caused increase of liver-glycogen were such as produced no conspicuous hyperglycaemia. An attempt was made to produce similar conditions in young rabbits.

Exp. Two series were taken, 8 from 1 litter and 6 from another, 4 and 3 being kept as controls. The treatment of the others was exactly the same as in the former experiment, with the exception that the 4 larger rabbits of the first series received 0.15 to 0.17 mg. adrenaline each, and the 3 smaller ones of the second series received 0.1 mg. each. Table XIV shows the combined results.

Table XIV.

Wt. of rabbit g.	Controls Percentages			Wt. of rabbit g.	After adrenaline Percentages			Adrenaline dose mg.
	Liver-glycogen	Muscle-glycogen	Blood-glucose		Liver-glycogen	Muscle-glycogen	Blood-glucose	
1020	0.45	—	0.135	1015	1.87	—	0.168	0.17
686	0.46	0.55	0.141	164	0.92	0.36	0.215	0.15
576	0.46	0.44	0.145	798 (1)	0.46	0.22	0.252	0.16
722 (Lost)		0.40	0.138	886	1.60	0.19	0.155	0.17
500	0.10	0.46	0.148	492	1.94	0.16	0.163	0.10
535	0.13	0.40	0.120	509 (2)	0.61	0.20	0.148	0.10
495	0.10	0.34	—	558	1.40	0.13	0.163	0.10
Av.	0.28	0.43		Av.	1.26	0.21		

It will be seen that in every case except one, which is marked (1) in the table, the liver-glycogen is definitely higher than in any of the controls. In this instance it will be seen that the blood-glucose is raised to the level of a definite hyperglycaemia, only slightly below the range of those in Table XII.

In another instance, marked (2) in the table, the increase of liver-glycogen is relatively small in relation to the highest value of all the controls, but it is still some 5 or 6 times as abundant as in the controls of its own small group. In this case the blood-sugar is hardly above the normal range, and imperfect absorption of the injected adrenaline may, perhaps, be suspected. Even when these cases are included, the average liver-glycogen content of the 7 rabbits treated with adrenaline is 4 or 5 times as great as that in 6 available controls. The increase is almost as striking as that recorded by Goldblatt, and by myself, with insulin. The muscle-glycogen in the treated rabbits is again reduced to about one-half that in the controls.

DISCUSSION.

If we look at the results here recorded as a whole, it is obvious that they cannot easily be interpreted on Goldblatt's supposition that inhibition of the discharge of glycogen from the liver is the primary and essential effect of insulin, to which all others are secondary. They show that the effect on which he based this suggestion, namely the increase of glycogen in the liver of the young rabbit, is an unusual rather than a constant and typical effect of insulin on the normal animal. In none of the other species here under experiment have I succeeded in detecting it at all: in the mouse, in which the most systematic effort was made to discover it as a temporary phase, I failed, even when sufficient glucose was given to prevent a fall of blood-sugar.

A comparison of the results of Tables I and XIV might suggest another possibility. Results immediately similar to those in Table XIV were obtained by the Coris in young rats also, with small doses of adrenaline; but in rats and in the species other than rabbits here examined insulin has been shown to produce a shift of the glycogen balance in the other direction, the muscles gaining and the liver losing glycogen. Is it possible, then, that the glycogen changes seen in the rabbit are due not to insulin at all, but to adrenaline secondarily secreted in response to it? There are many facts which forbid the assumption of so complete a paradox. Adrenaline, given in such a dose that we can safely regard it as acting in substantial excess, causes not storage but discharge of glycogen from the liver (cf. Table XIII). In an animal without a pancreas, and therefore without insulin, glycogen disappears from the liver, to be stored again when insulin is given.

These anomalies and apparent contradictions are familiar to anyone who has followed the investigations which, during the past 8 years, have been stimulated by the discovery of insulin. With the evidence before us of the readiness with which secretion of insulin is provoked by a rise and adrenaline by a fall of the blood-sugar, we may well hesitate before too confidently attributing the effects on glycogen distribution, following the injection of either of these hormones into a normal animal of any species, to the uncomplicated action of the one injected. To obtain a safe basis, however, for even a speculative estimate of the respective parts played by the two hormones

in the production of any particular result, we must turn to the evidence provided by experiments of a kind enabling the action of each to be studied in isolation. For the effects on muscle-glycogen such evidence is relatively complete, and simple in its significance. Insulin by itself causes increase of the muscle-glycogen at the expense of circulating glucose [Best, Dale, Hoet and Marks, 1926]. Adrenaline by itself, as suggested by experiments of Cori and Cori on rats, and confirmed in experiments on the eviscerated spinal cat by Marks and myself, which are being separately published, causes discharge of glycogen from the muscle, but not as glucose. Accordingly, when injection of insulin causes hypoglycaemia accompanied by increase of muscle-glycogen, we may reasonably conclude that insulin itself is the predominant agent; when insulin hypoglycaemia, in the absence of convulsions, is accompanied by loss of muscle-glycogen, as in the young rabbit, we may reasonably suspect that secretion of adrenaline has caused glycogen to be discharged from the muscles faster than it is stored under the action of insulin.

The evidence as to the action of insulin and of adrenaline separately on the liver is not so easy to interpret. The restoration of glycogen to the diabetic liver by insulin naturally aroused the expectation that such an effect would be demonstrable on the isolated liver with artificial perfusion. With insulin alone attempts to detect this action have uniformly failed. It may properly be suggested that the maintenance of a physiological condition under artificial perfusion is peculiarly difficult in the case of the liver cells. This, however, will hardly account for the fact that a slow increase in the glycogen of a perfused liver may be retarded or converted into a decrease, but never accelerated, by addition of insulin to the blood [Bodo and Marks, 1928]. On the other hand, it is a matter of common experience that adrenaline accelerates the discharge of glycogen as glucose from a perfused liver. If the facts are valid, they seem to lead to the difficult conclusion that both insulin and adrenaline cause disappearance of glycogen from the liver, and that neither promotes its storage. There are, however, certain observations on record which, though they need critical repetition and extension, are at least suggestive.

v. Issekutz [1924, 1927] detected no direct action of insulin alone on the rate of breakdown of glycogen into sugar in the perfused livers of frogs and rabbits. Certain of his experiments, however, suggest an antagonism of insulin to the mobilising effect of adrenaline on liver-glycogen, and some even appear to show that, when insulin alone has failed to affect the output of glucose, adrenaline in addition may retard it. Cori and Cori have interpreted the storage of glycogen in the liver of the normal rat, following the injection of small doses of adrenaline, with concurrent loss from the muscles, as due to passage of lactic acid from the muscles, and its resynthesis to glycogen in the liver; but it is at least open to doubt whether this gain by the liver would occur in the absence of the pancreas. We are entitled, then, to consider the possibility that, when either insulin or adrenaline acts alone, or in great

excess, it causes a diminution of liver-glycogen—either by increasing the rate of discharge in relation to that of new formation, as we can reasonably assume in the case of adrenaline, or by depressing the rate of new formation in relation to that of discharge, which is compatible with what is known of the action of insulin—but that some kind of balanced action of both is needed for the normal storage of glycogen in the liver. Such a view would certainly meet the case of the diabetic animal, where the liver, emptied of its glycogen, in spite of extravagant gluconeogenesis, under the unbalanced action of adrenaline¹, regains its normal store and rate of production when insulin is given in the dosage needed to restore the balance. In the normal young rabbit, again, we have found reason, from the effect of an insulin injection on the muscle-glycogen, to suspect that the action of insulin itself is complicated, to a greater extent than in other animals, by that of adrenaline secreted in response to it; and it is in this species alone, of those here examined, that storage of liver-glycogen has been found to follow injection of insulin. It may be doubted whether the whole of an effect following the injection of insulin into any normal animal can safely be attributed to insulin alone; but the storage of glycogen in the rabbit's liver seems to be more clearly under suspicion of secondary complication than most. The possibility that yet other hormones than adrenaline may play a part in effects following an injection of insulin must be kept in view, but there are no experimental data for its discussion.

SUMMARY.

1. The accumulation of glycogen in the liver of the young, fasting rabbit as the result of injecting insulin, described by Goldblatt, is confirmed.
2. Under such conditions the musculature loses at least as much glycogen as the liver gains.
3. Similar effects have not been observed in other species. Insulin causes no deposition of glycogen in the liver of the fasting chicken, but prevents its deposition there as the result of giving glucose, while causing its deposition in the muscles. In the ferret it causes formation of glycogen in the muscles and has little effect on that in the liver. In the mouse it causes rapid discharge of glycogen from the liver; when glucose is also given, glycogen is deposited in the muscles, but still disappears from the liver.
4. Injections of adrenaline too small to cause glycosuria produce, in the young, fasting rabbit, a change in glycogen distribution closely similar to that following injections of insulin.
5. It is argued that the effects of insulin on the normal animal are complicated by effects of adrenaline secreted in response to its action, especially in the rabbit.

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¹ The term "adrenaline" is used for convenience, to include also possible similar actions by sympathetic nerves to the liver.

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