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INSULIN AND ADRENALINE.

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THE suggestion has been put forward recently that certain effects following the administration of insulin to the intact animal might be due to the secondary secretion of adrenaline (Cori [1931], Corkill [1930]). Whilst the evidence appears good that the secretion of adrenaline is stimulated when the blood sugar falls to hypoglycæmic levels, such a view would suggest almost as a corollary that certain effects of adrenaline might be due to insulin and that, as far as the intact animal is concerned, an impasse would be reached in elucidating the essential actions of these hormones. These matters become particularly involved when it is found that certain results of the injections of both adrenaline and insulin appear to be identical, at least qualitatively. Thus, both hormones can, under properly controlled conditions, produce an accumulation of glycogen in the liver of the starving young rabbit (Corkill [1930], Sahyun and Luck [1929], Bischoff and Long [1930], Goldblatt [1929]). This effect was found by some workers to be accompanied by a loss of muscle glycogen in both cases.

General agreement has been reached that insulin stimulates the peripheral oxidation of carbohydrate, and there appears to be good evidence that adrenaline depresses it. This latter view is based on the fact that considerable and long-lasting hyperglycæmia can be produced in the presence of small quantities of hepatic glycogen, such hyperglycæmia in the uninjected animal requiring the infusion intravenously of amounts of glucose far in excess of that available in the liver. It is therefore concluded that two factors make persistent adrenaline-hyperglycæmia possible, viz. inhibition of peripheral oxidation and resynthesis of the products of the breakdown of peripheral glycogen when they

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reach the liver, so that, provided no loss occurs by way of the kidneys, there is a cycle of changes which sustains the blood sugar above normal. The claim that certain effects of insulin are due to adrenaline is particularly directed to those effects which do not accord well with the generally accepted view of the action of insulin. There is no doubt that, in the intact animal, the effects obtained at any moment from any treatment with a hormone is necessarily complicated by the simultaneous action of other internal secretions. Even the injection of an excess of one hormone does not remove the difficulty of quantitative interpretations. The conception of homoiostasis implies that the internal adjustments in the organism make the intact animal unsuitable for the investigation of the essential action of hormones. Qualitatively, however, much can be obtained by the use of the intact animal. For example, no complication of the action of adrenaline by the secondary secretion of insulin could explain adrenaline hyperglycæmia, nor could the rise in oxygen utilization and R.Q. following the injection of insulin be regarded as due to the simultaneous action of adrenaline. In the majority of animals the effect of insulin is to deplete the hepatic glycogen, even when the animal is actively absorbing sugar. Now insulin without doubt brings about a deposition of glycogen in the liver of the depancreatized or phlorizinized animal, but it would not be suggested that this is due to a secondary secretion of adrenaline. Indeed such secondary secretion might be invoked to explain those cases in which insulin depletes liver glycogen, this hormone being of importance in making the glycogen available for the increased oxidation produced by the insulin. A view which has not received attention is that increased peripheral oxidation might reasonably stimulate the endogenous formation of glycogen in the liver.

The questions which will be considered in this paper are:

(1) Is the redistribution of glycogen in young rabbits after the injection of insulin similar to that after the injection of adrenaline?

(2) Is the loss of muscle glycogen after insulin sufficient to account for the increase in liver glycogen which occurs in the same conditions?

(3) Is the action of insulin in these animals associated with a definite increase in blood lactate, the latter being regarded as evidence of the secondary secretion of adrenaline?

(4) Can the effect of insulin be dissociated from that of adrenaline by means of ergotamine or iodoacetic acid?

Experimental.

Glycogen was estimated by a modification of Pflüger's method similar to that described by Lovatt Evans and his co-workers [1931]. The lactate in blood was estimated by the method of Friedmann, Cotonio and Shaffer and blood sugar by Maclean's method.

The animals used in these experiments were mainly young rabbits. The results were compared for litter mates only and, unless otherwise stated, the values for muscle glycogen represent those for mixed samples taken from all four limbs. Where values are given for the total glycogen in the animal, liver and muscles only are meant, the assumption being that the muscles constitute about 50 p.c. of the body weight. All estimations of tissue glycogen are subject to some doubt if referred back to the living animal. The recent findings of Anderson and Macleod [1930] indicate, however, that the rate of loss of glycogen from the muscles post-mortem is very slow if the muscles are uninjured. To test this point in young rabbits they were killed by a sharp blow at the base of the skull, extended and the lumbar spine transected immediately. The gastrocnemius, tibialis anticus and vastus internus were carefully dissected out from the right limb and worked up for glycogen; the animal was left at room temperature (unless otherwise stated in the table) and the corresponding muscles on the left side removed after the times stated in Table I. As Anderson and Macleod found in cats, there is a definite discrepancy between the glycogen of the muscles both on the same side and on opposite sides, but the averages are very close. As is seen in Rabbit No. 6, injury to the muscles leads to marked loss in glycogen. Rather a large loss occurred in No. 7 when the limb was kept immersed in saline at 37° C. It is also seen that the effect of adrenaline on muscle glycogen ceases immediately after death. It is clear that reliance may be placed on glycogen values for muscle even up to an hour after death, provided that (1) averages are taken, (2) the muscles are not injured, and (3) the temperature be kept well below body temperature.

As to the loss of liver glycogen after removal of the liver from the body, Lovatt Evans *et al.* [1931] have shown that, when kept at room temperature, the sliced liver loses 5 p.c. of its glycogen in 1 min. and 40 p.c. in about 8 min., after which the rate of loss is very slow. In similar experiments we have found a similar rate. In the experiments in this paper the liver samples were in the hot potash in about a minute after removal from the body, so that it was not considered necessary to correct.

TABLE I. Post-mortem changes in glycogen content of mammalian muscle.

Rabbit No.	Wt. kg.	Right p.c.	Left p.c.	Remarks and time between muscle removal
1	1·26	0·177	0·165	1 hour
		0·206	0·211	
		0·190	0·231	
		Av. 0·191	Av. 0·202	
2	1·50	0·211	0·332	1 hour
		0·251	0·208	
		0·185	0·159	
		0·219	0·233	
3	1·35	0·139	0·093	0·25 mg. adrenaline intravenously
		0·114	0·065	2 min. before death 1 hour
		0·151	0·126	
		0·135	0·095	
4	1·65	0·202	0·140	As in No. 3
		0·189	0·151	
		0·117	0·175	
		0·169	0·155	
5	1·65	0·368	0·408	0·5 mg. adrenaline intravenously
		0·396	0·461	2 min. before death ½ hour
		0·348	0·457	
		0·371	0·442	
6	1·00	0·639	0·450	Muscles on left side were bruised
		0·534	0·394	1 hour
		0·397	0·451	
		0·523	0·432	
7	1·20	0·323	0·250	Left limb kept at 37° C. after
		0·262	0·154	death 1 hour
		0·234	0·199	
		0·276	0·201	

The effect of adrenaline on glycogen distribution in young rabbits.

The suitability of these animals for comparative work on glycogen distribution has been demonstrated by the present writer [1929]. It is important that such comparisons be made between litter mates, preferably bred in captivity. All the animals we have used have been so bred. A specially constructed light wooden collar was used during starvation of the animals, to make ingestion of fæces impossible. No difficulty has been found in obtaining animals with empty stomachs in this way if the diet during a day or two before the experimental period was somewhat restricted. In Table II are given the results of subcutaneous injection of adrenaline into these animals after starvation for various periods.

It is seen that in the starving young rabbit there is a considerable uniformity in the total glycogen per unit mass, even after greatly varying

TABLE II. Effect of adrenaline on glycogen distribution in young rabbits.

Litter No.	Time of starvation (days)	Animal No.	Wt. g.	Blood sugar mg./100 c.c.	Glycogen: Liver p.c.	Glycogen: Muscle p.c.	Glycogen: Total g./100 g. body wt.	Dose and time of action of adrenaline
1	2	1	587	175	0·55	0·41	0·22	Control
		2	675	120	0·50	0·36	0·20	Control
		3	653	360	0·75	0·24	0·14	0·2 mg. 3 hours
		4	640	390	0·26	0·16	0·09	0·3 ,, 3¼ ,,
		5	660	380	0·32	0·35	0·18	0·4 ,, 3½ ,,
		6	677	360	0·37	0·21	0·11	0·5 ,, 4 ,,
2	2	1	785	156	0·31	0·34	0·18	Control
		2	767	102	0·27	0·27	0·14	Control
		3	615	340	0·28	0·26	0·14	0·5 mg. 3¼ hours
		4	775	300	0·60	0·18	0·11	1·0 ,, 3½ ,,
		5	710	364	0·43	0·15	0·18	1·0 ,, 3¼ ,,
		6	853	330	0·16	0·15	0·08	2·0 ,, 3½ ,,
3	2	1	495	200	0·52	0·26	0·15	Control
		2	432	109	0·50	0·26	0·14	Control
		3	437	41	1·60	0·38	0·25	Insulin 12 units in 6 doses over 6 hours
		4	485	171	0·29	0·18	0·11	1·0 mg. repeated after 2 hr. 25 min. 6 hours
4	3	1	445	77	0·41	0·36	0·19	Control
		2	408	—	0·15	—	—	0·2 mg. 1¼ hours
		3	457	350	0·26	0·19	0·10	1·1 mg. in 4 doses 4¾ hours
5	5	1	550	95	1·30	0·33	0·20	Control
		2	518	264	0·24	0·28	0·15	1·1 mg. in 4 doses 4¼ hours
		3	490	350	0·35	0·27	0·15	As in 2

periods of starvation. The average of the controls was $0{\cdot}18 \pm 0{\cdot}04$ g. per 100 g. animal, whilst the average for the animals injected with adrenaline was $0{\cdot}13 \pm 0{\cdot}05$. We have introduced in Litter 3 an animal injected with large doses of insulin in order to illustrate the vastly different effect produced in these animals by this hormone. The average weight of these rabbits is about 500 g. and the average loss of glycogen per 100 g. is about 0·05 g., *i.e.* an average absolute loss of about 0·25 g. per animal. There is no reason to believe that this is brought about by increased oxidation or by loss in the urine (very little urine is as a rule excreted in these experiments). We are led, therefore, to the probability that there is a storage of the degradation products of glycogen actually in the tissues after the injection of adrenaline. Whilst the acute effect of adrenaline is a loss of both liver and muscle glycogen, the process of recovery is a peculiar one. It has been known from the time of Claude Bernard that muscle glycogen does not produce glucose but lactic acid.

Hence, loss of muscle glycogen is due either to increased oxidation or to increased formation of lactic acid. That adrenaline produces an increase in circulating lactate has been shown by several workers, notably by Cori. Since it is known that lactic acid can give rise to hepatic glycogen, the following cycle of changes is taken as occurring after the injection of adrenaline:

Muscle glycogen → Lactic acid → Hepatic glycogen → Blood glucose → Glycosuria if threshold is exceeded.

Using rats which (*a*) had very small initial liver glycogen values, and (*b*) were injected with small doses of adrenaline, Cori found that the liver glycogen was very considerably increased and the muscle glycogen fell. Pollak [1909] had already observed that such an end-effect could occur after adrenaline injected in large and repeated doses during starvation.

The following is an experiment on the same lines as Pollak's: Two rabbits from the same litter were starved for 3 days and then for a further 2 days, during which one of them received injections of adrenaline and the other was kept as control. The injected animal received 2 mg. adrenaline a day and at the time of death had excreted 1·5 g. glucose. On analysis the control gave a blood sugar of 0·056 p.c. and a liver glycogen 0·30 p.c.: the injected animal gave a blood sugar of 0·40 p.c. and a liver glycogen of 2·5 p.c.

More recently Corkill [1930], using small doses of adrenaline, demonstrated the same effect in young rabbits. Sahyun and Luck [1929]

TABLE III.

Rabbit No.	Wt. g.	Blood: Sugar mg./100 c.c.	Blood: Lactate mg./100 c.c.	Glycogen: Liver p.c.	Glycogen: Muscle p.c.	Glycogen: Total g./100 g. body wt.	Time after adrenaline hours
			Controls				
1	775	117	76	0·490	0·158	0·096	—
2	800	113	87	0·859	0·126	0·092	—
3	950	102	71	0·489	0·187	0·106	—
						Av. 0·097	
			Adrenaline 0·15 mg.				
4	875	150	63	1·058	0·101	0·108	2·0
5	800	216	93	1·524	0·101	0·103	2·2
6	950	164	74	1·352	0·144	0·120	2·4
						Av. 0·110	
			Adrenaline 0·15 mg. and pituitrin 1 c.c.				
7	750	213	89	1·457	0·079	0·092	2·3
8	900	177	79	1·920	0·083	0·112	2·5
9	800	187	111	1·528	0·094	0·103	2·7
						Av. 0·102	

found that 1 mg. of adrenaline produced a rapid fall in the glycogen of the liver and the muscles, the former recovering and attaining very high values, whilst the latter remained at extremely low levels for as long as 42 hours. This must mean that the lactic acid produced from the muscle lies dormant until such time as the liver is able to work it up to glycogen. If, however, small doses of adrenaline are used it is possible to obtain an almost quantitative redistribution of glycogen from the muscles to the liver in between 2 and 3 hours. This is shown in Table III.

The effect of a non-glycosuric dose of adrenaline.

A litter of nine young rabbits was starved for 24 hours. Three were taken as controls, three received 0·15 mg. adrenaline subcutaneously and the remaining three received 1 c.c. pituitrin and 25 min. later 0·15 mg. adrenaline. The last three animals were so injected to see if, as has been stated by some workers, pituitrin exerts an antagonistic effect on adrenaline hyperglycæmia: such an effect was not found.

It is clearly seen that, with this dose of adrenaline, there was no loss in glycogen, a quantitative redistribution having occurred (Table III).

Time factor in the resynthesis of liver glycogen.

The time factor in the glycogen redistribution is shown in Table IV, where it is seen that the fall in muscle glycogen after 0·5 mg. adrenaline had reached a maximum in about 1½ hours and from that time on the

TABLE IV. Litter of five young rabbits. 48 hours' starvation before injection.

Rabbit No.	Wt. g.	Blood sugar mg./100 c.c.	Glycogen Liver p.c.	Glycogen Muscle p.c.	Glycogen Total g./100 g. body wt.	Dose of adrenaline and time of action
1	942	380	0·51	0·28	0·16	0·5 mg. 1½ hours
2	985	139	2·87	0·23	0·20	,, 4½ ,,
3	865	125	2·59	0·29	0·24	,, 12 ,,
4	980	129	1·14	0·28	0·18	,, 18 ,,
5	655	152	2·53	0·35	0·26	,, 24 ,,

products of the breakdown of glycogen were being resynthesized in the liver, the rate of glycogenolysis in the liver having become normal in about 4½ hours.

Summary of experiments on the effect of adrenaline.

We find that the end-effect of adrenaline depends on the dose and the time of action. With a large enough dose the effect in young rabbits even up to 3 hours after the injection may be loss of glycogen from both

the liver and muscles, but after this time the liver glycogen recovers and far exceeds the initial value although the muscle glycogen may fall no further. This is clearly due to a lag between the liberation of the products of the breakdown of muscle glycogen and their resynthesis in the liver. With a suitable smaller dose of adrenaline the glycogenolytic effect in the liver may be so slight that resynthesis proceeds so rapidly that quantitative redistribution is attained in a relatively short time. This would involve the resynthesis of the glucose liberated from the liver when it reaches the muscles, an effect which is usually associated with the action of insulin.

We proceed now to present results illustrating the effects of insulin in these animals and to examine how far it is justifiable to consider that adrenaline, secondarily secreted, is responsible for them.

The action of insulin in young rabbits.

The injection of insulin is followed by an increase in the peripheral oxidation of carbohydrate and in the majority of normal animals this leads to a loss in both muscle and liver glycogen. Corkill [1930] found this to be the case in several different species. To his list of animals we may add the guinea-pig with which the results in Table V were obtained. Ten small guinea-pigs were starved for 24 hours. The muscles used for glycogen estimation were the tibialis anticus and the gastrocnemius; usually about 2 g. could be obtained.

The results shown in Table V are puzzling. The effectiveness of the insulin is clear from the blood-sugar values. No significant change in the

TABLE V. Young guinea-pigs starved 24 hours.

Rabbit No.	Wt. g.	Blood sugar mg./100 c.c.	Glycogen		Dose of insulin and time of action
			Liver p.c.	Muscle p.c.	
1	214	135	0·440	0·619	Controls
2	258	144	0·366	0·619	
3	600	97	0·312	0·552	
4	550	85	0·292	0·631	
5	350	58	0·509	0·310	
6	183	47	0·457	0·412	1 unit 3 hours
7	287	27	1·074	0·377	1½ ,, 3¼ ,,
8	550	35	0·365	0·770	1½ ,, 2¾ ,,
9	550	60	0·396	0·559	1 ,, 3¾ ,,
10	400	0	0·412	0·681	2 ,, 2¼ ,,
		Averages: Controls ...	0·384	0·546	
		Treated animals	0·408	0·559	

(Anomalous liver value for No. 7 omitted in average.)

liver or muscle glycogen was found in spite of a fall in average blood sugar from 104 to 34. Further study of this species will be necessary.

It is, however, with the young rabbit that the striking and apparently paradoxical action of insulin is observed, viz. a very marked increase in liver glycogen, which exceeds any loss which may simultaneously occur in the muscles. In Table VI are shown the results in a typical experiment. Calculated in the usual way, the average total glycogen per 100 g. body

TABLE VI. A litter of eight young rabbits starved for 24 hours. The dose of insulin was 1 unit injected subcutaneously.

		Blood		Glycogen			
Rabbit No.	Wt. g.	Sugar mg./100 c.c.	Lactate mg./100 c.c.	Liver p.c.	Muscle p.c.	Total g./100 g. body wt.	Time of action
1	800	168	44	0·549	0·202	0·118	Controls
2	625	156	37	0·552	0·198	0·116	
3	700	143	53	0·613	0·181	0·110	
							hr. min.
4	825	88	63	1·804	0·130	0·130	1 50
5	750	106	50	2·647	0·139	0·150	2 10
6	800	84	68	5·042	0·141	0·245	2 55
7	775	96	44	2·824	0·129	0·162	3 15
8	725	106	68	3·426	0·098	0·166	3 30

weight for the controls in Table VI is 0·115 g. and for the injected animals 0·171 g. Certain workers, confirming the increase in liver glycogen here demonstrated, attribute it to the secondary secretion of adrenaline. It will be seen in Table VI that the blood sugar of the injected animals was at an average normal level, this being due to the fact that this litter was of the kind, sometimes met with, in which the blood sugar is high. Can we on the basis of the blood sugar consider that adrenaline was being secreted at a rate sufficient to account for the liver glycogen? In the dog the secondary secretion of adrenaline occurs according to Houssay, Lewis and Molinelli [1924] at a blood-sugar level of 0·05 p.c., and according to LaBarre and Houssa [1932] at 0·075 p.c. The rabbit does not lend itself to determinations of this sort, but it is improbable that the critical level is as high as that of the injected animals in this experiment. On the other hand, the average blood lactate rose from 45 to 59, which might be regarded as slight evidence of an increased secretion of adrenaline; but Matakas [1932], in a recent paper, reports that the rabbit is not reliable in this connection. Averages from young litter mates are, in our experience, reasonably reliable.

The decisive experiment would be to observe the effect of insulin in the adrenalectomized young rabbit. We have, after many attempts,

failed to obtain clear results by this method, since the animals do not survive the operation for a sufficiently long time.

Two other ways of attacking the problem seemed possible, viz. by using ergotamine and iodoacetate. The former is known to inhibit adrenaline hyperglycæmia, and might conceivably act similarly on the peripheral liberation of lactate. If it did so, then it might be possible to dissociate the characteristic effects of adrenaline from those of insulin. Iodoacetate is known to prevent the post-mortem liberation of lactic acid in muscle and might, in adequate dose, prevent the liberation of lactic acid produced by adrenaline in the living animal.

Ergotamine and the action of insulin.

The ergotamine was used in the form of "ergotamine methan sulphonate" [Sandoz] injected intravenously and the insulin was injected as usual subcutaneously. The animals were from one litter and were starved for 24 hours. The results are given in Table VII, and it will

TABLE VII. A litter of four young rabbits, starved for 24 hours.

				Blood		Glycogen			
Rabbit No.	Wt. g.	Insulin unit	Ergotamine mg.	Sugar mg./100 c.c.	Lactate mg./100 c.c.	Liver p.c.	Muscle p.c.	Total g./100 g. body wt.	Time of action
1	784	0	0	134	33	0·267	0·148	0·081	—
									hr. min.
2	715	1·0	0	65	36	0·933	0·118	0·091	2 45
3	638	0·8	4·0	23	33	1·293	0·158	0·130	2 7
4	592	0·8	2·5	14	30	0·967	0·237	0·153	2 2

suffice to say that the effect shown in Table VI is not inhibited by ergotamine. The effectiveness of the ergotamine is shown by the much greater and more rapid fall in blood sugar in the animals injected with it. It will also be observed that the blood lactate remained the same in all the animals.

Ergotamine and the action of adrenaline.

The results of the combined actions of ergotamine and adrenaline are shown in Table VIII. It is sufficient to state that ergotamine, in doses large enough to inhibit adrenaline hyperglycæmia, does not prevent the redistribution of glycogen which has been demonstrated in Table III. It will also be observed that, whilst as a result of the ergotamine the adrenaline only raised the average blood sugar by about 16 p.c., the rise in lactic acid in the blood was over 80 p.c.

TABLE VIII. A litter of ten young rabbits, starved for 24 hours.

Rabbit No.	Wt. g.	Ergotamine mg.	Adrenaline mg.	Blood mg./100 c.c. Sugar	Blood mg./100 c.c. Lactate	Glycogen Liver p.c.	Glycogen Muscle p.c.	Glycogen Total g./100 g. body wt.	Time of action of adrenaline
1	625	0	0	135	23	0·754	0·430	0·204	—
2	625	0	0	115	17	0·567	0·202	0·119	—
3	525	0	0	111	16	1·164	0·193	0·130	—
4	700	0	0	137	39	1·275	0·204	0·204	—
			Averages	125	24	0·940	0·292	0·164	
									hr. min.
5	650	1·3	0·15	171	40	1·751	0·154	0·140	2 15
6	625	1·25	0·15	129	43	3·126	0·231	0·219	2 35
7	750	1·5	0·15	137	40	2·354	0·125	0·138	2 55
8	650	3·3	0·15	139	28	1·043	0·130	0·098	2 30
9	900	5·0	0·30	152	56	2·192	0·120	0·126	2 50
10	750	4·2	0·20	146	56	2·041	0·188	0·158	3 15
			Averages	146	44	2·085	0·158	0·147	

Nos. 5, 6, 7 received the adrenaline 10 min. after the ergotamine, Nos. 8, 9, 10, 1 hour after it.

Summary of experiments on the influence of ergotamine.

Ergotamine does not prevent the increase in liver glycogen produced by insulin in the starving young rabbit, this increase being greater than can be accounted for by losses of glycogen from the muscles. Ergotamine, in doses sufficient to inhibit adrenaline hyperglycæmia, does not prevent the liberation of lactic acid from the muscles and the quantitative redistribution of glycogen which follows the injection of a small dose of adrenaline. It appears, therefore, that the mechanism of glycogenolysis in the liver is more rapidly paralysed by ergotamine than is that in the muscles. If, however, the dose of ergotamine is sufficient demonstrably to paralyse the peripheral vaso-constrictors in the anæsthetized animal, then the increase of lactate in the blood caused by adrenaline is also prevented (Goldblatt [1933]). Thus, with the doses of ergotamine we have used, we have been unable to dissociate the effects of insulin and adrenaline.

Iodoacetate and the actions of insulin and adrenaline.

The dose of the neutralized acid which could safely be given was first determined. The acid was neutralized with $N/2$ NaOH, using methyl orange as indicator.

(*a*) Rabbit. 1·1 kg.
5 mg. intravenously ... ... No symptoms
15 mg. 1 hour later ... ... No symptoms
40 mg. 1½ hours later ... ... Convulsions and death in 15 min.

(*b*) Rabbit. 1·0 kg.
10 mg. intravenously ... ... No symptoms
15 mg. 55 min. later ... ... No symptoms

It was decided to use doses up to 15 mg.

The results are set out in Tables IX and X and may be summarized as follows:

(1) Iodoacetate in doses up to 10 mg. does not seriously interfere with the accumulation of liver glycogen or the increase in total body glycogen which we have seen constantly to occur in these animals after insulin.

(2) With 15 mg. this effect is inhibited to a very great extent but not abolished, the total glycogen per unit body weight remaining unchanged. This amount of iodoacetate does not interfere with the rate of fall in blood sugar, nor is there any appreciable change in blood lactate.

(3) 15 mg. iodoacetate prevent the rise in liver glycogen which normally follows the injection of small doses of adrenaline but do not prevent the hyperglycæmia, the rise in lactic acid in the blood or the fall of muscle glycogen. The inability of the liver to synthesize the liberated lactic acid is reflected in the persistence of a high blood lactate. The total glycogen per unit body weight is very greatly diminished (cf. Table III).

TABLE IX. Fifteen young rabbits from two litters of approximately the same age were starved for 24 hours. Of these six were used as controls, two were injected with insulin alone, and seven with both insulin and acetoacetate as detailed in the table. Except for one animal in the last group, the litters were equally represented in all the groups.

Rabbit No.	Wt. g.	Iodo-acetate mg.	In-sulin unit	Blood mg./100 c.c.		Glycogen			Time of action of insulin hours
				Sugar	Lactate	Liver p.c.	Muscle p.c.	Total g./100 g. body wt.	
1	700	0	0	77	40	0·304	0·181	0·100	—
2	700	0	0	95	39	1·057	0·189	0·130	—
3	550	0	0	100	84	0·397	0·152	0·087	—
4	700	0	0	90	68	1·949	0·267	0·199	—
5	500	0	0	67	62	0·514	0·197	0·117	—
6	600	0	0	72	63	1·096	0·226	0·150	—
			Averages	83	59	0·886	0·202	0·131	
7	750	0	1	30	40	2·169	0·283	0·210	2·3
8	925	0	1	54	46	3·947	0·402	0·323	2·3
			Averages	42	43	3·008	0·342	0·267	
9	800	5	1	58	59	2·587	0·250	0·216	2·3
10	850	10	1	30	89	2·156	0·212	0·179	1·3*
			Averages	44	74	2·372	0·231	0·198	
11	650	15	1	30	31	1·526	0·258	0·178	2·3
12	750	15	1	48	68	0·809	0·160	0·108	2·3
13	725	15	1	62	48	1·135	0·136	0·109	2·3
14	700	15	1	48	46	0·875	0·173	0·116	2·3
15	700	15	1	45	74	1·469	0·138	0·130	2·3
			Averages	48	53	1·163	0·173	0·128	

* Convulsion.

TABLE X. Eight young rabbits from the same litter were starved for 24 hours. Three were used as controls and five were injected with iodoacetate and adrenaline.

Rabbit No.	Wt. g.	Iodo-acetate mg.	Adrena-line mg.	Blood mg./100 c.c. Sugar	Blood mg./100 c.c. Lactate	Glycogen Liver p.c.	Glycogen Muscle p.c.	Glycogen Total g./100 g. body wt.	Time of action of adrena-line hours
1	650	0	0	137	56	Lost	0·400	—	—
2	650	0	0	118	28	2·139	0·574	0·358	—
3	650	0	0	113	26	1·065	0·428	0·255	—
			Averages	123	37	1·602	0·467	0·307	
4	600	15	0·15	229	51	1·098	0·194	0·136	2·3
5	600	15	0·15	236	39	1·055	0·245	0·161	2·3
6	750	15	0·15	168	64	2·457	0·239	0·226	2·3
7	600	15	0·15	187	46	1·205	0·258	0·181	2·3
8	625	15	0·15	227	85	1·164	0·232	0·162	2·3
			Averages	209	57	1·396	0·234	0·173	

It is clear that the increase of lactate in the blood caused by adrenaline is not affected by iodoacetate in doses of 15 mg. Hence, were the accumulation of liver glycogen shown in Rabbits Nos. 7 and 8 in Table IX due to resynthesis from lactate released from the muscles, we should have obtained a great rise in blood lactate in Rabbits Nos. 11 to 15, in which the formation of new glycogen in the liver was practically abolished. (Rabbit No. 10, Table IX, illustrated that the peripheral release of lactate was easily possible.) We consider that this failure of the lactate to be increased constitutes evidence against the adrenaline hypothesis. In Table IX the last group of animals, in spite of the fall in blood sugar, showed no change in total body glycogen, whereas in the litter of Table X the total fell by over 40 p.c., no appreciable loss having occurred by the urine. Iodoacetate, whilst not giving the unequivocal evidence sought for, served to emphasize the quantitative differences between the effects of insulin and adrenaline in these animals.

DISCUSSION.

The most recent contribution to the problem presented by these young animals is that of Corkill [1932], in which it is shown that diphtheria toxin, in doses that were too small to abolish the power of the liver to make glycogen from glucose, completely prevented the deposition of liver glycogen which ordinarily follows the injection of insulin or adrenaline. The toxæmia is accompanied by a distinct resistance to insulin as far as the blood sugar is concerned, but this resistance can be overcome by ergotoxine, which supports the view that adrenaline secretion is

increased during the toxæmia. But ergotoxine does not allow of a deposition of liver glycogen by the action of insulin in the toxæmic young rabbit. Corkill has thus also reached an impasse in the elucidation of the source of the new glycogen. In regard to these very interesting experiments it would be important to determine whether in toxæmia there occurred a large rise in blood lactate, and whether injected lactate gave rise to liver glycogen. By intravenous injection of *d*- or *i*-lactate it is easy to produce a deposition of liver glycogen in normal young rabbits, and it may be that the toxæmic liver may have lost the power to form glycogen from lactate whilst retaining that from glucose. Should this prove to be the case, the necessary link in connecting the anomalous action of insulin with the adrenals might become clearer. The fact that, as we have seen, ergotamine permits both the insulin and adrenaline effects to take place and that iodoacetate and diphtheria toxin [Corkill] prevent them, undoubtedly gives qualitative support to the hypothesis that there is some link between the two; but on quantitative grounds we consider that there are outstanding differences between the observed changes and what might reasonably be expected if the effects following the injection of insulin in these animals were really due to adrenaline. Further facts must be obtained before a definite decision can be reached.

Conclusions.

1. Insulin and adrenaline under properly controlled conditions produce a very marked increase in liver glycogen in the starving young rabbit.

2. In the case of insulin this increase may or may not be associated with a fall in muscle glycogen. The effect on the total glycogen of the body is most frequently an increase.

3. In the case of adrenaline in non-glycosuric doses, the increase in liver glycogen is always associated with a fall in muscle glycogen. With a properly adjusted dose there is a quantitative redistribution of glycogen between the muscles and the liver, the total remaining unchanged. With larger doses there is a loss in both situations.

4. Ergotamine, in doses up to 5 mg. intravenously, does not prevent the action of insulin or of adrenaline on the distribution of glycogen.

5. Iodoacetate, in doses of 15 mg., prevents the accumulation of liver glycogen usually produced by insulin and adrenaline in young rabbits. The final result in the case of adrenaline is a loss of glycogen from both liver and muscles; in the case of insulin the total glycogen was not changed.

6. The action of insulin in these animals is not accompanied by such an increase in blood lactate as would justify the belief that the adrenal secretion is the cause of the increase in liver glycogen which always follows such action.

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