LXI. A COMPARISON OF THE EFFECTS OF GLUCOSE AND DIHYDROXYACETONE ON METABOLISM.

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THE question as to whether dihydroxyacetone is or is not directly oxidised in the animal body is one which is being keenly debated. Campbell and Markowitz [1927], working with depancreatized dogs, found that the triose administered to such animals is excreted quantitatively as glucose and is not metabolised in the absence of insulin. Insulin, however, is not necessary for its conversion to glucose. Further, in these experiments the R.Q. was not raised and ketosis was not prevented even when insulin was administered. These authors conclude that there is no evidence that dihydroxyacetone is an intermediary compound in the metabolism of glucose.

Embden, Schmidt and Wittenberg [1913] found that glucose is formed when the surviving liver of the dog is perfused with blood containing dihydroxyacetone. It has also been shown that this substance can give rise to glycogen. Markowitz and Campbell [1926] conclude that it must be transformed to glucose in the liver before it can be metabolised. It has further been shown that it can overcome the hypoglycaemia following an excessive dose of insulin [Campbell and Hepburn, 1926; Kermack, Lambie and Slater, 1926]. The fact, however, that this triose brings about, both in animals and man, a much more rapid and much greater rise in the respiratory metabolism than an equivalent dose of glucose, made it probable that it can be, at least in part, directly oxidised without previous transformation into glucose [Mason, 1926; Lambie and Redhead, 1927].

Cathcart and Markowitz [1927, 1] compared the effects of glucose, sucrose, laevulose, galactose, maltose, lactose and dihydroxyacetone on the respiratory metabolism in man, and found, in agreement with Higgins [1916] and others, that laevulose, galactose and sucrose cause a sharper and higher rise in R.Q. than glucose. They also confirm Mason's findings with dihydroxyacetone that the R.Q. following this substance exceeds unity. They then argue that, since dihydroxyacetone cannot be used directly but must first be transformed to glucose, these results cannot be interpreted as indicating preferential oxidation. They suggest that the conception of the respiratory quotient as a combustion quotient must be revised and that it represents rather the algebraic sum of

the transformations carbohydrate $\rightleftharpoons$ fat plus the oxidation of carbohydrate for energy purposes. The direction of the reaction carbohydrate $\rightleftharpoons$ fat is conceived as dependent on the "tension" of carbohydrate in the liver; thus, when this tension is so high that gluco-neogenesis from fat and protein is no longer necessary, one obtains a respiratory quotient greater than unity and fat is formed from the sugar.

It is not, however, at all obvious why certain of the sugars normally foreign to the blood should give us a high carbohydrate tension and hence a R.Q. greater than unity, whilst glucose does not do this. Cathcart and Markowitz suggest that when dealing with sugars foreign to the organism the liver rapidly removes them from the circulation and so brings about a great rise in carbohydrate tension.

As judged, however, by glycogen determinations in the liver and muscles of animals following the administration of the various sugars, one is led to conclude that glucose is the sugar *par excellence* for increasing carbohydrate tension. To take the case of glucose and galactose, the administration of 50 g. of these sugars to rabbits weighing 2 kg. will, in the case of glucose, produce about 10 % of glycogen in the liver, whilst in that of galactose only about 2.5 % is obtained, 4 or 5 g. of galactose being found in the urine.

Further, glucose is certainly the best of all sugars for combating ketosis induced by whatever means, and it seems fairly certain that this antiketogenic action is due to a deflection of metabolism from a preponderance of fat to a preponderance of carbohydrate. Thus the effectiveness of a sugar as an antiketogenic agent would be proportional to the carbohydrate tension which it could bring about in the liver and tissues. On Cathcart's view we would expect the best antiketogenic sugars to be those which produce the greatest rise in R.Q., but in point of fact this is not the case, galactose, sucrose and laevulose being less effective in this respect than glucose, in spite of the fact that the former sugars all give higher R.Q.s than the latter. We shall see that dihydroxyacetone also is not very effective in the matter of antiketogenesis.

In the case of dihydroxyacetone the rise in R.Q. is so greatly over unity that it becomes necessary to suppose a considerable synthesis of fat to account for it.

Now it seemed to us that a method of testing certain aspects of the problem was presented by the effect of the administration of the triose to the starving human subject. In such a subject, after a period of about 2 days without food, a condition is attained in which the carbohydrate tension of Cathcart and Markowitz [1927, 2] is considerably lowered and the organism is thrown back on to its protein and fat for its carbohydrate and energy. The occurrence of a ketosis in these conditions indicates the preponderance of what may be called the fat type of metabolism [Raper and Smith, 1926] and the administration of any substance throwing extra fat on the metabolism is accompanied by an increase in the excretion of the ketonic acids. This is well shown in the following experiment.

Exp. 1. The writer was the subject of experiment. After 37 hours of complete starvation 50 g. of melted butter were ingested. The urine was collected at the times given in the table and the ketonic acids estimated by a modification of the Shaffer-Marriot method.

Time	Urine cc.	Acetoacetic acid as mg. acetone		β -Hydroxybutyric acid mg.	
		Total	Per hr.	Total	Per hr.
8.0 a.m.	270	17.2	1.4	45.2	3.8
9.0	Butter taken				
10.0	25	7.6	7.6	10.4	10.4
11.15	22	8.0	6.4	8.3	6.6
12.00	16	6.5	8.6	7.1	9.5
1.0 p.m.	22	8.7	8.7	8.1	8.1
2.10	23	11.4	9.8	14.8	12.7
3.30	24	13.8	10.3	18.4	13.8
4.30	18	12.7	12.7	19.5	19.5
6.0	31	24.1	16.1	40.9	27.3
6.30	15	10.5	21.0	28.2	56.4

These figures leave no doubt at all that at least the fatty acids found in butter readily bring about a large increase in the excretion of ketones in these conditions. Now in the matter of dihydroxyacetone it was argued that if it were all converted to glucose before being metabolised it should be at least as effective as glucose as an antiketogenic agent. If, on the other hand, it is to any great extent converted to fat, it should be demonstrable as a ketogenic substance: and, lastly, if both oxidation and transformation to fat are proceeding, its effect should be intermediate between ketogenesis and ketolysis.

In a previous communication [Goldblatt, 1925] it was shown that, in the case of the writer, after a period of about 40 hours' complete starvation 50 g. of glucose readjusted the keto-antiketogenic balance in 1 hour and no further excretion of ketonic acids occurred for $3\frac{1}{2}$ hours more. It was further demonstrated that the ketosis was accompanied by a marked loss of tolerance to sugar, high blood-sugar curves and glycosuria occurring after doses of glucose normally easily tolerated. It was also found that the R.Q. in these conditions rose only very slightly compared to the findings in the usual post-absorptive state.

In the following experiments the effects of dihydroxyacetone are compared with those of glucose in similar conditions. The respiratory metabolism was determined by the Douglas-Haldane method after a period of complete rest of about 1 hour. The blood-sugar was estimated by MacLean's method. With regard to the effect of any circulating dihydroxyacetone on the blood-sugar as estimated by this method, it is rather surprising that the effect is in fact negligible, in spite of its powerful reducing properties. If, instead of using the usual 0.2 cc. of blood, one employs 0.2 cc. of a solution containing 0.1 % of dihydroxyacetone, the difference in the thiosulphate values of the alkaline copper solution before and after reduction does not exceed 0.10 cc. *N*/400. This seems to be due to the degree of alkalinity of the copper reagent, for considerably greater reduction values are obtained if a few drops of strong alkali are added.

The writer was the subject of experiment, except where otherwise stated.

Exp. 2. The effect of 50 g. glucose 16 hours after food. The sugar was taken immediately after the first R.Q.

Time mins.	Blood- sugar g. %	O ₂ cc. per min.	CO ₂ cc. per min.	Rise in O ₂ intake %	R.Q.
0	0.102	177	135	—	0.76
30	0.150	189	155	6.8	0.82
60	0.113	187	164	5.6	0.88
90	0.091	176	159	—	0.90
120	0.075	149	120	—	0.81

Exp. 3. The effect of 50 g. glucose 20 hours after food.

0	0.081	212	167	—	0.79
30	0.146	220	169	3.8	0.77
60	0.156	226	185	6.6	0.82
90	0.122	210	176	—	0.84
120	0.100	184	159	—	0.86

Exp. 4. The effect of 50 g. glucose 41 hours after food.

Time mins.	Blood- sugar g. %	O ₂ cc. per min.	CO ₂ cc. per min.	Rise in O ₂ intake %	R.Q.	Acetoacetic acid in urine as mg. acetone per hr.
0	0.113	241	176	—	0.73	5.7
25	0.104	238	176	—	0.74	6.1
40	0.184	—	—	—	—	—
55	0.189	264	198	9.5	0.75	5.6
70	0.187	—	—	—	—	0.0
85	0.189	256	207	6.2	0.81	0
105	0.141	—	—	—	—	0
215	0.099	—	—	—	—	0
265	—	—	—	—	—	2.3

Exp. 5. The effect of 50 g. dihydroxyacetone 15 hours after food.

Time mins.	Blood- sugar g. %	O ₂ cc. per min.	CO ₂ cc. per min.	Rise in O ₂ intake %	R.Q.
0	0.100	197	170	—	0.86
30	0.097	234	292	18.8	1.25
60	0.067	221	195	12.2	0.88
90	0.081	230	182	16.8	0.79
120	0.095	231	164	17.3	0.71
150	0.077	—	—	—	—

Exp. 6. The effect of 50 g. dihydroxyacetone 40 hours after food.

Time mins.	Blood- sugar g. %	O ₂ cc. per min.	CO ₂ cc. per min.	Rise in O ₂ intake %	R.Q.	Acetone bodies in urine as mg. acetone per hour	
						Aceto- acetic acid	β-Hydroxy- butyric acid
0	0.072	208	160	—	0.77	13.14	9.72
15	0.072	—	—	—	—	25.96	6.04
25	—	—	—	—	—	—	—
35	0.095	275	249	32.2	0.903	23.11	3.16
45	—	—	—	—	—	—	—
55	—	—	—	—	—	9.60	7.74
60	0.095	253	222	21.6	0.88	—	—
87	—	—	—	—	—	—	—
95	0.095	228	162	9.6	0.71	1.56	2.27
115	—	—	—	—	—	—	—
125	0.077	223	164	7.2	0.74	2.41	6.84
150	0.056	—	—	—	—	3.90	3.90
215	—	—	—	—	—	3.56	2.58

Exps. 2, 3 and 4 show the diminishing tolerance to glucose which occurs as the starvation period is increased. The R.Q. shows definitely less rise, following the administration of the sugar, the longer the starvation, the maxima being 0.90 in Exp. 2, 0.86 in Exp. 3 and 0.81 in Exp. 4. The maximum rises in the oxygen intake in the successive cases were 6.8 %, 6.6 % and 9.5 %. Thus we cannot suppose that the lower R.Q.s found after starvation are to be interpreted as indicating diminished oxidation. The truth seems to be rather that the relative depletion of glycogen in the liver and the muscles brings about the necessity to attack administered carbohydrates as quickly as possible for energy purposes; so that the R.Q. in this case, on Cathcart's view given above, does not rise as high as ordinarily through the masking effect of simultaneous gluco-neogenesis from fat or protein or both. With such considerable increases in oxygen absorption as, for example, occur in Exp. 4 there is no question that oxidation has increased, so that if the R.Q., as at present usually interpreted, does not indicate increased oxidation, we can only conclude that the interpretation must be modified on some such lines as those laid down by Cathcart.

The high and prolonged blood-sugar curve obtained after starvation must mean that an effort is being made to satisfy the needs of the muscles for carbohydrate. As the transference of blood-sugar from the liver to the tissues is progressing, oxidation is going on simultaneously, a large increase in oxygen consumption occurring at once, but the maximum rise in R.Q. occurring when gluco-neogenesis is at a minimum. Thus we expect high R.Q.s during the fall in blood-sugar when the tissues are supplied with an effective head or tension of carbohydrate. This is in fact what occurs, as was found by Bornstein and Holm [1922] and Goldblatt [1925].

If this analysis of our findings in starvation is correct then the main disturbance in carbohydrate metabolism in that condition is a diminished power of storage in the liver, a condition which is determined by the carbohydrate depletion of the muscles. It seems, further, probable that when the carbohydrate tension of the tissues is lowered, there is a compensatory diminished activity on the part of the pancreas. Thus when highly fatty diets are fed, sufficient to produce ketosis, abnormally high and delayed blood-sugar curves are obtained [Kohn, Fries and Felshin, 1927]. On the above view, such diets would lead to a struggle for carbohydrate on the part of the muscles with a compensatory diminished secretion of insulin, thus delaying the fall in blood-sugar after a dose of glucose, until such time as the tissue-sugar were re-established.

Recent work by Grafe and Meythaler [1927] and Dietrich [1927] makes it very probable that glucose acts as a direct stimulant to insulin secretion, which would corroborate the view that after a period of starvation the response of the islet mechanism is more sluggish than under ordinary post-absorptive conditions.

We must now examine Exps. 5 and 6 to see if any similar results are obtained with dihydroxyacetone.

In Exp. 5, 15 hours after food, 50 g. of the triose produced hypoglycaemia in 1 hour, then a rise occurred in the blood-sugar and 2½ hours after the solution was taken there was again a tendency to hypoglycaemia. At no time did there occur a rise above the fasting blood-sugar level. These findings confirm those of Cathcart and Markowitz [1927, 2] and others.

This sort of effect can be obtained with less than 50 g. and in our experience much more rapidly if the triose be taken about 5 hours after a meal. The following figures give the blood-sugar findings, in the case of the writer, following 30 g. of dihydroxyacetone taken 5 hours after food.

Time p.m.	2.00	2.10	2.25	2.40	3.10	3.50
Blood-sugar g. %	0.097	Triose taken	0.109	0.067	0.083	0.104

If we regard this rapid removal of sugar from the blood as the result of a stimulus to the insulin-secreting mechanism, then it appears that even after 15 hours' fasting this stimulus has become less effective. If now we examine the blood-sugar values in Exp. 6, it is seen that for 120 minutes they are consistently above the fasting level and hypoglycaemia only occurs 150 minutes after the triose was taken. Here, then, there is a definite loss in the rapidity of response to the stimulus which dihydroxyacetone undoubtedly exerts on the mechanism which removes sugar from the blood.

In addition to this the R.Q. in Exp. 6 rose to 0.903, whilst in Exp. 5 it reached 1.25. In each case a large increase in oxygen utilisation occurred, in Exp. 5 amounting to a maximum of 18.8 % and in Exp. 6 to 32.2 %. It is thus again made clear that the starving organism shows much less tendency to form fat, at least as judged by the R.Q. But what is the function of the large increase in oxygen intake? There is no question of overbreathing, for this would become more manifest in the starving condition, when the subject is already somewhat acidosed, whereas the R.Q. is much lower in this case. Whatever interpretation be adopted for the R.Q. an increased intake of oxygen can only mean increased oxidation or conversion of fat or protein to carbohydrate. It is true that this increased oxygen intake is not of long duration, but this must, it seems to us, be regarded as due to a readjustment to the metabolism of a different proportion of fat, protein and sugar with the same oxygen need as was necessary in basal conditions, gluco-neogenesis being at a minimum, whilst carbohydrate is both being oxidised and changed to fat. The fact that we can never account for more than a small fraction of administered carbohydrate by the rise in oxygen consumption must mean that other reactions are occurring which mask the oxidation of the sugar.

This difficulty in interpreting the significance of the large though temporary increase in oxygen utilisation is occasionally absent in certain pathological cases which give R.Q.s exceeding unity even with glucose. In these cases there occurs a steady fall in oxygen intake clearly shown in the following two examples. In each case the analysis was carried out 15 hours after food, the dose of glucose was 50 g. given in 100 cc. water.

Subject <i>M.</i> Eunuchoid.				
Time hrs.	Blood-sugar g. %	R.Q.	O ₂ cc. per min.	CO ₂ cc. per min.
0	0.072	0.88	259	228
$\frac{1}{2}$	0.100	1.01	240	242
1	0.104	1.02	212	217
$1\frac{1}{2}$	0.113	0.99	204	202
2	0.081	0.95	249	236
Subject <i>C.</i> Cretin.				
0	0.087	0.90	86	78
$\frac{1}{2}$	0.085	1.00	82	82
1	0.133	1.07	78	80
$1\frac{1}{2}$	0.125	1.16	79	91
2	0.099	1.16	77	89

In such cases it must be supposed that the excess carbon dioxide is produced from the change of carbohydrate to fatty acid. In the case of dihydroxyacetone, however, we must at present regard the very high R.Q. associated with a large rise in oxygen consumption as pointing to powerful oxidation going on simultaneously with the formation of fat. The rapidity with which the oxygen absorption takes place points to direct oxidation in spite of the mass of evidence against it. The findings of Lambie and Redhead [1927] indicate that there occurs a rapid rise in the blood-dihydroxyacetone and, in the normal subject, a fall to low values in about an hour, corresponding approximately to the rise and fall in the oxygen used. It seems to us difficult to escape from the view that direct oxidation can go on at least to some extent, whilst simultaneously glycogen is being formed.

We must now consider the effect of dihydroxyacetone on the ketosis in Exp. 6 as compared with the effect of glucose in Exp. 4. In Exp. 4 the ketosis disappeared in about 1 hour 10 minutes after the sugar was taken and only the faintest traces of acetoacetic acid could be found during the succeeding $3\frac{1}{2}$ hours, when the excretion of ketonic acids began definitely to increase. This is a finding which has been made on many occasions.

With the dihydroxyacetone the results were quite different. First of all, there was no period throughout the whole experiment in which acetoacetic acid and β -hydroxybutyric acid could not be easily estimated. There was, indeed, a marked fall, which began about $1\frac{1}{2}$ hours after the triose was taken and lasted for $\frac{1}{2}$ hour, when the excretion per unit time began to increase again. This result is so definitely different from anything we have ever obtained under similar conditions with glucose, that we are forced to the conclusion that dihydroxyacetone in these conditions forms glycogen much less readily than glucose or that it is forming fat at the same time.

Again comparing Exps. 4 and 6 we note that in the case of glucose the disappearance of the ketones is simultaneous with the increase in oxygen intake, whilst in the case of dihydroxyacetone the rise in oxygen took place about an hour before the marked fall in ketosis. As there appears no reason to doubt that effective oxidation of glucose leads to antiketogenesis or ketolysis very rapidly, we suggest that the initial rapid rise in oxygen absorption in Exp. 6 was not for the purpose of oxidation of glucose. Later on, as glycogen was formed, antiketogenesis became obvious, although at no time was there

sufficient to establish complete keto-antiketogenic equilibrium; which may in part be due to the formation of fat. The fact that during the half-hour or so after the dihydroxyacetone was taken there occurred a marked rise in the ketone substances would make this latter assumption not improbable, since, as we saw in Exp. 1, this is what occurs when fat is fed in similar conditions.

Our results, then, suggest that dihydroxyacetone forms glycogen, at least in the starving organism, much less readily than glucose does and is less effective as an antiketogenic substance. Further, our findings do not lend themselves to the conception that this triose is an intermediate stage in the breakdown of the glucose molecule.

Preferential oxidation is always very difficult to demonstrate directly and interpretations of experiments on the respiratory metabolism are beset with difficulties. However obvious it may seem that direct oxidation is taking place, it is nearly always possible to give alternative explanations of any changes in R.Q. or oxygen absorption which may occur. In reporting the following three experiments on a case of extreme non-diabetic glycosuria of the obese type, we are sensible of the danger in interpreting the data on lines of preferential oxidation, but we suggest that the results are at least suggestive of it. When dealing with the sugars, most of which give rise to glycogen, which in its turn can only give rise to glucose on hydrolysis, the idea is naturally developed that glycogen is a necessary intermediate step before any of them can be metabolised. It seems probable that ultimately all the sugars are utilised by way of glycogen, but it is difficult to avoid the conclusion that some of them may be oxidised directly.

In the following experiments the conditions were similar to those usually employed in estimating the effects of sugars on the respiratory metabolism, viz. 15 hours without food, 1 hour complete rest, estimation of the fasting metabolism and then administration of the sugar.

Subject V. Age 50. Weight 15 st. 10 lbs. Obese glycosuric. No ketosis at any time.

50 g. glucose.					
Time mins.	Blood-sugar g. %	R.Q.	O ₂ cc. per min.	CO ₂ cc. per min.	Urinary sugar as g. glucose
0	0.336	0.84	258	216	24.46 in 150 mins.
30	0.327	0.82	255	209	
60	0.396	0.79	255	203	
93	0.400	0.75	275	205	
125	0.376	0.75	270	203	
150	0.312	—	—	—	
50 g. laevulose.					
0	0.268	0.87	297	259	19.92 in 270 mins.
40	0.286	0.89	316	281	
68	0.291	0.95	285	271	
106	0.304	0.94	297	286	
139	0.286	0.81	292	237	
178	0.271	0.83	291	240	
50 g. galactose.					
0	0.289	0.75	283	213	19.08 in 260 mins.
35	0.336	0.78	307	239	
65	0.375	0.98	276	271	
100	0.341	0.91	269	245	
135	0.310	0.77	273	210	

In such a case as this the suggestion made by Cathcart and Markowitz [1927, 2], that the higher R.Q.s obtained after laevulose and galactose may bear some relationship to the rapid removal of sugar from the circulation, does not apply, for here we have an extreme deficiency in the power to store carbohydrate in the liver. So much is this the case that this type of patient shows a remarkable resistance to insulin even in the fasting condition. This being so, it seems to us likely that the higher R.Q.s obtained after laevulose and galactose are true indices of at least some preferential oxidation. There seems no reason to suppose that these sugars have led to a diminished gluco-neogenesis to a greater extent than glucose and even if this be insisted upon it would still be something in the nature of a preferential metabolism.

In view of the fact that dihydroxyacetone brings about a marked hypoglycaemia in man, it was of interest to see if a similar effect could be demonstrated in the rabbit. The following results were obtained in two rabbits which have been frequently used for experiments with glucose, and the blood-sugar curves of which have often been determined and found of the same nature. The figures were obtained in each case after 24 hours without food.

Rabbit 1. 2.5 kg.			
4 g. glucose in 20 cc. water, <i>per os</i>		4 g. dihydroxyacetone in 20 cc. water <i>per os</i>	
Time mins.	Blood-sugar g. %	Time mins.	Blood-sugar g. %
0	0.109	0	0.111
30	0.162	20	0.129
60	0.137	35	0.115
90	0.106	65	0.175
120	0.109	95	0.156
		125	0.135
		155	0.146
		185	0.133
		215	0.129
		245	0.117
Rabbit 2. 2.7 kg.			
0	0.104	0	0.117
30	0.166	30	0.122
60	0.129	60	0.143
90	0.113	90	0.135
120	0.113	120	0.131
		150	0.110
		180	0.131

It would thus appear that in the rabbit there does not occur the same hypoglycaemic sequence of blood-sugar values as occurs in man. Further experiments are necessary before any interpretation can be offered.

SUMMARY.

1. The metabolic effects of glucose and dihydroxyacetone in the normal and starving human subject are compared. The much greater oxygen intake and higher R.Q.s following the administration of dihydroxyacetone as compared with glucose, found by other observers, are confirmed.

2. Dihydroxyacetone is not an effective antiketogenic substance. Although it produces a definite diminution in the ketosis of starvation, its power in this direction is much less than that of glucose. It is pointed out that such anti-ketogenesis as it does exert does not occur simultaneously with the maximum rise in oxygen intake, which is the case with glucose.

3. The question of preferential oxidation is discussed.

4. The mechanism of the production of carbohydrate intolerance in starvation is discussed.

5. The hypoglycaemia which occurs in man after administration of dihydroxyacetone does not occur in the rabbit.

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