XI. THE EFFECT OF INSULIN ON CARBOHYDRATE FORMATION IN THE LIVER

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In a previous communication one of us [Gemmill & Holmes, 1935] demonstrated an increase in yeast-fermentable reducing substance in slices of rat's liver, shaken for 2 hours in bicarbonate-buffered Ringer's solution at 37°. It was presumed that the yeast-fermentable reducing substance investigated was carbohydrate: we have not, however, as yet attempted to work up the large quantity of tissue necessary for chemical identification, and while we shall, for the sake of brevity and convenience speak of "fermentable carbohydrate", it must be understood that we do so with the reservation just implied.

The starting-point of the present work was the observation that the carbohydrate production of the liver slices *in vitro* was diminished, sometimes slightly, sometimes to a considerable degree, by the addition of insulin to the medium in which the slices were shaken. This observation seemed to us to be of considerable interest, first because it accords with the view of Best *et al.* [1926] that insulin suppresses gluconeogenesis, and second because it has hitherto been extremely difficult to demonstrate (except in the case of the beating heart) any effect of insulin on isolated tissues.

The fact that the action of the hormone was never completely to inhibit the production of carbohydrate strongly suggested that carbohydrate was formed in the liver slices from more sources than one, and that formation from one or more of these remained unaffected by insulin. We have obtained strong evidence that this is indeed the case, and we have also been able to throw some light upon the point at which, in our experiments at least, insulin exerts its action.

Methods

Young rats and, in a few cases, young rabbits were used for the work. The rats were chiefly albinos, though black and white rats were occasionally used. They were almost all males, and their weight was usually 100–200 g., though sometimes animals as heavy as 300 g. were used. They were always starved for 10–24 hours before the experiment. If this were not done, the amount of carbohydrate initially present in the liver was so high that the increases observed fell close to or even within the limits of experimental error. Before starving, the animals were kept on the ordinary stock laboratory diet.

In cutting the tissue slices, the blade was moistened in Ringer's solution from time to time and then blotted, merely to prevent it from becoming sticky. The slices were accumulated in a moist chamber. As they were not wetted with Ringer's solution they could be weighed before being placed in the vessels used for the experiment; it was impracticable to dry and weigh them at the end of the experiment. A convenient vessel in which slices may be shaken is a Barcroft manometer cup, fitted with a side-tube for irrigation with gas mixture, and shaken

120 times per min. in a water thermostat at 38°. The Ringer's solution in which they were suspended, the volume of which was 3·0 ml. for the Barcroft cup, or 5 ml. when larger vessels were used, contained 0·025*M* $NaHCO_3$ and was equilibrated with a gas mixture containing 95% O_2 and 5% CO_2.

In experiments in which the oxygen uptake and R.Q. were observed, the method of Dixon & Keilin [1933] was employed. In all cases care was taken that equilibrium was obtained after tipping HCl into the right-hand cup. The rat's liver always forms some acetoacetic acid, which is only slowly decomposed by HCl [Gemmill & Holmes, 1935]. The figures which we give for the R.Q. are thus not vitiated by the fact that CO_2 has been retained as acetoacetic acid.

Total fermentable carbohydrate was estimated as follows. The tissue was ground in a mortar and, together with the fluid in which it had been shaken, hydrolysed in approximately 0·5*N* H_2SO_4 for 3 hours; the hydrolysate was then treated with H_2SO_4 and $HgSO_4$, followed by $BaCO_3$, according to the method of Cori & Cori [1933], except that for samples of tissue of 0·5–1·0 g., and a total volume of 9 ml. of hydrolysate, only 1·0 ml. of the $HgSO_4$-H_2SO_4 reagent was employed; the final volume was adjusted to 40 ml. This procedure gave good results and saved time and material, since precipitation with $BaCO_3$ was quicker and easier, and the precipitate was less bulky than when larger amounts of mercuric reagent were used; less fluid was therefore lost by centrifuging.

Fermentation was carried out according to the method of Cori & Cori [1933]. Some of the sugar estimations were performed by the Shaffer-Hartmann method, but later that of Hagedorn & Jensen [1923] was uniformly employed.

Urea was estimated as follows. The tissue and fluid were washed into a centrifuge cup graduated at 10 ml. Ten drops of 3% acetic acid were added, and the tube was placed in a boiling water-bath for 2 min., the contents being stirred with a glass rod. After cooling, the volume was adjusted to 10 ml. and the tube centrifuged. Ammonia was estimated in a suitable amount (1 or 2 ml.) of the supernatant fluid by the method of Parnas & Heller [1924]. A similar amount was then treated with urease and the ammonia again estimated, urea being calculated from the difference in ammonia content observed. The free ammonia content was always very low and tended to be less at the end of the experiment.

The insulin used was obtained as insulin hydrochloride from British Drug Houses, Ltd. The potency varied slightly in different specimens but was always close to 20 units per mg. The required amount was weighed out on a microbalance, and except when otherwise stated, was added to the Ringer's solution just before the tissue.

Whenever insulin, amino-acids or other substances were added to the tissue, suitable control experiments were performed to show that these did not themselves reduce the sugar reagents. All solutions added to the Ringer were first adjusted to *p*H about 7·4.

Results

The results of a series of experiments which demonstrate the effect of insulin on the formation of fermentable reducing substance by the liver slices are given in Table I. This table also shows that Gemmill & Holmes's experiments are confirmed and that satisfactory duplicate estimations are obtained. It will be observed that the effect of insulin is variable; that within wide limits it does not depend on the amount of insulin added (separate experiments indicated that the minimum effective amount is in the neighbourhood of 0·0065 mg. (0·13 unit) per ml. of fluid in which 0·5–1·0 g. of tissue is suspended) and that on the average

Table I. *Effect of insulin on carbohydrate synthesis*

Quantities: mg. fermentable carbohydrate per g. fresh liver

Duration of exp. hours	Initial	Increase without insulin	Inhibition by insulin of the increase	Insulin added, units per ml.
3	4·05 3·95	4·45	—	—
3	4·24	1·50	—	—
2½	7·47	2·03	—	—
3	4·51	2·30	—	—
3	1·35 1·39	2·82	0·38	0·5
3½	4·35 4·68	2·69	1·26	0·6
3	5·04 5·01	2·09	0·36	1·9
3	5·71	2·30	1·38	2·9
4	17·34	−0·08	1·20	3·0
4	3·54	2·35	1·39	2·4
4	0	5·14	1·41	3·2
5	4·36	0·70	0·94	3·3
6	3·40	2·79	1·58	2·8
4	3·19	3·22	0·74	3·2
4*	2·14	2·49	—	—
4	3·78	1·72	0·53	0·2
1½	4·15	0·91	1·29	0·6
1	18·80	0·79	0·12	0·15
1	—	—	0·61	0·22
2½†	7·77	2·83	1·48	0·60
Average	5·23	2·28	0·98	

* Rabbit. † Female.

it causes about 40 % inhibition of carbohydrate formation. The effect is complete in 2½ hours. There was no evidence of any deleterious effects, even with much larger concentrations of insulin. That this new formation of carbohydrate is an aerobic process was shown by two experiments in which N_2/CO_2 gas mixture, carefully freed from traces of O_2, was substituted for O_2/CO_2. In these no synthesis occurred.

It has been shown repeatedly by various workers by means of experiments on the whole animal, that carbohydrate can be formed in the body from non-carbohydrate sources. These sources include among others amino-acids, keto-acids, lactate and glycerol. Whether or not fatty acids can be converted into carbohydrate is still a matter of controversy. Gemmill & Holmes [1935], among others, have lately brought forward evidence which they believe to support such a view and which, in spite of certain criticism [Cori, 1935], they believe to be valid. In any event, it is to be anticipated that liver slices may be forming carbohydrate from more sources than one, and Table I suggests that the gluconeogenesis from one or more of these is unaffected by insulin. If this be so, it would explain also why the "insulin effect" varied in magnitude, since every animal examined would not necessarily, even after 24 hours' starvation, be in an identical condition of nutrition and its liver might therefore be expected to contain either more or less of some particular carbohydrate-forming substance. Our problem has been to try to determine which of the possible sources of gluconeogenesis was affected by insulin.

It is possible, by means of the Dixon-Keilin apparatus, to determine the R.Q. of slices of tissue *in vitro*. If the gaseous exchange so observed represents the sum of the respiratory exchanges of the tissue (and the validity of the isolated tissue technique depends upon the fact that such is the case) it is reasonable to

argue that, if the changes in metabolism brought about by insulin are such as to affect the respiratory processes and particularly the R.Q., they will be detectable by this technique, provided, of course, that they are of sufficient magnitude. The R.Q. of liver slices taken from a starved rat is always low, even allowing for the production of acetoacetic acid which occurs [Gemmill & Holmes, 1935]. Calculation makes it plain that a suppression of the conversion of protein into carbohydrate of the order of magnitude observed would have only a small, and probably an undetectable, effect on the R.Q.

A suppression of the conversion of lactate into carbohydrate, which involves neither the absorption of O_2 into, nor its elimination from, the molecule, would be without any effect, and a suppression of gluconeogenesis from pyruvate or glycerol would again produce only a small change. On the other hand, the suppression of gluconeogenesis from fatty acids (supposing such a process to occur) would result in a rise of R.Q. It will be observed from Table II that no

Table II. *Effect of insulin on respiration*

Duration of exp. hours	O_2 consumption per hour μl. per g. fresh liver		R.Q.		Insulin added, units per ml.
	Without insulin	With insulin	Without insulin	With insulin	
1	2360	2320	0·675	0·676	2·7
1	2525	2430	0·616	0·649	0·22
1	2275	2070	0·640	0·585	0·22
2½	1975	1965	0·614	0·606	0·23
2½	2310	1900	0·634	0·616	0·23
2½	2170	—	0·601	—	—
2	—	1425	—	0·58	0·27
2	1450	1280	0·617	0·559	0·24
2	1830	1570	0·573	0·577	0·24
2	2425	2475	0·560	0·550	2·7
Average	2147	1937	0·614	0·599	

such rise occurs, though it is apparent that the R.Q. is lower than could be accounted for even by the assumption that only fats were being oxidized. The failure of insulin either to increase the oxygen consumption (it lowers it slightly) or to raise the R.Q. seems also to dispose of the possibility that its apparent inhibition of carbohydrate formation is really the result of an increased combustion of carbohydrate. Reinwein & Singer [1928] also failed to observe an increase in the oxygen consumption of rat liver in the presence of insulin.

Table II shows that the R.Q. of the liver slices is not raised by insulin. The only change observed is a slight decrease in oxygen consumption. This makes it unlikely that insulin suppresses gluconeogenesis from fat. Whether or not gluconeogenesis from lactate and pyruvate is affected can be tested by direct experiment. Tables III and IV show that, while the liver slices form carbohydrate freely from these substances and to a closely similar extent in each case, the process is quite unaffected by the presence of insulin. In the experiments with lactate parallel determinations of the effect of insulin on carbohydrate formation by the tissue without lactate were carried out. It will be seen that the "insulin effect" appears both in the presence and in the absence of lactate, but that it exercises no appreciable influence on the extra carbohydrate synthesis due to lactate, showing that gluconeogenesis from some source is still depressed by insulin, though that from lactate remains unaffected.

Table III. *Effects of* dl-*lactate and* dl-*lactate plus insulin on carbohydrate synthesis*

Quantities: mg. fermentable carbohydrate per g. fresh liver

No. of exp.	Duration of exp. hours	Without insulin: Final	Without insulin: Final with lactate	Without insulin: Rise due to lactate	With insulin: Final	With insulin: Final with lactate	With insulin: Rise due to lactate
1	1½	1·91	3·54	1·63	0·90	2·45	1·55
2	2	1·70	5·97	4·27	—	4·73	—
3	2	3·64	6·66	3·02	3·37	6·37	3·00
4	2	7·59	10·35	2·76	6·79	10·31	3·52
Average of comparable Exps. 1, 3, 4		4·38		2·47	3·69		2·69

Note. Concentration of lactate: *M*/50. Exp. 1: 0·3 unit insulin per ml. Exps. 2 and 4: 1·7 units insulin per ml. Exp. 3: 0·9 unit insulin per ml.

Table IV. *Effects of pyruvate and pyruvate plus insulin on carbohydrate synthesis*

Quantities: mg. fermentable carbohydrate per g. fresh liver

Final plain	Additional increase with pyruvate	Additional increase with pyruvate and insulin
5·17	2·11	—
4·78	4·22	—
12·43	1·41	1·00
3·48	3·27	2·90
Average	2·75	

Note. Concentration of pyruvate: *M*/50. Insulin added: 3 units per ml. Duration of exp.: 2 hours in every case.

Table V shows that insulin affects neither the oxygen consumption nor the R.Q. of liver slices to which *dl*-lactate has been added. Another point of interest which appears is that with concentrations of lactate of the order of 0·18% no

Table V. *Effects of lactate and lactate plus insulin on respiration*

No. of exp.	Weight of rat	Duration of exp. hours	O_2 consumption per hour μl. per g. fresh liver: Without lactate	With lactate	With lactate plus insulin	R.Q.: Without lactate	With lactate	With lactate plus insulin
1	108	1½	—	1730	2265	—	0·595	0·667
2	(Rabbit)	1½	—	1670	1735	—	0·635	0·673
3	(Rabbit)	1½	—	1605	1620	—	0·716	0·627
4	143	2	1658	2650	—	0·573	0·657	—
5	143	2	1790	2680	—	0·483	0·630	—

Note. Exps. 1, 2 and 3: 0·18% *dl*-lactate (*M*/60). Exps. 4 and 5: 0·27% *dl*-lactate (*M*/30). Exp. 1: 0·3 unit insulin per ml. Exps. 2 and 3: 0·17 unit insulin per ml.

increase in oxygen consumption appears. It is only with higher concentrations than this that we were able to observe increases in oxygen consumption and R.Q. such as were observed by Meyerhof & Lohmann [1926].

Experiments with added pyruvate on the other hand (Table VI) regularly give an increase in oxygen consumption and a rise in R.Q., but the extent to which the R.Q. (though not the oxygen consumption) is raised depends upon the concentration of pyruvate added.

Table VI. *Effect of pyruvate on respiration*

Average values in μl. of O_2 per g. fresh tissue per hour

	Without pyruvate		With pyruvate	
	O_2 consumption	R.Q.	O_2 consumption	R.Q.
Pyruvate *M*/50 (4 exps.)	1969	0·505	3355	1·111
Pyruvate *M*/100 (4 exps.)	1949	0·535	3229	0·818

The next possibility which we explored was that insulin affected the gluconeogenesis from protein or amino-acids. As a first essay, we determined whether or not insulin affected the liberation of ammonia or production of urea in the slices. It was not, of course, certain that even if insulin did affect gluconeogenesis from this source, the fact would be revealed by these experiments, for it might well have been that insulin exercised its effect on the carbon moieties after deamination. Our attempt, however, proved a fortunate one. Table VII shows

Table VII. *Effect of insulin on urea formation*

	mg. urea-N per g. fresh liver						mg. ammonia-N per g. fresh liver		
Duration of exp. hours	Initial	Final plain	Increase without insulin	Final plain plus insulin	"Insulin effect" (final plain minus final plain plus insulin)	Insulin added, units per ml.	Initial	Final plain	Final plain plus insulin
2	0·117	0·422	**0·305**	0·309	**0·113**	1·0	0·157	0·174	0·167
2	0·130	0·415	**0·285**	0·156	**0·259**	0·5	0·128	0·07	0·053
2	0·366	0·543	**0·177**	0·492	**0·051**	0·7	0·061	0·077	0·049
2	0·355	0·474	**0·119**	0·442	**0·032**	1·0	0·073	0·032	0·028
3	—	0·327	—	0·088	**0·239**	0·5	—	0·06	0·090
2	0·307	0·507	**0·200**	0·111	**0·396**	0·7	0·112	0·126	0·189
2	0·196	0·560	**0·364**	0·260	**0·300**	0·5	0·077	0·081	0·065
3½	—	0·102	—	0·062	**0·040**	1·3	—	0·043	0·031
2¼	0·052	0·540	**0·488**	0·301	**0·239**	1·2	0·073	0·050	0·046
2½	—	0·267	—	0·314	**−0·047**	0·6	—	0·111	0·088
2¾	0·091	0·205	**0·114**	0·121	**0·084**	2·5	0·072	0·077	0·055
2	0·176	0·362	**0·186**	0·272	**0·090**	0·5	0·102	0·047	0·052
2	—	0·290	—	0·258	**0·032**	0·6	—	0·068	0·100
		Average	**0·248**		**0·140**				

that the urea production which occurs in the slices during shaking is cut down by insulin, although it is never completely abolished. The ammonia content of the tissue is always low and tends if anything to diminish when the tissue has been shaken in O_2/CO_2. There is therefore no question of insulin having affected the mechanism responsible for the synthesis of urea from ammonia. Insulin, apparently, prevents the deamination of part of the amino-acids, which otherwise would form carbohydrate.

The fact that there is only partial inhibition by insulin of urea production in liver slices seems to fit, at first sight, with the accepted fact that only a fraction of the amino-acids making up the protein molecule is capable of giving rise to carbohydrate.

From Table VII we see that insulin inhibits the appearance of 0·140 out of 0·248 mg. of urea-N per g. of fresh tissue in the experimental period; that is, of 56% of the urea-N appearing in the absence of insulin. On the basis of many experiments which have been performed on the intact animal, it is generally considered that about 58% of the protein molecule is capable of forming sugar.

Thus, in our experiments, insulin seems to prevent the conversion of protein into carbohydrate in about the proportion which, on other grounds, we should anticipate. Again, 0·140 mg. of urea-N (the amount of the observed "insulin effect" on urea-N) is equivalent to 0·86 mg. of protein (0·0140 × 6·12). Assuming the conversion of N into urea and S into sulphate, 0·86 mg. of protein, if wholly converted into carbohydrate, should yield 0·97 mg. of the latter substance.

Table I shows that the observed "insulin effect" on carbohydrate is 0·95 mg.

There is, however, an obvious difficulty when we try to picture exactly what is happening in the present instance. If insulin inhibits the deamination of amino-acids, why should it affect only those which are able to form carbohydrate? We may suppose that the structure of the insulin molecule is such that it is able to "cover" only certain amino-acids, or only those portions of the amino-acid molecule which are capable of forming sugar. It may be, on the other hand, that the ammonia which contributes to that fraction of the urea formation which is not sensitive to insulin is not derived from amino-groups of

Table VIII. *Effects of amino-acids and amino-acids plus insulin on carbohydrate synthesis*

Quantities: mg. fermentable carbohydrate per g. fresh liver.

The amino-acids used were optically inactive. All amino-acid concentrations 0·4%, except for the first three experiments with glutamate, in which it was 0·2%.

Duration of exp. hours	Initial	Final plain	Increase	Final amino-acid only	"Amino-acid effect" (final amino-acid minus final plain)	Final amino-acid plus insulin	"Insulin effect" (final amino-acid minus final amino-acid plus insulin)	Insulin added, units per ml.
Experiments with glutamate:								
3	2·21	2·775	0·565	3·82	1·045	—	—	—
4	0·865	1·980	1·115	2·78	0·800	—	—	—
2½	1·830	2·780	0·950	2·84	0·06	1·92	0·92	1·2
2½	1·64	4·69	3·05	5·12	0·43	4·44	0·68	1·0
2	2·90	4·48	1·58	4·97	0·49	4·74	0·23	1·3
		Average	1·45		0·56		0·61	
Experiments with aspartic acid:								
2	2·77	5·39	2·62	5·97	0·58	5·79	0·18	2·3
2	1·55	6·03	4·48	6·94	0·91	6·61	0·33	1·3
2	2·29	3·94	1·65	4·78	0·84	4·90	−0·12	2·0
Experiments with arginine:								
2	2·075	3·25	1·175	3·82	0·57	3·56	0·25	2·0
2	2·17	4·45	2·28	4·96	0·51	4·88	0·08	2·0
Experiments with glycine:								
2½	4·32	4·41	0·09	4·22	−0·19	3·83	0·39	1·6
2½	3·625	4·475	0·850	3·94	−0·535	3·70	0·24	2·4
3	2·85	4·62	1·77	3·94	−0·68	3·15	0·79	2·3
2½	2·45	4·42	1·97	3·4	−1·02	5·22	−1·82	1·7
2½	1·96	6·72	4·76	5·77	−0·95	—	—	—
Experiments with alanine:								
2	2·56	4·06	1·50	7·23	3·17	6·10	1·13	1·4
2	9·01	10·14	1·13	12·82	2·68	9·08	3·74	4·0
2	2·31	2·82	0·51	5·46	2·64	2·72	2·74	3·7
1¾	2·09	3·86	1·77	4·40	0·54	4·08	0·32	2·7
2	2·49	4·26	1·77	5·02	0·76	4·82	0·20	2·8
2	1·97	3·47	1·50	4·12	0·65	2·98	1·14	2·2
	Average for alanine		1·36		1·74		1·55	

amino-acids, but from nitrogenous groups attached to other compounds. These considerations naturally led us to investigate carbohydrate formation by liver slices in the presence of added amino-acids and the effect of insulin upon the process. The results have been interesting and unexpected: much more work must be done before a coherent picture can be obtained, or all the difficulties cleared up. Except, therefore, for the observations included in Table VIII, and for experiments with glycine, we have confined our attention to the problems presented by gluconeogenesis from alanine.

The results of our preliminary experiments are shown in Table VIII.

It will be seen that small increases in gluconeogenesis are found with *dl*-glutamic acid, *dl*-aspartic acid and *dl*-arginine, a considerably larger rise with *dl*-alanine and a definite decrease with glycine. All these amino-acids are reputed to give rise to carbohydrate when administered to the intact animal.

The results with glycine are particularly interesting. Whether or not glycine can be converted into carbohydrate in the animal body has been the subject of controversy [Lusk, 1908; Greenwald, 1918]; but there seems to be no doubt that when it is added to liver slices, so far from there being an increase, there is actually a decrease in carbohydrate synthesis.

It is very interesting that Reid [1936] has found that, when glycine is injected into the anaesthetized animal, there is a decrease, rather than an increase, of glycogen in the liver, although lactate, pyruvate and alanine all give rise to increases. Reid did not find increases of glycogen after injecting glutamic and aspartic acids, though we have found some increases in total carbohydrate with these substances. At present, we have no certain explanation to offer for these different effects. Glycine exerts a marked specific dynamic action in the intact organism, and it appears (Table IX) to increase the oxygen consumption of the

Table IX. *Effect of glycine on respiration*

Glycine 0·4% in all cases. Average values of five experiments

O_2 consumption per g. fresh tissue per hour	Without glycine	2231
	With glycine	3100
R.Q.	Without glycine	0·560
	With glycine	0·640

liver slices. It may be therefore that, in the special conditions both of Reid's experiments and of our own, the increased metabolism caused by glycine is sufficient to give rise to an actual decrease (by combustion) of any extra carbohydrate which might be formed. In favour of this explanation is the fact that glycine raises the R.Q. (Table IX).

Table X shows that, whilst alanine gives rise to an increased formation of urea by the slices which is inhibited by insulin, no extra urea is formed in the presence of glycine. Kisch [1936] finds that glycine is deaminated by kidney extracts and by other enzyme preparations much less readily than are other amino-acids. It is optically inactive, and behaves exceptionally in other ways.

Table XI gives the results of a number of experiments with the Dixon-Keilin apparatus upon the effects of added alanine and of alanine plus insulin on the respiratory metabolism. Like glycine, alanine gives rise to an increase in oxygen consumption, but unlike glycine, it does not raise the R.Q. Another point of interest arises here. It is reasonable to suppose that alanine, when deaminated, gives rise to pyruvic acid. Both alanine (0·2%, i.e. 0·0225M) and pyruvic acid (0·02M) give rise to carbohydrate, and both also give rise to extra oxygen consumption when added to slices of liver. But whilst pyruvate increases

Table X. *Effects of amino-acids and amino-acids plus insulin on urea formation*

mg. urea-N per g. fresh liver							mg. ammonia-N per g. fresh liver			
Initial	Final plain	Increase	Final amino-acid only	Final amino-acid plus insulin	"Amino-acid effect" (final amino-acid minus final plain)	"Insulin effect" (final amino-acid minus final amino-acid plus insulin)	Initial	Final plain	Final amino-acid	Final amino-acid plus insulin
Experiments with alanine:										
0·160	0·288	**0·128**	0·425	0·179	**0·137**	**0·246**	0·027	0·070	0·040	0·080
0·103	0·214	**0·111**	0·245	0·196	**0·031**	**0·049**	0·036	0·028	0·035	0·039
0·069	0·101	**0·032**	0·752	0·355	**0·651**	**0·397**	0·062	0·035	0·036	0·048
0·113	0·111	**−0·002**	0·121	0·284	**0·010**	**−0·163**	0·055	0·053	0·032	0·023
0·248	0·493	**0·245**	1·175	0·815	**0·682**	**0·360**	0·092	0·053	0·102	0·126
0·381	0·735	**0·354**	1·545	1·210	**0·810**	**0·335**	0·073	0·056	0·121	0·141
	Average	**0·144**			**0·387**	**0·204**				
Experiments with glycine:										
0·137	0·341	**0·234**	0·343	0·301	**0·002**	**0·042**	0·058	0·036	0·037	0·039
0·143	0·321	**0·178**	0·333	0·279	**0·012**	**0·054**	0·048	0·033	0·040	0·044

Note. Amino-acid concentration 0·4%. Insulin added: 2 units per ml. Duration of all exps.: 2 hours.

Table XI. *Effects of alanine and alanine plus insulin on respiration*

O_2 consumption per hour μl. per g. fresh liver			R.Q.			
Without alanine	With alanine	With alanine plus insulin	Without alanine	With alanine	With alanine plus insulin	Insulin added, units per ml.
2310	3300	—	0·508	0·542	—	—
2080	4500	—	0·488	0·597	—	—
1550	4460	—	0·573	0·520	—	—
1460	4160	—	0·647	0·562	—	—
—	2710	4130	—	0·448	0·498	3·2
—	3550	4155	—	0·459	0·419	3·2
—	2275	3000	—	0·620	0·420	3·0
—	1910	3050	—	0·589	0·379	3·0
—	2460	2480	—	0·507	0·515	3·0
2425	1800	1940	0·558	0·497	0·510	2·7
2450	2700	3750	0·517	0·503	0·376	4·0
2170	2865 2740	2495	0·542	0·506 0·445	0·465	3·0
1597	3060	3435 2500	0·602	0·542	0·526 0·445	2·7
Av. 2005	3035	3093	0·554	0·524	0·455	

Duration of all exps.: 2 hours.

the R.Q. (Table VI) alanine does not do so, although the increases in O_2 consumption are of the same order in both cases.

Calculating on the basis of the observed effect of insulin on urea production, the effect of insulin on the R.Q. and oxygen consumption of slices to which alanine was added would amount to about 350μl. of O_2 per g., provided that in the absence of insulin a correspondingly greater number of alanine molecules would have undergone complete combustion; or, if oxidative deamination were the only process concerned, the effect of insulin would have been to diminish oxygen consumption by about 89μl. per g. No diminution in oxygen consumption was detected; moreover, either change should have tended to raise the

R.Q., whereas, in fact, it is lowered. Calculating from the extra urea-N formed in the presence of alanine (0·420 mg. per g. in 2 hours, Table X), the extra oxygen consumption per hour should be in the neighbourhood of 1000 μl., which is near the value found. But there should also be a rise in R.Q., which we did not find.

The results shown in Tables VIII and X represent experiments performed each on a separate animal. The "insulin effects", therefore, on urea-N and on carbohydrate respectively, cannot be expected to correspond closely in individual experiments. If the prevention of the appearance of two atoms of urea-N represents the suppression by insulin of the formation of a molecule of carbohydrate, the ratio

$$\frac{\text{Insulin effect on carbohydrate}}{\text{Insulin effect on urea-N}}$$

should be $\frac{180}{28}$ or 6·43. The ratio of the two average figures from these tables is $\frac{1{\cdot}54}{0{\cdot}204}$ or 7·5. In an attempt to confirm this and to eliminate possible errors caused by using different animals, a number of experiments were made, in which the "insulin effects" on carbohydrate and urea-N respectively were determined by parallel experiments on slices obtained from the same liver. The results are shown in Table XII.

Table XII. *Simultaneous determinations of the effects of alanine and alanine plus insulin on urea and carbohydrate synthesis carried out on samples of the same liver*

Quantities: mg. fermentable carbohydrate and urea-N respectively per g. fresh liver

Duration of exp. hours	Final alanine only		Final alanine plus insulin		"Insulin effect" (final alanine minus final alanine plus insulin)		Ammonia-N per g. fresh liver	
	Carbohydrate	Urea-N	Carbohydrate	Urea-N	Carbohydrate	Urea-N	Final plain plus alanine	Final plain plus alanine plus insulin
2	5·68	0·163	4·07	0·015	**1·61**	**0·148**	0·076	—
2	5·48	0·735	4·86	0·534	**0·62**	**0·201**	0·058	0·072
2½	8·35	0·214	7·64	0·114	**0·71**	**0·100**	0·121	0·049
3	5·17	0·696	4·57	0·594	**0·60**	**0·102**	0·045	0·078
2	5·27	0·294	4·27	0·076	**1·00**	**0·218**	0·076	0·060
				Average	**0·91**	**0·154**		

Note. Alanine concentration: 0·2%. Insulin added: 3 units per ml.

The ratio obtained here is $\frac{0{\cdot}91}{0{\cdot}154} = 5{\cdot}9$. If, on the other hand, we compare the averages from Tables I and VII, we see that the figures are $\frac{0{\cdot}980}{0{\cdot}140}$, giving a ratio of 7·0.

This degree of agreement between the ratio found experimentally and the theoretical ratio is perhaps a fortunate accident: we would claim only that the observed figures are of the order which might be expected on the supposition that, for every molecule of alanine which was prevented by insulin from forming carbohydrate, one atom of nitrogen was also prevented from forming urea. The fact that a closely similar ratio is found when we consider the insulin effects on carbohydrate and urea-N in the absence of added amino-acids may mean no more than that, in general, three carbon atoms only of amino-acids can form carbohydrate, while the removal of only one of the amino-groups (in the case of amino-acids which contain more than one) is influenced by insulin. But these results make us confident that insulin does depress gluconeogenesis from amino-acids, and not that from other sources.

There is an extensive literature dealing with the formation of carbohydrate in the animal body from all the substances, the effects of which we have examined upon carbohydrate synthesis in liver slices. Reference may be made to the following among many papers: alanine to carbohydrate [Dakin & Dudley, 1914]; pyruvic acid to carbohydrate [Dakin & Janney, 1913]; lactic acid to carbohydrate [Parnas & Baer, 1912]. Ringer & Lusk [1910] showed that "extra glucose" is formed when *dl*-alanine, aspartic acid and glutamic acid are administered to the phloridzinized animal. Dakin & Janney [1913] suggest that pyruvic acid has probably to be converted into lactic acid before it can form carbohydrate.

Discussion

The experiments which we have described fall into line with the current conception that one of the important effects of insulin is to diminish the new formation of carbohydrate in the liver from non-carbohydrate substances, and they show that among a number of the substances which are concerned in gluconeogenesis and which we have tested, this effect is confined to amino-acids. It remains to be considered how far the effects which we have observed can be supposed to account, on quantitative lines, for the action of insulin on the intact animal. In the first place, the use of isolated tissue slices, valuable as it is as a means of delimiting the problem—for it enables one to deal with the metabolism of one organ only, and ensures that the products of metabolism cannot escape—obviously introduces complicating factors. The condition of a liver lobule in a tissue slice must be very different from that of a lobule with its elaborate arrangements intact for the supply of portal and hepatic blood.

If the process of gluconeogenesis is controlled on the one hand by insulin, the effect of insulin is, in the intact animal, almost certainly balanced by that of other hormones. As a matter of fact, we have evidence that adrenaline at least, as well as insulin, affects the process *in vitro*. The actions of this and other hormones will form the subject of a further paper.

Another relevant point is that we have found it necessary (as we pointed out earlier in this paper) always to use starved animals. But starvation alone alters the R.Q. of the isolated tissue from about 0·78 to about 0·60: it obviously modifies the whole metabolic balance of the organ, presumably by greatly increasing the amount of fat metabolized. Now Bainbridge [1925] has pointed out that fat feeding renders animals more resistant to the action of insulin. In this connexion the low R.Q. which we obtain, and the fact that there is always a considerable residuum of carbohydrate formation which is not affected by insulin, seem to us to be significant.

Another difficulty in the quantitative interpretation of our data lies in the fact that carbohydrate combustion no doubt goes on at the same time as gluconeogenesis, and to an extent which it is difficult to assess. The increase in carbohydrate which we observe must represent the balance between these two processes and our only guide as to the extent of carbohydrate combustion is the amount of extra oxygen used, and the alteration, if any, in the R.Q. The addition of alanine increases the oxygen consumption, but lowers the R.Q. For this result we have at present no satisfactory explanation.

In the case of glycine, the increased oxygen consumption is equally marked and we have been unable to show any increased production of urea. The R.Q., on the other hand, is distinctly raised, presumably indicating an increased combustion of carbohydrate. Here we can at present only invoke "specific dynamic action" to explain our results.

If we examine the data of Reid [1936] we see that intravenous infusion of alanine into the anaesthetized cat gives rise to a deposition of 1·5 mg. of glycogen per g. of liver in 3 hours. Presumably the machinery both of synthesis and of oxidation works at higher speed in the rat. All that we can say therefore is that his figures and ours are of the same order of magnitude.

In considering how far our results can explain the action of insulin on the animal as a whole, it must also be borne in mind not only that deamination of amino-acids occurs in tissues other than the liver, but that the liver is not even the tissue which, weight for weight, deaminates most rapidly. The effect of insulin on kidney deaminase is at present being investigated in this department. If this mechanism too should prove to be insulin-sensitive the point will be one of great interest.

Summary

1. Slices of liver of starved rats shaken *in vitro* form carbohydrate from non-carbohydrate sources. The following substances, added to the slices increase gluconeogenesis; lactate, pyruvate, alanine, aspartic acid, glutamic acid, arginine. Glycine gives rise to no increase and indeed actually causes some diminution.

2. The carbohydrate synthesis is partially (about 56 %) inhibited by insulin *in vitro*. Insulin simultaneously inhibits partially the formation of urea (by preventing deamination) in the slices. Urea formation is increased in the presence of alanine and this increase is inhibited by insulin. There is quantitative agreement between the effects of insulin on the formation of carbohydrate and of urea-N. It is concluded that insulin acts, at least in these experiments, by inhibiting synthesis of carbohydrate from amino-acids.

3. Insulin has no effect on carbohydrate synthesis from lactate or pyruvate. Its effect is therefore probably on the process of deamination.

4. It slightly lowers the oxygen consumption of the tissue slices in the absence of added substrate. It does not affect the respiratory metabolism in the presence of lactate.

5. Alanine and glycine, as well as pyruvate and lactate, give rise to increased oxygen consumption. Insulin lowers the R.Q. in the presence of added alanine.

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