XXIX. THE EFFECT OF INSULIN ON GROWTH, NITROGEN EXCRETION AND RESPIRATORY METABOLISM¹.

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THE clinical observation that insulin therapy in diabetes is frequently associated with a rapid increase in weight has led to considerable discussion as to whether the hormone promotes the formation of fat from carbohydrate or allows of a greater degree of sparing of fat and protein by a more complete metabolism of carbohydrate. In the diabetic subject the increase in weight is frequently brought about even when the diet is maintained at a level not necessarily in excess of what would be regarded as normal for age and weight. With the normal animal the matter is not clear. Whilst Macleod *et al.* [1930] refer to rabbits which gain weight rapidly during utilisation for the assay of insulin, Long and Bischoff [1930] could find no effect of insulin on the growth curves of these animals. The former investigators using young pigs and doses of insulin from 1.5 to 2 units per kg. could observe no very striking effect on the rate of increase in weight.

In view of these findings it is perhaps of interest to report our findings with young albino rats. In the following experiments very much larger doses of insulin were used than would *a priori* be considered compatible with the survival of the animal.

The animals were bred in the laboratory from a single pair and were of an extremely healthy and uniform strain. Comparisons were made between litter-mates only.

The method of experiment was to subject the animals of one series to a restricted diet, making certain by hand feeding that each individual received the same quantity daily. Another series was investigated with an unlimited quantity of food. Thus the appetite factor was of significance only in the second series. The diet consisted in every case of a mixture of white bread and fresh whole milk. No attempt was made to keep the diet of absolutely constant composition, but the average composition was 0.74 g. N and 17.74 g. carbohydrate (as glucose after hydrolysis) per 100 g. mixture and each animal of the restricted series received the same amount on any given day.

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In the case of the animals kept on an unrestricted amount of food, care was taken that the container was always well filled. The animals were all isolated in separate compartments. The doses of insulin, administered subcutaneously, varied in the animals on a restricted diet from 0.25 units to 40 units daily, always injected in one dose. In the case of the series on an unrestricted diet, the doses varied from 2 to 50 units daily, also given at one time.

The insulin used was that prepared by Messrs Burroughs Wellcome and Co.

Considerations of space allow only averages to be given, but it is to be understood that the growth curves of individual animals were practically identical.

Table I.

Litter 1. Restricted diet. Born 16. ii. 30. Weights given in g.													% increase in wt.
Age in days	26	27	30	32	34	37	40	43	46	50	55	61	—
Controls (wt. in g.) (1 ♀, 1 ♂)	49	53.5	55.5	58	59.5	65.5	70	74	81.5	87	100	111	126
Insulinised (1 ♂, 1 ♀)	45.7	50	50.3	50.3	53	63	69	73	83	89	107	109	138
Litter 2. Restricted diet. Born 18. ii. 30.													
Age in days	24	25	28	30	32	35	38	41	44	48	53	59	—
Controls (wt. in g.) (1 ♂, 3 ♀)	47	48	48.5	50	50	63	71	77	83.5	89	112	113	139
Insulinised (♀♀, ♂♂)	47	48	51	50	61	69	69	73	84	88	101	106	136
Insulin dosage for rats on restricted diet.													
Days	0-4	5-9	10-12	13	14-18	19-20	21-24	25-26	27	28	29-35		
Units per day	0.25	0.5	1.0	2	8	12	16	20	30	40	30		
Litter 3. Unrestricted diet. Born 28. vi. 30.													
Age in days	20	24	25	30	36	42							—
Controls (wt. in g.) (3 ♂, 3 ♀)	42	64	80	98	111	107							198
Insulinised (2 ♂, 4 ♀)	37	56	72	90	105	115							212
Litter 4. Unrestricted diet. Born 28. vi. 30.													
Controls (wt. in g.) (2 ♂, 1 ♀)	38	62	82	101	114	131							249
Insulinised (1 ♂, 2 ♀)	36	60	74	95	106	123							238
Insulin dosage for rats on unrestricted diet.													
Days	0-4	5-7	8	9	10-11	12-13	14-19	19-22					
Units per day	0	2	4	6	10	20	40	50					

Note. Of the insulinised animals, two in litter 1 and one in litter 2 were killed on the 61st and 55th days respectively by allowing them to consume all their diet in a short time and then injecting the insulin.

It will be observed from the results in Table I that the rate at which the animals put on weight was very much greater in the case of those on an unrestricted diet. Whereas the controls on the restricted diet showed an increase of about 130% in 35 days, those on the unrestricted diet increased by over 200% in 22 days. In this way a large range of growth activity was

obtained and any effects which insulin might have been able to produce could have been interpreted as having a broad application.

That no tolerance to large doses of insulin had developed was shown by the fatal effect of a dose of 50 units of insulin after withdrawal of food (see *Note*, Table I).

In administering such large doses of insulin, one has to consider the question of the degree to which it is eliminated in the urine. Whilst this may have accounted for a certain amount of loss, there can be little doubt that the animals were liable to a fatal hypoglycaemia during the greater part of the day, and were able to prevent its occurrence only by frequent feeding.

It is therefore not a little surprising that the hormone had, in fact, no effect whatever on either the rate of growth or on the final percentage increase in weight. Whatever theory may be held as to the essential action of insulin in the normal animal, these results appear difficult to reconcile with it. If the essential effect be regarded as a stimulus to peripheral oxidation of carbohydrate with a simultaneous drain on the glycogen of the liver, then it would have been reasonable to expect that at least in the case of the animals on the restricted diet, either a loss of weight or a diminished rate of increase in weight would have occurred. If, on the other hand, we regard the action of insulin as an inhibition of glycogenolysis, then we should have to suppose a temporary inhibition of the release of glycogen from the liver but a complete and adequate readjustment when the excess of the hormone had been eliminated. The experiments of Chaikoff and Macleod [1927] showed that rabbits on a high carbohydrate diet exhibited no increase in metabolism under the action of insulin. Lublin [1926], however, stated that, in man, simultaneous administration of carbohydrate and insulin might cause the R.Q. to exceed unity, and this he interpreted as evidence of a transformation of carbohydrate to fat. In the many experiments one of us (M. W. G.) has carried out on human subjects he has never seen such a phenomenon. Insulin indeed cannot increase the rate of metabolism and R.Q. above certain levels. When such increase cannot occur, a fall in blood sugar would only be possible if the release of carbohydrate from the liver were inhibited. Such a view is however not borne out by the fact that administration of insulin to a rat causes no increase in its hepatic glycogen, and may indeed, during active absorption, actually produce a fall.

In the present state of our knowledge it would be premature to attempt to reconcile these matters with our total inability to demonstrate a modified rate of growth by the action of insulin. Experiments on the effect of insulin on the protein metabolism of rats during starvation have demonstrated no increased protein catabolism. Since the largest dose of insulin which could be safely given was 0.5 unit it was however difficult to obtain a conclusive result. In the following experiments the plan was to subject rats to a period of 3 days' starvation, making daily collections of urine. The animals were then allowed to feed until the original weight was regained (always much more

than 3 days) after which a second period of starvation was begun during which insulin was administered. The fatalities were sufficiently frequent to make the experiment rather tedious. The urine was in most cases analysed for total N, inorganic P and creatinine.

Table II.

Day	Average initial wt. g.	Average daily excretion per 100 g. initial wt.			Remarks
		Nitrogen g.	Inorganic P mg.	Creatinine mg.	
(a) Control periods of starvation. Results from 14 rats in pairs. Average:					
1	424	0.070	5.9	4.6	Starvation
2	—	0.057	6.5	4.1	"
3	—	0.064	6.5	3.9	"
(b) Periods of starvation and insulin. Results from 3 pairs from above:					
1	389	0.099	4.4	3.5	Starvation
2	—	0.107	8.3	3.1	Starvation and insulin
3	—	0.060	5.2	2.2	"

The doses of insulin were 0.5 unit per animal daily.

On the second day one of the animals died in hypoglycaemia; exp. continued with the remaining two pairs. On the third day all the animals were hypoglycaemic; it was thought necessary to give one pair a few drops of sugar solution *per os*; hence drop in N excretion. No convulsions at any time.

Table III.

Larger doses of insulin.

Three adult rats were used and subjected to a preliminary starvation of 3 days, allowed to recover their original weight and then starved and treated with insulin.

Day	Initial period of starvation			Insulin period		
	1	2	3	1	2	3
Insulin daily (units)	—	—	—	1.5	1.0	1.0
Average N per 100 g. initial wt.	0.070	0.057	0.057	0.040	0.062	0.083

On the first day one animal died in hypoglycaemia, continued with remaining two.

We think that these results (Tables II and III) justify the conclusion that, in the starving normal rat, even in hypoglycaemic doses, insulin does not increase the catabolism of protein. When, however, as in pair 3 on the third insulin day (Table II), glucose is given, there occurs a very sharp drop in excreted nitrogen. The incidence of convulsions would of course increase the general catabolism, but in the above experiments these did not occur, so that our remarks apply to the primary effect of insulin and not to the secondary manifestations of hypoglycaemia.

In spite of the very large literature on the effects of insulin on metabolism, it cannot be said that we are clear as to its essential action. The difficulty of dissociating its effects from those of other hormones acting simultaneously in the intact animal has encouraged some workers, notably Dale and his collaborators, in the use of eviscerated and decapitated preparations. The relationship of their preparation to the living animal must however be remote. Effects can be observed to follow the injection of insulin into normal animals which

seem to be irreconcilable with the results obtained in the perfused preparation. Thus, in the latter glucose disappears from the perfusate, the oxygen utilisation rises very considerably and synthesis of glycogen occurs in the muscles, the R.Q. of the preparation remaining unity throughout. In the intact animal on a high carbohydrate diet, however, administration of insulin produces no rise in R.Q. or oxygen utilisation, whilst in the starving animal, insulin injection is followed by a rise in R.Q. and a definite rise in oxygen utilisation. Further in certain species of animal a very large rise in liver glycogen can be demonstrated to follow relatively large doses of insulin during starvation, whereas in others a definite loss occurs. The matter becomes even more confusing when we add the above findings that enormous doses of insulin do not affect the rate of growth of normal rats on restricted or unrestricted diet or their excretion of nitrogen in starvation. These latter facts, it seems to us, make it clear that the rat possesses mechanisms by means of which the tendency for insulin to increase the peripheral oxidation of sugar is effectively balanced by a diminished utilisation of other substances, probably fat. In other words, the effect of insulin is to produce a diversion of metabolism in the direction of carbohydrate. This causes a rise in R.Q. and may or may not produce an increase in total metabolism according to the readiness with which these mechanisms are brought into action. Thus, as will be demonstrated in the human subject, a rapid increase in heat production and R.Q. can occur after injection of insulin; but a progressive fall in blood-sugar may also occur without any such rise in R.Q. or metabolic rate (*v. infra*). It seems to us that this can only be explained by attributing to insulin the double action of inhibiting glycogen release from the liver and stimulating peripheral oxidation, these effects not occurring simultaneously. The rat appears normally to possess extraordinarily active mechanisms for the mobilisation of its hepatic glycogen for the needs of its peripheral tissues, so that the administration of insulin is never followed by a demonstrable rise in liver-glycogen, such as has been found both in the young cat and rabbit [Goldblatt, 1930]. It is perhaps relevant here to describe certain experiments carried out on young dogs to illustrate that the latter effect is not as unusual as the recent work of Corkill [1930] might lead us to believe.

The methods used were those described by one of us (M. W. G.) in previous communications. Comparisons were made on litter mates. The insulinised animals were always killed before any symptoms occurred. The values for muscle-glycogen were obtained from a mixed sample from all four limbs.

It is to be observed that the effect here described is by no means as striking as that found in young rabbits nor are the control values as uniform as is desirable in such comparison experiments. But it is clear that, as in young cats and rabbits, a dangerous hypoglycaemia can occur even when large amounts of glycogen are still available in the liver and before any marked change has occurred in the muscle glycogen. In so far as we are justified in taking averages of these rather divergent figures, the conclusion may be drawn

The effect of insulin on young dogs.

Animal No.	Wt. g.	Blood sugar g. per 100 cc.	Plasma inorg. P mg. per 100 cc.	Glycogen			Remarks
				Liver		Muscle %	
				%	Total mg.		
Litter 1. 24 hrs. starvation.							
1	530	0.102	8.60	0.84	140	0.82	Control
2	510	0.104	7.93	1.47	265	0.69	"
3	375	0.095	7.86	2.06	258	0.44	"
			Av. 1.46		Av. 0.65		
4	576	"0"	6.46	2.21	417	0.53	11.20 a.m. 0.5 unit 12.45 p.m. 0.5 1.40 1.0 2.25 Killed
5	600	0.037	—	2.22	460	0.66	11.23 a.m. 0.5 12.45 p.m. 0.5 1.40 1.0 2.10 Killed
6	560	0.077	6.60	1.73	347	0.78	12.05 p.m. 0.5 1.10 2.0 1.30 Killed
			Av. 2.05		Av. 0.66		
Litter 2. 48 hrs. starvation.							
1	662	0.087	7.81	0.28	56	0.59	Control
2	790	0.090	7.86	0.79	209	0.40	"
			Av. 0.53		Av. 0.49		
3	732	0.018	6.28	0.87	216	0.55	11.30 a.m. 1 unit 1.00 p.m. 1 " 2.15 Killed
4	654	"0"	6.00	1.09	242	0.51	11.30 a.m. 1 unit 1.00 p.m. 1 " 2.00 Killed
5	417	"0"	—	0.48	72	0.60	11.30 a.m. 1 unit 1.00 p.m. 1 " 1.15 Killed
			Av. 0.81		Av. 0.55		

that an increase was occurring in liver-glycogen without any attendant decrease in peripheral glycogen. In the publication of Corkill, above referred to, the increase in the liver-glycogen of insulinised young starving rabbits was associated with an average fall in muscle-glycogen and it was calculated that the peripheral loss was at least as great as the gain in the liver. His emphasis on this matter seems to suggest that it was considered that a transference was occurring from the muscles to the liver, an assumption for which there is at present no experimental evidence. In our experiments such large peripheral losses have not been found and the increase in liver-glycogen may certainly occur when no loss at all has been sustained by the muscles.

There seems to be no question that insulin stimulates peripheral oxidation and hence we must consider that in the above experiments and in those on young rabbits and cats, either there was proceeding a simultaneous replenishment of glycogen in the muscles or the effect on oxidation is a later one. Indeed Macleod [1930] has recently suggested that this accumulation of glycogen in the liver after insulin is an early effect of the hormone; but it is

sometimes difficult to accept this because the animal may die in hypoglycaemia with an immensely increased liver-glycogen and a very considerable though diminished muscle-glycogen. In some cases a synthesis of glycogen could be demonstrated in muscle as well as in the liver [Goldblatt, 1930].

It is clear that no theory of the action of insulin can approach the truth which does not take into account that it can produce a fall in blood-sugar in the perfused decapitated eviscerated cat. But this action of the hormone seems to be influenced in the intact animal by the presence of the liver and other mechanisms, which are available to different extents in different species. Further we have no evidence as to the amount of insulin which is normally operative in different species; so that when we superimpose doses of insulin in acute experiments, the normal mechanism or its velocity is probably entirely disturbed.

In the following experiments we propose to show that even in the human subject the effect of insulin on the respiratory metabolism may vary with the conditions.

One of us (M. W. G.) was the subject of experiment. Starvation was for a period of 16 hours before the administration of the insulin. Estimations of metabolism were carried out by the Haldane and Douglas methods. The estimations of the basal values were made after 2 hours' complete rest.

Table IV. *Effect of insulin on the respiratory metabolism of a starving human subject.*

10 units insulin injected subcutaneously at 1.16.

Time	Blood-sugar g. per 100 cc.	Ventilation per min. litres	O ₂ per min. cc.	CO ₂ per min. cc.	Total R.Q.	Non- protein R.Q.
11.20	0.109	—	—	—	—	—
12.05	—	5.66	226	186	0.83	0.84
12.50	—	5.16	206	165	0.80	0.80
1.46	—	4.93	179	145	0.81	0.82
2.05	0.045	—	—	—	—	—
2.10	—	6.78	246	242	0.98	1.01
2.20	0.062	—	—	—	—	—
2.42	—	5.72	200	176	0.88	0.92
2.50	0.067	—	—	—	—	—

At 2 p.m. a moderately severe hypoglycaemic reaction was experienced, apprehension, sweating and hunger being marked. It will be observed that at the time of greatest fall in blood-sugar, the R.Q. had reached unity and the O₂ utilisation had increased very definitely. This increase in oxygen utilisation was the result of the greater ventilation and not of an increased difference in the compositions of the inspired and expired air. The CO₂ difference, however, rose from 3.6 to 3.9, so that it may be considered that there was a true increase in oxidation of carbohydrate with a secondary stimulus to the respiratory centre from the rise in CO₂ production. Recovery was spontaneous and the rise in blood-sugar was accompanied by a fall in R.Q. and total metabolism.

This type of metabolic change after injection of insulin is, in our experience, always reproducible in the starving organism. The apparent drop in protein metabolism is unusual and is quickly balanced by the subsequent rise. Such results give considerable support to the view that insulin provides a stimulus to the oxidation of carbohydrate, but the matter is more complicated than this.

In the following experiments (certain of which were carried out with Dr William Maclean) it will be demonstrated that:

(1) The fall in blood-sugar produced by insulin is not necessarily accompanied by a rise in R.Q.

(2) The rise in R.Q. which follows the injection of insulin in the fasting state can be inhibited by giving glucose simultaneously.

(3) The reduction of blood-sugar to negligible proportions may be accompanied by an acute fall in R.Q.

These experiments were performed on diabetic patients and on glycosurics without ketosis.

The effect of insulin on a glycosuric without ketosis.

The patient was a woman of 50 years and 15 st. weight. In Exp. A a light meal was taken at 8.30 a.m. In B starvation for 16 hours before the experiment.

	Time	Blood-sugar g. per 100 cc.	R.Q.	O ₂ per min. cc.	Urine-sugar g. per 100 cc.
Exp. A	10.56	0.304	0.89	275	3.80
	11.08	10 units insulin subcutaneously		—	—
	11.35	0.328	0.85	286	—
	12.05	0.286	0.84	271	2.74
	1.05	0.230	0.83	255	0.41
	2.05	0.200	0.81	258	0
	2.38	0.181	0.85	252	0
	4.20	0.130	0.83	244	0
	5.30	0.117	—	—	—
Exp. B	11.25	0.348	0.74	304	3.34
	12.25	5 units insulin subcutaneously		—	—
	12.55	0.301	0.68	296	2.00
	1.35	0.275	0.77	262	1.76
	2.15	0.240	0.72	281	0.66
	3.00	0.212	0.76	272	0.18
	4.26	0.177	0.75	261	0

The subject was a woman of active life (a washerwoman) and complained only of obesity. We were unable by any therapeutic measures to influence her obesity, partly because she did not reduce her intake of food. She was losing regularly about 100 g. sugar per day in the urine. Her threshold for sugar was definitely raised, as can readily be seen above.

It is clear from the above figures that the prevention of the glycosuria was not accompanied by any change in the degree or type of metabolism, so that we must suppose an inhibition of release of sugar from the liver to be taking place under the action of the hormone. It is important to note that this type of patient rarely shows evidence of ketosis and, in fact, in this case there was never any excretion of acetone which could be estimated. The conditions

usually taken as evidence of gluconeogenesis were absent in spite of the great loss of sugar and the high fasting blood-sugar.

It thus appears that a steady fall in blood-sugar may be induced without any observable change in metabolism. Of course the doses were small and give no information of what may occur in severe hypoglycaemia. In the next experiments very much larger amounts of insulin were used and very low levels of blood-sugar were obtained; the subjects were diabetics.

The effect of insulin on diabetic subjects.

Case 1. A young woman of 19 years. Very severe diabetic with all the classical symptoms. Always strong ketosis present. Before each experiment food was stopped for 16 hours and insulin not injected after the morning dose of the previous day.

We first give the reaction of this subject to glucose and fructose.

Time after sugar (hrs.)	Blood-sugar g. per 100 cc.	R.Q.	O ₂ per min. cc.	Urine-sugar	
				g. per 100 cc.	Total during experiment g.
Effect of 50 g. glucose <i>per os</i> .					
0	0.286	0.79	260	3.3	
$\frac{1}{2}$	0.390	0.71	264	6.1	
1	0.420	0.74	281	6.6	
$1\frac{1}{2}$	0.410	0.75	259	8.5	
2	—	0.74	248	7.1	43.31
Effect of 50 g. fructose.					
0	0.373	0.69	227	7.25	
$\frac{1}{2}$	0.403	0.80	232	6.42	
1	0.423	0.68	259	7.25	
$1\frac{1}{2}$	0.443	0.76	233	7.62	
2	0.414	0.78	219	7.75	40.2

These results present the classical features of a very severe diabetic. A certain amount of support is given by these findings to the view that the diabetic organism can make somewhat better use of fructose than of glucose.

In the following two experiments the effect of insulin was examined.

Time after insulin (hrs.)	Blood-sugar g. per 100 cc.	R.Q.	O ₂ per min. cc.	Urine-sugar	
				g. per 100 cc.	Total g.
Effect of 50 units insulin (subcutaneously) followed 1½ hours later by 50 g. fructose.					
0	0.343	0.77	209	8.30	
0	—	0.74	235	—	
1	0.252	0.75	215	6.6	
1½	0.209	0.91	211	6.05	33.5
50 g. fructose <i>per os</i> .					
2	0.187	0.88	227	2.80	
2½	0.206	0.85	235	1.50	
3	0.166	0.73	227	1.03	
3½	0.162	0.80	236	0.50	0.98
Effect of 50 units insulin and 50 g. glucose <i>per os</i> at same time.					
0	0.187	0.76	258	—	
½	0.388	0.68	257	6.60	
1	0.398	0.72	241	7.10	
1½	0.353	0.76	238	8.50	
2	0.302	0.72	229	9.00	
3	0.209	0.73	237	5.30	
4½	0.104	0.76	235	0.85	34.64

The peculiar fact emerges from these findings that whereas insulin produced a rapid rise in R.Q. whilst the patient was starving, this effect was either diminished by giving fructose or completely inhibited if glucose were given simultaneously with the insulin (of course this is not specific). Further no significant change in the respiratory metabolism occurred even when the blood-sugar reached normal levels in the latter case.

Thus again it seems that insulin may promote a synthetic action without any simultaneous change in the oxidative processes.

In the following case similar observations were made.

Case 2. Patient was a girl of 14 years. Severe diabetic. Experiments were performed 16 hours after food and insulin.

Time after sugar (hrs.)	Blood-sugar g. per 100 cc.	R.Q.	O ₂ per min. cc.	Urine-sugar	
				g. per 100 cc.	Total g.
Effect of 30 g. glucose <i>per os</i> .					
0	0.420	0.69	215	5.65	
$\frac{1}{2}$	0.505	0.72	208	6.30	
1	0.465	0.77	201	6.82	
$1\frac{1}{2}$	0.408	0.83	200	7.10	
2	0.371	0.75	204	6.82	34.7
Effect of 30 g. fructose <i>per os</i> .					
0	0.353	0.78	183	4.75	
$\frac{1}{2}$	0.420	0.86	186	5.12	
1	0.381	0.76	206	6.38	
$1\frac{1}{2}$	0.383	0.88	197	6.56	
2	0.381	0.93	189	6.38	28.0

In this case we again see some evidence that the diabetic may make a more successful use of fructose than of glucose.

The findings in this case serve to show how various may be the reactions

Time after insulin (hrs.)	Blood-sugar g. per 100 cc.	R.Q.	O ₂ per min. cc.	Urine-sugar	
				g. per 100 cc.	Total g.
Effect of 50 units insulin followed by 30 g. fructose 1½ hours later.					
0	0.388	0.78	142	7.10	
1	0.201	0.96	155	5.40	
1½	0.133	0.84	178	1.03	35.0
30 g. fructose <i>per os</i> .					
2	0.067	0.85	162	0.30	
2½	0.022	0.77	205	0	0.30
The very low blood-sugar reached at this time made it necessary to give some carbohydrate. 50 g. glucose were accordingly given <i>per os</i> .					
3	0.090	0.90	204	0	0
Effect of 50 units insulin and 30 g. glucose 1½ hours later.					
0	0.374	0.75	201	5.65	
½	0.338	0.77	—	5.25	
1	0.302	0.78	204	5.77	
1½	0.333	0.79	198	6.30	30.0
30 g. glucose <i>per os</i> .					
2	0.328	0.82	204	6.17	
2½	0.339	0.84	202	7.35	
3	0.344	0.78	192	8.17	
3½	0.296	0.76	201	7.76	14.6

of the respiratory metabolism to insulin even in the same subject. For whereas on one occasion 50 units produced an acute rise in R.Q. to 0.96 with a fall in blood-sugar from 0.388 to 0.201, on another occasion the same dosage and conditions only raised the R.Q. from 0.75 to 0.78 and the blood-sugar fell only from 0.374 to 0.302.

Very striking was the sharp drop in R.Q. (0.77) which occurred when the blood-sugar fell to 0.022. We have observed this on several occasions and can only attribute it to a retention of CO_2 in the tissues as a result of some unknown factor. There is of course the possibility that at so low a level of glycaemia there may be so great a deficiency of circulating carbohydrate that other substances are brought into the metabolic field, thus lowering the R.Q. As we have reason to believe that these substances cannot be protein, they might possibly be fat, a view which would be in harmony with the increased excretion of ketones which occurs during insulinisation of fat-fed rats.

Attention may be drawn here to the fact that it has been found that in hypoglycaemia the venous blood is highly oxygenated [Klein, 1930; Klein and Holzer, 1929] and approximates in its O_2 content to arterial blood. The arterio-venous CO_2 difference was also found by these authors to be very much diminished. They conclude that the O_2 utilisation by the tissues in insulin shock is very greatly diminished. This is, however, a conclusion which seems to us quite untenable, seeing that estimations of respiratory metabolism will almost always demonstrate a greatly increased utilisation of O_2 and especially in hypoglycaemia. If through some interference with capillary function the removal of CO_2 were interfered with, then the sudden fall in R.Q. might be accounted for. In the following experiment we show another example of the same phenomenon of fall in R.Q. in extreme hypoglycaemia.

Case 3. The patient was a mildly diabetic man. All experiments carried out after 16 hours without food or insulin.

Time after sugar (hrs.)	Blood-sugar g. per 100 cc.	R.Q.	O ₂ per min. cc.	Urine-sugar	
				g. per 100 cc.	Total g.
Effect of 50 g. glucose <i>per os</i> .					
0	0.146	0.76	208	0.20	
$\frac{1}{2}$	0.171	0.74	224	0.20	
1	0.206	0.95	194	0.85	
$1\frac{1}{2}$	0.241	0.89	185	2.15	
2	0.246	0.87	197	2.45	3.51
Effect of 50 g. fructose.					
0	0.241	0.84	244	1.95	
$\frac{1}{2}$	0.261	0.87	257	1.50	
1	0.281	0.84	238	1.95	
$1\frac{1}{2}$	0.277	0.85	275	2.07	
2	0.271	0.85	297	2.70	15.16
Effect of 25 units insulin and 50 g. glucose at same time.					
0	0.129	0.73	209	0.24	
$\frac{1}{2}$	0.146	0.72	240	0.25	
1	0.206	0.83	209	1.25	
$1\frac{1}{2}$	0.231	0.82	209	2.05	
2	0.222	0.83	211	1.64	
$2\frac{1}{2}$	0.166	0.80	204	0.95	3.54

Effect of 20 units insulin followed 1 hour later by 50 g. fructose *per os*.

Time after insulin (hrs.)	Blood-sugar g. per 100 cc.	R.Q.	O ₂ per min. cc.	Urine-sugar	
				g. per 100 cc.	Total g.
0	0.156	0.74	242	0.70	
$\frac{1}{2}$	0.166	0.76	252	0.37	
1	0.150	0.84	239	0.27	2.80
	50 g. fructose <i>per os</i> .				
$1\frac{1}{2}$	0.109	0.92	254	0.25	
2	0.104	0.92	272	0.34	
$2\frac{1}{2}$	0.060	0.91	277	0.26	
3	"0"	0.79	271	0	0.60

Thus it is seen that the administration of glucose simultaneously with the insulin did not produce as great a change in R.Q. as when the glucose was given alone.

Again we find that very low levels of blood-sugar (in this case the symbol "0" indicates that the thiosulphate equivalent of the Cu-reducing substances in 0.2 cc. blood is less than 0.2 cc. (N/400)) were associated with an extraordinarily rapid fall in R.Q. not accompanied by any change in oxygen utilisation and in spite of the large amount of available sugar.

In case 2 the sudden fall of R.Q. at the blood-sugar level of 0.022 was associated with a large rise in oxygen absorption (43 cc. per min.) but the increase in CO₂ output (20 cc. per min.) was not sufficient to keep the R.Q. up. In case 3 just considered there was no rise in oxygen utilisation at the critically low blood-sugar but the CO₂ output suddenly fell. It seems to us highly improbable that this sudden change in R.Q. is due to a change in type of metabolism and we consider it much more likely that we are dealing with some retention of CO₂ not at present explicable. Such low levels of blood-sugar as we have reached have not, to our knowledge, been reported in the literature in association with estimations of respiratory metabolism and as it is not often that one dares, in the human subject, continue to such low levels, the phenomenon may be readily overlooked.

Results of such a kind raise the old difficulty which assails every attempt to interpret respiratory quotients. However, from the above results we think that the conclusion may be drawn that insulin may bring about a definite stimulus to carbohydrate oxidation but that this is not the only mechanism by which the blood-sugar is lowered, since the effect may quite certainly be produced without any perceptible change in respiratory metabolism.

A complete knowledge of the action of insulin must involve the power to foretell exactly what will occur in given conditions. It must be admitted that we cannot as yet do this. In one condition, however, *viz.* in starvation, insulin seems always to produce an increase in R.Q. but not necessarily a rise in heat production. When the conditions are made more complicated, as by administration of carbohydrate or the production of extreme hypoglycaemia, it appears that secondary influences enter which make it difficult to interpret the findings of respiratory metabolism. Where, however, in the presence of

large amounts of sugar, the R.Q. does not change after the administration of insulin in large doses, it seems reasonable to accept glycogen synthesis as the most probable explanation.

Further experiments on the effect of insulin on nitrogen excretion.

In certain of the above experiments the effect of insulin on the protein metabolism of rats in starvation was examined and it was found that no appreciable effect occurred. In the following series an attempt was made to see if the injection of the hormone during periods of exclusively fatty diets could modify the excretion of nitrogen.

Ketones were estimated by a method described by one of us [Goldblatt, 1925]. The animals were albino rats bred in the laboratory and kept previously to the experimental period on a diet of bran, oats and occasionally green stuff.

Exp. 1. An adult rat was starved during 3 days and treated as below.

Day	Wt. g.	Urine		Remarks
		Total N g. per 100 g. initial wt.	Total ketones as mg. acetone	
1	207	0.072	1.10	Starvation
2	197	0.063	0.80	Starvation
3	185	0.058	0.80	Starvation and 0.4 unit insulin at 2.30 p.m. and 4.50 p.m.

Clearly no significant change in either nitrogen or ketones occurred.

Exp. 2. This experiment was carried out during the summer, which will explain the type of ketone excretion which occurred. An adult rat was taken and put on a diet of lard and 5 % salt mixture (Wigglesworth) and water. Treatment as below.

1	163	0.047	0.80	Fatty diet commenced
2	160	0.068	3.20	
3	154	0.040	21.00	
4	152	0.042	4.50	
5	152	0.027	0.80	Adaptation complete
6	152	0.040	2.50	2 doses 0.4 unit insulin
7	158	0.046	1.10	1 dose 0.8 unit insulin

Exp. 3. The same rat and the same diet as in the last experiment.

1	—	0.039	1.00	Fat diet commenced
2	—	0.075	2.40	
3	—	0.080	17.50	
4	—	0.053	10.90	
5	—	0.045	8.60	
6)	—	0.065	8.20	Adaptation almost complete
7)				
8	—	0.038	3.10	2 doses 0.4 unit insulin
9	—	0.032	1.10	
10	—	0.040	0.90	2 doses 0.4 unit. Animal weak and hypoglycaemic about 2 hours after the second injection. No convulsions

On neither diet was the nitrogenous excretion modified by insulin. Excretion of ketones was increased by insulin in the rats on the fatty diet in the summer [cf. Burn and Ling, 1928].

In the next experiment similar estimations were made with the addition

that the period was extended, the exact amount of fat eaten per day was determined and rather greater doses of insulin were administered.

Exp. 4. Two rats were used in the same cage. The fat diet was the same as that used in the previous cases. The diet was fed by hand, so that the exact amount eaten per day was known.

Day	Wt. g. (combined)	Fat eaten g.	Urine		Remarks
			N per 100 g. animal g.	Ketones as mg. acetone	
1	287	0	0.074	1.30	Starvation
2	259	17.53	0.092	1.60	
3	266	17.95	0.061	2.00	
4	270	8.92	0.040	2.20	Ketosis of winter type
5	266	3.02	0.041	1.20	
6	254	4.97	0.048	0.80	Adaptation complete
7	245	7.32	0.047	2.10	0.28 g. NaHCO ₃ taken by animals from a solution put in place of water
8	242	19.96	0.042	3.50	0.24 g. NaHCO ₃
9	237	6.12	0.037	1.90	Each 3 doses of 0.3 unit insulin—no symptom
10	—	8.14	—	6.30	Each 0.5 unit—both hypoglycaemic
11	226	9.20	0.038	2.10	No treatment
12	225	7.36	0.049	3.90	Each 0.5 unit
Total		110.49	1.549	29.00	

The total fat excreted in the faeces was <2 g.

This experiment demonstrates clearly the existence of a basal nitrogen excretion on a nitrogen-free diet, in this case about 0.04 g. N per 100 g. body weight. Further this quantity is independent of the actual amount of fat ingested daily. The insignificant excretion of ketones confirms the findings of other workers, who find that the commonly held views on ketosis are in no sense applicable to the rat.

The changes in the excretion of nitrogen after insulin were clearly of no significance and again confirm the findings in the previous experiments. It will also be seen that the excretion of ketones was, after adaptation was complete, increased to a greater extent by insulin than by NaHCO₃ administered under similar conditions. These sudden rises in ketosis after insulin are not readily explicable. The view taken by Hirschhorn and Pollak [1927] as to the action of adrenaline on ketosis may be partly applicable to that of insulin, *viz.* that there occurs a sudden drain on the hepatic glycogen and that it is the suddenness of this drain which determines the degree of increase of ketosis. On this view a quantitatively equal loss of glycogen more gradually released would be followed by a smaller degree of ketosis.

The interesting fact that rats can continue for so long as in the above experiments in health and relatively slow rate of loss in weight on a diet consisting solely of fat and salts raises the contested question as to whether fat can give rise to carbohydrate. There can be no doubt that rats killed after prolonged periods of exclusively fatty feeding show very considerable quantities of glycogen in the liver and tissues. This matter will however be dealt with in a further communication.

CONCLUSIONS.

From these experiments it appears justifiable to consider that, in the normal animal, starving or consuming a diet containing no nitrogen, there is no change in the excretion of nitrogen as a result of the injection of insulin. The effect of insulin therefore appears to be limited to the metabolism of fat or carbohydrate or both.

The effect of insulin on peripheral oxidation is not one which, as far as our evidence goes, can exert any profound influence on the general total metabolism, as was seen in our growth experiments with rats. Nor is an increase in oxidative processes a necessary accompaniment of the fall in blood-sugar which follows the injection of the hormone.

SUMMARY.

1. Litters of white rats, kept on restricted or unrestricted amounts of food, showed no difference in rate of growth from that of litter mates, when injected with daily doses of insulin varying from 2 to 50 units.

2. Insulin was found to have no effect on the nitrogen excretion in the urine of rats consuming an exclusively fatty diet or during starvation. This was the case whether hypoglycaemia did or did not occur; convulsions were absent.

3. Insulin hypoglycaemia in a starving human subject is associated with a marked increase in R.Q. and hence presumably an increased carbohydrate- and diminished fat-oxidation. This effect passes over as the blood-sugar recovers.

4. Progressive fall in blood-sugar and disappearance of glycosuria may be induced in a glycosuric patient without any change in R.Q. or calorie output. It is considered that this must indicate glycogen synthesis in the liver.

5. Insulin can increase the liver-glycogen of young dogs during starvation without any change in muscle-glycogen. This effect may be accompanied by profound hypoglycaemia.

6. The attainment of blood-sugar values of vanishing proportions may be accompanied by an acute fall in R.Q. It is considered improbable that this fall indicates a true change in metabolism.

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