CXVIII. THE METABOLISM OF CARBOHYDRATE AFTER STARVATION.

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In a recent paper Dann and Chambers [1930] have reopened the question of the intolerance to carbohydrate which follows a period of starvation. The phenomenon has been examined by one of us [Goldblatt, 1925] and it was found that, after a period of some 40 hours' complete starvation in human subjects, administration of a dose of glucose, normally easily tolerated, was followed by high and delayed blood-sugar curves, glycosuria and a rise in R.Q. very much less than that occurring in ordinary post-absorptive conditions. The possible causes of this have been discussed by various authors. Thus, Brailsford Robertson [1923] suggested that the period of starvation was associated with a diminished secretion of insulin, so that the intensive administration of glucose found the mechanism for control of blood-sugar temporarily ineffective. Other workers have taken the view that it is due to the appearance of ketosis or to the tendency to acidosis. The fact, however, that the phenomenon is easily demonstrable in animals which do not develop a ketosis during starvation seems to rule out effectively its attribution to this cause. To show that the tendency to acidosis might be responsible, attempts have been made to readjust the disturbed mechanism by the administration of alkalis.

Dann and Chambers [1930], using dogs starved for about 3 weeks, have found well marked intolerance to glucose as manifested by a failure of the R.Q. to rise after the administration of sugar by more than 0.06. On the day following such an experiment a further test with glucose produced marked rises in R.Q. These results are similar to those found by one of us in human subjects, when it was shown that the administration of 50 g. of sugar readjusted the disturbed mechanism so that a subsequent dose gave a normal tolerance curve. Dann and Chambers have further made the interesting observation that complete readjustment of carbohydrate metabolism cannot be obtained even when 20 units of insulin are injected in addition to the ingestion of glucose. They conclude therefore that starvation-intolerance to carbohydrate may be associated with some as yet unknown change in the tissues, following upon disuse of the sugar-oxidising mechanism. In one of their experiments they found that after a 19-day fast, ingestion of 50 g.

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glucose and injection of 20 units insulin, the blood-sugar $4\frac{1}{2}$ hours later was 0.220 %, whilst on the next day in the same animal the blood-sugar in similar circumstances was 0.116 %, and on the succeeding day 0.087 %. They show clearly that the insulin response became progressively better as the metabolism of the animal was readjusted by the carbohydrate. *Pari passu* with these findings the utilisation of sugar as calculated from respiratory data increased steadily, the values being 2.13, 4.34, and 4.50 g. glucose per hour. Although these authors made no determinations of alkali reserve of the blood, there can be no doubt that their animals were in a state of more or less compensated acidosis. The relative ineffectiveness of the insulin injection may be referable to this acidosis and is comparable with the clinical finding that severely acidotic diabetic subjects are resistant to the action of the hormone [Koehler, 1926]. It has further been found by several workers that administration of alkali to such severely acidotic subjects brings about a better response to insulin. It is therefore not necessary, without further knowledge, to attribute this failure of the starved dog to respond adequately to insulin to specific tissue changes, changes in tissue reaction being perhaps sufficient to account for this observation.

Using dogs which had been starved for from 3 to 7 days, Hines *et al.* [1929] found that intravenous injection of 4 g. glucose per kg. per hour produced blood-sugar values and glycosuria which indicated diminished tolerance, but the R.Q. was high and the heat production rose by 50 %. These results are entirely out of harmony with those of Dann and Chambers [1930] and of Goldblatt [1925]. It seems improbable that the discrepancies can be explained by the shorter period of starvation of the dogs since definite intolerance had been established, unless we suppose that it is possible to dissociate the glycogen-storing power of the liver from the oxidising function in the peripheral tissues. Such a dissociation is met with in the human subject in those cases of glycosuria without ketosis in which a dose of glucose produces a considerable oxidation response. In view of the differences of opinion on these matters we present the following experiments.

Experimental methods.

Blood-sugar was determined by MacLean's method, respiratory metabolism by the Douglas and Haldane technique, urinary nitrogen by Kjeldahl's, and urinary sugar by Bertrand's method. Acetone substances were estimated by a method described by one of us [Goldblatt, 1925]. Plasma-bicarbonate was determined by Van Slyke's method after equilibration with normal alveolar air; plasma-inorganic phosphorus by Briggs's modification of the Bell-Doisy method; blood-lactic acid by Friedemann, Cotonio and Shaffer's method after precipitation with $\text{CuSO}_4\text{-Ca(OH)}_2$ in the Folin-Wu tungstic acid filtrate.

Exp. 1. The effect of starvation on the carbohydrate tolerance of normal rabbits. The rabbit is an animal which in our experience does not produce ketones on starvation. The demonstration of starvation intolerance in these animals

would therefore dispose effectively of the view that ketosis is a necessary accompaniment of this phenomenon. Three rabbits (3 kg.) were taken and blood-sugar curves obtained after the administration of 4 g. glucose in 20 cc. water by stomach tube. The animals had been accustomed to handling and all gave low initial blood-sugar values. During starvation they were allowed water *ad lib*.

Table I. *Blood-sugar values (mg. per 100 cc.) were obtained $\frac{1}{2}$ -hourly after 4 g. glucose per os.*

	Diet preceding starvation period—bran, oats, cabbage.			Average blood-sugar	Mg. increase above fasting value
	Rabbit 1	Rabbit 2	Rabbit 3		
	(a) After 27 hours' starvation.				
Fasting	104	109	104	106	—
	166	162	162	163	57
	129	137	159	142	36
	113	106	159	126	20
2½ hours	113	109	129	117	11
	(b) After 72 hours' starvation.				
Fasting	65	81	102	83	—
	83	175	115	124	41
	115	199	143	152	69
	106	187	137	143	60
	129	206	171	169	86
2½ hours	106	139	191	145	62
	111	92	125	109	26
	92	—	—	—	—
	(c) After 72 hours' starvation. 1 g. NaHCO ₃ administered with the glucose.				
Fasting	69	90	109	89	—
	100	171	191	154	65
	144	201	213	186	97
	83	154	129	122	33
	156	175	197	143	54
2½ hours	135	152	141	143	54
	125	115	120	120	31

The last column of Table I shows the definitely diminished rate of re-adjustment of blood-sugar after 3 days' starvation. Further it will be seen that the addition of 1 g. NaHCO₃ to the glucose was not followed by any improvement in tolerance. Such a dose of alkali is sufficient to produce a neutral urine in rabbits of this size. Du Vigneaud and Karr [1925] found that the administration of 1 g. NaHCO₃ daily to rabbits during a 4-day fast prevents the abnormal response to sugar. Their results appear to resolve the problem into a carbohydrate intolerance due to a diminution in available base. Our results do not bear this out nor did our animals show any tendency to acidosis, but we do not assert that the intolerance is unrelated to acidosis. The matter with which, however, we are immediately concerned is that starvation intolerance is not necessarily accompanied by ketosis. Eisner [1926], confirming the findings of others in the matter of the abnormal blood-sugar curve after starvation, also found that during the 3 hours following the administration of 35 g. glucose to rabbits starved for 10–11 days the deposition of glycogen in the liver and muscles was very poor compared with that found in ordinary

conditions. Twenty-four hours after the sugar was given the glycogen deposition was normal. These results seem to leave little doubt that starvation leads to a diminished power to store glycogen in the tissues. The later adequate deposition of glycogen is simply the expression of the readjustment to normal which carbohydrate is able to effect. In an experiment [Goldblatt, 1925] with starving human subjects it was found that readjustment to normal carbohydrate metabolism was established $2\frac{1}{2}$ hours after a dose of 50 g. fructose even though at this time ketosis was again appearing.

The remaining experiments in this paper were performed on two normal human subjects, whose metabolism had been determined on several occasions and found to remain steady during many hours of experiment. Starvation was undergone during full working days in the laboratories and the wards. During the experimental investigations of the results of starvation the subject was kept at complete rest in bed. Collections of blood and urine were made with the minimum of disturbance, the subject being well used to the procedure.

Experiments with Subject I. (Normal adult man, 150 lbs. in wt.)

For comparative purposes it will be necessary to give the blood-sugar curves obtained with this subject after administration of 50 g. glucose in ordinary conditions.

Table II. *Blood-sugar curves of Subject I.*

3 hours after a light meal		16 hours after food	
Minutes after glucose	Blood-sugar mg. per 100 cc.	Minutes after glucose	Blood-sugar mg. per 100 cc.
0	122	0	102
5	129	30	150
25	159	60	113
45	150	90	91
65	125	120	75
85	117		

Glycosuria never occurred in these conditions. It will be seen that complete readjustment to fasting blood-sugar levels is reached in about 90 minutes.

Now, if the intolerance to carbohydrate which occurs after starvation is due to acidosis or deficiency of base, it should be possible to demonstrate this by some of the simple tests at our disposal. In the next experiment we used the principle of the very simple and very useful Sellard's test.

Exp. 2. Subject I underwent a period of 37 hours' starvation. After a period of rest 5 g. NaHCO_3 in 50 cc. water were taken. About 30 minutes later 50 g. glucose in 100 cc. water were ingested and the observations recorded in Table III were made.

At 3.20 p.m. the urine was again acid to litmus.

The delayed return to fasting blood-sugar levels and the glycosuria are sufficient indices of the disturbed tolerance to carbohydrate. As in the earlier experiment [Goldblatt, 1925], the ketosis disappeared in about an hour after the glucose was taken. The rise in ketone excretion (from about 5 to

Table III.

Time	Blood-sugar mg. per 100 cc.	Vol. cc.	Glucose mg.	Urine		
				Titratable acidity cc. N/10 per hour	p _H	Total ketones as mg. acetone per hour
10.30 a.m.	83	—	—	—	—	—
11.15 „	—	26.5	0	12.37	5.5	4.69
5 g. NaHCO ₃ taken.						
11.45 a.m.	81	17.0	0	12.75	5.5	5.16
50 g. glucose taken.						
12.15 p.m.	135	17.0	0	15.30	5.5	10.60
12.45 „	166	17.0	0	18.70	5.5	2.58
1.15 „	141	18.5	130	11.10	6.5	Trace
1.45 „	115	22.0	0	6.60	8.0	0
2.15 „	104	19.0	0	3.80	8.5	0
2.45 „	83	14.0	0	2.80	8.3	0

10 mg. per hour) is the invariable accompaniment of alkali ingestion during ketosis. The combination of bicarbonate and glucose produced a marked shift in urinary reaction to the alkaline side but the defective carbohydrate control was not corrected. Taking into consideration, however, that at the time of ingestion of the sugar the excretion of acid was actually greater than at the outset and further that the sugar alone is able to produce a reduction in acidity, we must conclude that 5 g. NaHCO₃ is insufficient to neutralise the incipient acidosis. This will be clearer in the next experiment.

Exp. 3. In this experiment two doses of bicarbonate were given and the glucose was taken when the urine was definitely alkaline (to litmus). Subject I underwent a period of 39 hours' starvation before the beginning of the observations recorded in Table IV.

Table IV.

Time	Blood-sugar mg. per 100 cc.	Plasma- bicarbonate cc. CO ₂ per 100 cc.	Urine			
			Vol. cc.	Glucose mg.	Titratable acidity cc. N/10 per hour	Ketones as mg. acetone per hour
9.00 a.m.	(overnight collection)	—	227	0	13.82	5.0
10.30 „	92	—	—	—	—	—
11.00 „	—	64.3	41	0	11.28	5.0
5 g. NaHCO ₃ taken.						
11.30 „	92	—	—	—	—	—
12.04 p.m.	—	69.0	29	0	10.15	6.5
12.45 „	75	—	—	—	—	—
1.04 „	—	—	34	0	9.35	6.8
1.34 „	—	—	12	0	12.00	5.5
5 g. NaHCO ₃ taken.						
1.45 „	87	—	—	—	—	—
2.30 „	—	—	21.5	0	9.21	7.5
2.55 „	87	86.5	—	—	—	—
3.15 „	—	—	28.5	0	2.85	8.0
50 g. glucose taken.						
3.25 „	131	—	—	—	—	—
3.55 „	—	—	19	0	4.43	8.0
4.00 „	—	—	12	0	11.76	—
4.25 „	168	—	21	462	7.88	—
4.55 „	222	—	—	—	—	Trace
5.25 „	162	—	—	—	—	—
5.33 „	—	—	23	97	7.66	7.5

It is clear from these results that 5 g. NaHCO_3 were sufficient to bring the urinary reaction from p_{H} 5 to p_{H} 6.8, *i.e.* just short of neutrality, in about 2 hours. The second dose of bicarbonate, given 30 minutes after the effect of the first dose had passed off, produced an alkaline urine in $1\frac{3}{4}$ hours, at which point the glucose was taken. Throughout the succeeding 2 hours the urine was at p_{H} 7.5–8. In spite of this a very abnormal tolerance curve was obtained and 0.56 g. sugar appeared in the urine. It is further to be observed that even at the beginning of the experiment the bicarbonate reserve of the plasma (equilibrated with normal alveolar air) indicated full compensation of any acidotic tendency. (The value 86.5 seems very high with these doses of NaHCO_3 .) The very large increases in ketone excretion following each dose of glucose are also noteworthy.

It seems justifiable, therefore, to conclude thus far that, whilst judged from Sellard's test there is a slight deficiency in available base, there is full compensation as far as the blood is concerned, that is to say that the production of abnormal acids after these periods of starvation is not more rapid than can be effectively buffered in the blood. This does not eliminate the possibility of local changes in the tissues. But when after the second dose of bicarbonate an alkaline urine is produced we may fairly certainly assume that tissue buffering has also been attained. When, therefore, we obtain definite evidence that the diminished tolerance to carbohydrate still persists we are forced to the view that it is not primarily due to lack of available base.

The increased elimination of ketones after alkali administration is well known and is associated with a great depression and even disappearance of ammonia formation. Even with the relatively small doses we used there occurred a very large increase in ketonuria accompanied by very marked falls in titratable acidity.

It has been demonstrated by Gamble *et al.* [1923] that during starvation (3–15 days) there was no depletion of fixed base in the plasma, although the plasma-bicarbonate fell from 66 vols./100 cc. before the fast to 41 vols. on the third day. In our experiments, however, the periods of starvation were much shorter, so that no depletion either of fixed base or of plasma-bicarbonate was demonstrable. Since in the experiments of Gamble *et al.* [1923] the plasma-bicarbonate fell without diminution of fixed base, it is probable that there was a transfer of the latter from the HCO_3 ion to the anions of the ketonic acids.

Hirsch [1927] investigated the effect of sodium acetate on the loss of carbohydrate tolerance of a normal man after 38 hours' starvation. 50 g. glucose given after the period of fasting raised the blood-sugar from an initial value of 84 mg./100 cc. to 169 mg./100 cc. in 98 minutes, and after 216 minutes the blood-sugar was still 93 mg. Administration of 2 g. sodium acetate to the same subject after another similar period of starvation led to the production of a neutral urine, and 50 g. glucose then raised the blood-sugar from 85 mg./100 cc. to 153 mg./100 cc. in 186 minutes and after 245 minutes the value was 70 mg./100 cc. Although Hirsch regards these values as supporting the view that the sodium acetate improved the tolerance, it is difficult to

reconcile this with a blood-sugar of 153 mg./100 cc. 186 minutes after giving the sugar. The value of 70 mg./100 cc. some 4 hours after giving the sugar, however, suggests a better utilisation. The R.Q. in the experiment with acetate rose from 0.756 to 0.854, whilst without acetate it rose from 0.756 to 0.808: the increase in oxygen utilisation was, however, rather better in the experiment without alkali. With starving rabbits (3 days) and dogs (4 days) this author obtained lower blood-sugar curves when alkali was administered.

To examine further whether starvation-intolerance to carbohydrate after relatively short fasts was associated with changes in acid-base balance we studied the urinary ammonia after various periods of starvation.

Exp. 4. This experiment was divided into two parts.

(a) Subject I starved for 18 hours, during the last 6 hours of which the urine was collected hourly. Total nitrogen, ammonia, and ketones were estimated (Table V).

Table V.

Time without food (hours)	Urine				NH ₃ /N
	Vol. cc.	Total N g. per hour	Ammonia-N mg. per hour	Ketones mg. per hour (acetone)	
13	75	0.615	28.40	4.51	1/20.9
14	75	0.599	30.98	5.97	1/19.3
15	70	0.524	30.87	6.11	1/17.0
16	55	0.451	30.03	5.19	1/15.0
17	60	0.440	26.88	6.65	1/16.4
18	70	0.473	26.95	3.46	1/17.6

Remembering that 1 g. ammonia is capable of combining with 6 mg. acetoacetic acid (3.4 mg. acetone) we see that the relatively large excretion of NH₃ is here a mechanism for dealing with acids other than the ketone bodies. It is also seen that as the period of starvation increases the fraction of the degradation products of protein which is transformed to ammonia tends to increase. For our present purpose the important point is that if at any of the times given in Table V a sugar tolerance test be performed a so-called normal curve will be obtained. In the second part of this experiment similar observations were made from the 40th to the 45th hour of starvation, 50 g. glucose being taken after determination of basal values.

Table VI.

Time	Urine				NH ₃ /N
	Vol. cc.	Total N g. per hour	NH ₃ -N mg. per hour	Ketones mg., per hour (acetone)	
11.30	—	—	—	—	1/23.5
12.30	50	0.499	21.00	3.44	1/23.8
1.30	40	0.376	20.16	5.32	1/18.6
1.40	50 g. glucose in 100 cc. water.				
2.33	25	0.412	23.45	4.70	1/17.6
3.00	12	0.427	27.89	0.00	1/15.3
4.00	42.5	0.557	29.16	2.82	1/19.8
5.00	97	0.543	23.09	3.73	1/23.6
5.15	9.5	—	—	—	—
5.45	11	0.255	11.55	3.97	1/22.1

(b) Subject I underwent a period of 40 hours' starvation prior to the beginning of the experiment in Table VI.

The degree of intolerance to sugar which developed in the experiment produced glycosuria and the half-hourly blood-sugar values were:

Time in hours	...	...	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3	$3\frac{1}{2}$
Blood-sugar, mg. per 100 cc.	74	141	166	141	129	117	120	87		

Glycosuria occurred between $\frac{1}{2}$ and $1\frac{1}{2}$ hours from taking the sugar, so that we may be certain that there was a temporary elevation above the threshold for glucose. The ammonia excretion was lower to start with than in Table V, so that from the point of view of acidosis we had no reason to suspect the existence of a low tolerance for sugar. The absolute amount of ammonia excreted rose steadily and began to fall when the fall in blood-sugar became marked, until, when the latter reached normal fasting values, the ammonia had fallen to 11.55 mg. per hour, and evidence of protein-sparing appeared in the much lowered nitrogen excretion.

We are thus constrained to doubt whether starvation intolerance to carbohydrate is primarily due to acid intoxication. It is well known that marked disturbances in acid-base equilibrium are associated with loss of tolerance to sugar, and so there can be no doubt that starvation of long enough duration to produce changes in tissue- and blood-reactions will in virtue of these changes lead to greater loss of tolerance. But that other and more subtle factors may be involved is indicated by our demonstration that the loss of tolerance may occur without associated acidosis, and that it is not completely corrected by such doses of alkali as are sufficient to produce neutral or alkaline urine. The fact that many observers have insisted that the lowered sugar tolerance in man begins to manifest itself when ketosis appears is not evidence that either ketosis or acidosis bring it about. Ketosis is an expression of a preponderance of fat metabolism, so that a possible view to take is that diminished tolerance to sugar after starvation is due to an inability of the liver cells to readjust rapidly from a preponderance of fat to a plethora of carbohydrate. It has been clearly shown by Kohn *et al.* [1927] and Ellis [1931] that during a period on a ketogenic diet children develop very great loss of tolerance to sugar but that following the ketogenic régime there is a period of very greatly increased tolerance before the return to normal. Thalhimar (quoted by Kohn *et al.*) tentatively suggests that over-secretion of insulin may be the cause of the increased tolerance in the *post*-ketosis period. There is, however, no direct evidence that during the ketosis induced either by ketogenic diet or by cyclical vomiting or by starvation there is any deficiency or excess of insulin. The experiments of Dann and Chambers [1930] suggest strongly that other factors than deficiency in insulin are concerned in the diminished sugar tolerance in starvation.

In the following experiments detailed examinations were made of the partition of heat production between the various nutritional constituents during starvation periods sufficient to induce a lowered tolerance to carbo-

hydrate. For this purpose we used Subject II. Comparisons were made between the metabolic state of this subject 16 and 39 hours after food, and the effect on metabolism of the ingestion of 50 g. glucose. Calculations of the partition of energy between fat, carbohydrate and protein were made by means of the Zuntz-Schumburg table as modified by Lusk, the ordinary corrections for nitrogen being made. In some cases estimations of the capillary and venous blood-sugar of the same arm were made.

Differences between capillary and venous blood-sugar are generally interpreted as evidence of sugar utilisation by the muscles, the fact that diabetics show very little difference between the two values providing corroboration of this view. But capillary-venous differences in blood-sugar may also indicate deposition of glycogen in the muscles, particularly if the latter have been depleted of their normal content by starvation or other means. Thus, in starvation, capillary-venous differences following glucose administration may be as marked or even more marked than in normal condition without this necessarily indicating as good or better oxidation of sugar. This will be shown in the experiments which follow in which great diminution in oxidation of carbohydrate was accompanied by capillary-venous differences in blood-sugar as high as occur without starvation.

The association of phosphorus with sugar metabolism led us to estimate plasma-inorganic phosphorus. The administration of glucose to a subject able to metabolise sugar normally is followed sometimes by a fall in plasma-inorganic phosphorus, but this returns to normal levels in an hour or two [Hartmann and Bolliger, 1925; McCullagh, 1931]. Insulin is also able to produce definite falls in plasma-inorganic phosphorus. Mild diabetic cases, however, show in similar circumstances a fall in phosphorus but there is a much slower return to normal values. Severe diabetics give no fall at all. In our experience the diminution in plasma-inorganic phosphorus is not always demonstrable after the ingestion of sugar, which may perhaps be due to the transitoriness of the change. Simultaneously with the fall in plasma-inorganic phosphorus there occurs a marked fall in urinary excretion of phosphate, normal excretion returning with the return to normal plasma values. If we regard the amount of circulating phosphate in the starving organism in the early stages as the resultant of the processes of formation and breakdown of hexosephosphoric esters and the excretion of urinary phosphate, then it appears probable that in the early stages of carbohydrate utilisation there is preponderance of synthesis of esters. In view of the fact that in the normal subject the fall in plasma-inorganic phosphorus is only of short duration whereas in the mild diabetic it is more prolonged, it is possible that the formation of phosphoric esters (regarded as intermediate steps in carbohydrate metabolism) is unimpaired in the mild diabetic but his oxidative capacity is deficient, so that the ester stage is only slowly passed through. In the very severe diabetic the fact that the blood-inorganic phosphorus does not fall at all is explicable on the view that both mechanisms are at fault so that he

neither esterifies his carbohydrate nor oxidises it. As will be seen later on, findings with Subject II seem to point to a response of plasma-inorganic phosphorus of the type associated with mild diabetes. Blood-lactic acid was estimated as an index of emotional and muscular rest and remained constant throughout.

Exp. 5. Metabolism of 50 g. glucose by Subject II, after 16 hours' fast (Table VII).

Table VII.

Blood analysis.

Time after glucose mins.	Blood-sugar mg. per 100 cc.		Plasma-CO ₂ combining power vols. %	Plasma-inorganic P mg. per 100 cc.	Lactic acid mg. per 100 cc. whole blood
	Capillary	Venous			
0	111	109 (3)	57.6	3.13	13.2
33	109	109 (5)	57.6	3.26	13.8
66	148	134 (2)	59.5	3.43	14.4
133	156	117 (5)	58.6	3.45	13.2

Venous blood taken () minutes after capillary blood.

Respiratory metabolism.

Time after glucose mins.	Urinary nitrogen mg. per min.	Non-protein R.Q.	O ₂ cc. per min.	g. metabolised per hour		
				Protein	Fat	Carbohydrate
Basal	11.56	0.81	258	4.34	3.34	4.76
Basal	12.70	0.88	248	4.76	2.25	7.39
28	9.70	0.78	268	3.64	4.78	3.64
58	10.51	0.93	244	3.94	1.39	10.08
93	10.51	0.93	285	3.94	1.70	12.31
130	10.51	1.04	270	3.94	0	15.72

The important points in this experiment are the following.

(1) There was some delay in the rise of blood-sugar; the value 156 (capillary) which appears rather late for a normal subject is referable probably to slow absorption¹.

(2) The plasma-CO₂ was normal and remained unchanged.

(3) The inorganic phosphorus in the plasma rose definitely during the 2 hours of the experiment.

(4) The respiratory metabolism gave unequivocal evidence of active oxidation of carbohydrate. Whilst the protein metabolism per unit time remained sensibly constant, the fat catabolism gradually diminished until when the non-protein R.Q. was 1.04 the usual assumptions led to the overwhelming preponderance of carbohydrate in the non-protein catabolism.

The attainment of a capillary-venous blood-sugar difference of 39 mg. per 100 cc. at the same time as an R.Q. of 1.04 may be regarded as additional evidence of active disposal of carbohydrate. Taking this as a typical reaction of Subject II to 50 g. glucose, we now proceed to give the results obtained after two periods of starvation of 39 hours each.

¹ This subject on several other occasions has given blood-sugar curves completely normal in every respect.

Exp. 6. Metabolism of 50 g. glucose by Subject II after 39 hours' starvation (Table VIII).

Table VIII.

Blood analysis.

Time after glucose mins.	Blood-sugar mg. per 100 cc.		Plasma-CO ₂ combining power vols. %	Plasma- inorganic-P mg. per 100 cc.	Lactic acid mg. per 100 cc. whole blood
	Capillary	Venous			
0	95	83 (2)	73.9	3.32	13.1
30	120	130 (3)	72.9	3.20	12.6
60	152	152 (5)	72.0	2.99	14.4
90	154	—	—	—	—
120	178	128 (10)	65.3	2.86	13.5
158	130	—	—	—	—
190	81	—	—	—	—

Venous blood taken () minutes after capillary blood.

Respiratory metabolism.

Time after glucose mins.	Urinary nitrogen mg. per min.	Non-protein R.Q.	O ₂ cc. per min.	g. metabolised per hour		
				Protein	Fat	Carbohydrate
Basal	13.04	0.71	252	4.89	5.30	1.70
Basal	11.25	0.76	248	4.22	4.59	2.34
25	11.09	0.73	268	4.16	5.69	1.15
55		0.77	270	4.16	4.95	3.12
88	15.00	0.81	254	5.63	3.38	4.19
118	12.12	0.81	270	4.55	4.00	4.96
140	11.35	0.86	263	4.26	2.84	7.58

Urinary findings.

Time after glucose mins.	Titrateable acidity in cc. N/10 per hour	Rothera's reaction
0	-4.5 (alk.)	Positive
36	-1.3 (alk.)	Strong
70	14.1	Trace
101	9.7	Negative
135	5.3	"
190	2.7	"

Attention may be drawn in this experiment to the facts that capillary-venous blood-sugar differences did not become established until well over an hour from the taking of the glucose, that the alkali reserve was high to begin with and at no time showed evidence of an acidosis, and further that the plasma-inorganic phosphorus fell steadily for 2 hours. The blood-lactic acid, as in the previous experiment, showed no change of note. It is also to be noted that the subject at the time of ingestion of the glucose was excreting an alkaline urine (morning "alkaline tide"). The cause of this phenomenon has been sought in the early morning hyperpnoea with resultant loss of CO₂ and hence the compensating loss of alkali, which sometimes occurs. It has also been attributed to secretion of gastric HCl on rising after sleep. The figures in Table VIII rather suggest that the morning alkaline tide, when it does occur, is associated with a high alkali reserve, which may be due to a temporary mobilisation of base, to balance a CO₂ retention during sleep. The fall in alkali reserve is at once reflected in a marked excretion of acid in the urine.

The ketonuria was not very marked and disappeared completely in about 70 minutes after the glucose was taken. In spite of these facts the respiratory findings show a persistently low oxidation response to carbohydrate.

Since the R.Q. was 0.86 at the end of this experiment it was necessary to repeat the experiment and continue until it was clear that no further rise in R.Q. was going to take place.

Exp. 7. Metabolism of 50 g. glucose by Subject II after 39½ hours' starvation (Table IX).

Table IX.

Time after glucose mins.	Capillary blood-sugar mg. per 100 cc.	Non-protein R.Q.	O ₂ cc. per min.	g. metabolised per hour		
				Protein	Fat	Carbohydrate
Basal	85	0.77	255	5.35	4.37	2.63
30	115	0.74	258	4.91	4.90	2.32
68	146	0.76	262	4.91	4.66	2.38
103	166	0.856	245	5.00	2.62	6.09
142	100	0.854	274	5.00	3.00	6.96
180	78	0.79	266	3.47	4.77	4.03
225	67	0.695	259	3.72	6.05	0

The initial ketosis in this experiment was very slight and rapidly disappeared after the glucose was taken. The blood-sugar curve was somewhat prolonged but not markedly abnormal. The fact that some 3½ hours after the glucose the blood-sugar was 67 mg. per 100 cc. shows that the mechanism for removal of sugar from the blood was now extremely active. The respiratory data are, however, unequivocal. The maximum rise in R.Q. was to 0.86 in 103 minutes, after which it fell to fat levels and showed no sign of rising again. Comparison of Tables VIII and IX with Table VII leaves no doubt that, on the usual assumptions as to the significance of the R.Q., there was a great depression of the power to deal immediately with carbohydrate.

The most obvious assumption in explanation of these facts is that in conditions of starvation there is a deficiency of insulin. If we take the view that insulin inhibits gluconeogenesis, then the fact that in starvation this process becomes more active would strengthen the belief that some inhibition of insulin secretion or activity occurs. It is known that insulin produces a marked rise in R.Q. in the normal subject starved for 16 hours [Goldblatt and Ellis, 1931] so that we should expect a rapid readjustment to normal reactions to glucose administration if both insulin injection and glucose ingestion were practised on the subject starved as in the previous experiment. The results of such an experiment are given in Table X.

Exp. 8. Subject II was starved for 39 hours. Basal determinations having been made, 10 units of insulin were injected subcutaneously; 15 minutes later 50 g. glucose were taken and the observations were made.

In this experiment there was considerable ketonuria at the beginning; this ceased completely about 1½ hours, but began to reappear some 4 hours after the glucose was taken. Glycosuria was absent throughout.

Table X.

Time after glucose mins.	Non-protein R.Q.	O ₂ cc. per min.	g. metabolised per hour			
			Protein	Fat	Carbohydrate	
Basal	0.85	249	4.84	2.80	6.10	
25	0.75	263	4.73	4.93	1.96	
59	0.78	263	6.02	4.40	3.35	
91	0.78	252	6.02	3.71	2.83	
130	0.86	251	4.77	2.66	6.61	
170	0.87	237	3.09	2.62	7.49	
206	0.84	246	3.09	3.65	6.31	
242	0.76	258	2.11	5.26	3.59	
Time after glucose (mins.)	0	35	70	100	150	195
Blood-sugar	...	...	81	109	143	139 109 51 (Hypoglycaemic symptoms with spontaneous recovery)

The peculiar fact emerges from this experiment that, whilst insulin completely prevented any manifestation of intolerance to sugar as far as the blood-sugar is concerned, it did not simultaneously readjust the oxidative processes.

Table XI gives the maximum rises in O₂ utilisation and maximum rises in heat production in the above experiments after 50 g. glucose.

Table XI.

	Maximum rise in O ₂ cc. per min.		Maximum rise in heat production cals. per hour		Maximum R.Q. (non-protein)
Control period	...	...	26.9	9.7	1.04
39 hours' starvation...	...	...	18.4	6.3	0.85
39½ hours' starvation	...	...	19.0	6.5	0.85
39 hours' starvation and 10 units insulin...	...	...	14.0	3.1	0.87

Percentage increase in heat production after giving glucose.

Control. Basal 73.5 Cals.		39 hours' starvation. Basal 71.3 Cals.		39½ hours' starvation. Basal 73.2 Cals.		39 hours' starvation. Insulin 10 units. Basal 72.6 Cals.	
Time mins.	% increase	Time mins.	% increase	Time mins.	% increase	Time mins.	% increase
28	4.6	25	7.0	30	0	25	3.2
58	0	55	8.7	68	2.3	59	4.3
93	14.7	88	2.8	103	0	91	0
130	10.9	118	9.2	142	8.9	130	0
—	—	140	7.4	180	4.2	170	-4.3
—	—	—	—	225	0	206	-1.8
—	—	—	—	—	—	242	1.6

In interpreting Table XI it is important to realise how steady the basal metabolism and oxygen consumption of this subject were at the beginning of each experiment. Of six determinations the mean percentage deviation from the mean (72.6) was 1.2 (max. 2.7—min. 0). There can thus be no reasonable doubt that some diminution in the so-called specific dynamic action of glucose becomes manifest in starvation. It is further evident that the injection of insulin does not immediately readjust the mechanism otherwise than to bring down the blood-sugar. This is the more strange since, as we found

in previous work [1931], insulin alone can, in a normal subject starved for 16 hours, raise the R.Q. from a basal non-protein value of 0.80 to 1.01 with a simultaneous increase in heat production of about 12 % in about an hour.

It is further of interest to note that whilst with glucose alone after starvation the calorie increase reached about 9 % above basal values, with insulin and glucose it attained only 4 % and that for a relatively short time.

The marked lowering of blood-sugar after insulin associated with a failure of the rate of oxidation of carbohydrate to rise to a greater extent than without insulin is extremely difficult to explain on the usually accepted theories of action of this hormone. We must seek the cause in the tissues.

If, as we believe, the diminished tolerance for carbohydrate observed in starvation is not explicable on the basis of an acidosis, then it is not admissible to suppose that the failure of insulin to readjust carbohydrate oxidation in these conditions is due to changes in tissue reaction. Dann and Chambers [1930] found that, after prolonged periods of starvation, even 60 units of insulin in a 10 kg. dog did not bring the oxidation of carbohydrate (50 g. glucose) to normal. Thus, whereas the normal dog after receiving this amount of sugar could metabolise about 4.7 g. glucose per hour, the starved animal oxidised only 0.6 g. glucose per hour, and when starved and given 60 units insulin with the sugar it oxidised only 2.1 g. carbohydrate per hour. There was obviously a marked improvement in the power to metabolise sugar but the value obtained fell far short of the normal. They also found that after about 3 weeks' starvation the rate of oxidation of carbohydrate remained deficient even during 3 days with daily doses of 50 g. glucose, whereas insulin simultaneously injected readjusted peripheral oxidation completely on the second day. Thus the failure of insulin to produce its typical effects was limited to the first day of the experimental period after starvation (19 days). In our experiment the preliminary period of starvation was just sufficiently long to make manifest the deficient power to oxidise glucose, and there was already a marked failure in oxidation response to insulin, in spite of the fact that the blood-sugar fell to hypoglycaemic levels. Since there is no reason to suppose that the absorption of glucose from the gut is deficient (the contrary is more probable) after starvation, we must suppose either that some essential stage in the metabolism of sugar is delayed or that the tissues after starvation differ fundamentally in some respect from normal tissues. The results of Eisner (*vide supra*) seem to us to offer strong evidence in favour of the former view, that is to say that there is a delay in the formation of that concentration of glycogen which permits of what is called normal metabolism. If we regard insulin as the physiological stimulus to the reactions which lead to both glycogen formation and carbohydrate oxidation, then it appears probable that in the conditions of these experiments the predominant effect of insulin is to promote the former of these results. Later, when adequate concentrations of glycogen are formed, the oxidative response to sugar becomes normal; a condition of things which may result, as we have seen, some

time only after the administration of sugar. The fact that even during starvation intolerance very considerable differences in capillary and venous blood-sugar are demonstrable even when oxidation of carbohydrate is inadequate, indicates that retention of sugar in the tissues must be actively proceeding. It is known that in animals the injection of insulin during carbohydrate absorption leads always to a smaller deposition of glycogen in the liver than when the carbohydrate is given alone. This fact is interpreted as due to the increased deposition and oxidation of carbohydrate peripherally. If this can be assumed to apply to the human organism then the results in Table X must indicate increased deposition of carbohydrate in the tissues, probably first as free sugar and later as glycogen, the oxidative response occurring considerably later.

SUMMARY AND CONCLUSIONS.

1. Evidence is presented to show that the intolerance for carbohydrate manifested after periods of about 40 hours' starvation is not due to either ketosis or acidosis.
2. The observation of Dann and Chambers that insulin does not completely re-establish normal carbohydrate oxidation in dogs after prolonged starvation, is shown to have its counterpart in human subjects even after only 40 hours' starvation.
3. In explanation of these facts it is considered unnecessary to assume any special changes in the tissues other than those incident to a fall in glycogen content. It is suggested that in the starving organism there is a temporary dissociation of the two mechanisms initiated by insulin, *viz.* the formation of peripheral glycogen and the oxidation of carbohydrate, the second effect being manifest only when the first has attained adequate proportions.

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