CLV. STUDIES ON KETOSIS: I. THE RELATION BETWEEN ALKALOSIS AND KETOSIS.

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THE appearance of acetone bodies in the urine (ketosis) occurs in man in many diverse conditions, and is usually associated with an upset in the metabolism of carbohydrate or with damage to the liver function. Davies, Haldane and Kennaway [1920] found that ketone bodies appear in small concentrations in the urine during experimental alkalosis; and it has recently been shown by Haldane, Wigglesworth and Woodrow [1924] that concurrently with this acetonuria there is a considerable disturbance of the normal metabolism of carbohydrate. The main object of the present work was to determine the relation between the ketosis of carbohydrate inanition and the acetonuria induced by alkalosis.

Methods.

The experiments have been carried out on rats. Rats of all ages have been employed at different times, but where accurate comparisons have been required, rats of about 100 g. selected from the same litter and reared under the same conditions have been used. The small rats have, as a rule, been used in pairs. Even under these conditions individual variations are considerable.

The urine of the rats has been obtained free from faeces in the usual way and collected over twenty-four hour periods in a small conical flask containing a few cc. of water covered with a layer of toluene. It was made up to 100 cc. with the washings of the collecting funnel and estimations made upon aliquot portions containing up to 15 mg. of ketone substances.

Acetone and acetoacetic acid (estimated together throughout as "total acetone") and β -hydroxybutyric acid have been determined on the same sample of urine by a method based on the Messinger iodometric titration and the Shaffer oxidation of β -hydroxybutyric acid with potassium dichromate.

The urine is placed in a 500 cc. round bottom flask, 25 g. of anhydrous sodium sulphate and 1 cc. of concentrated sulphuric acid are added and the volume made up to about 300 cc. with distilled water. The flask is fitted with a dropping funnel and a delivery tube leading to the bottom of another 500 cc. flask containing 100 cc. of 4 % caustic soda. The delivery tube from this

flask leads to a condenser fitted with an adapter which dips below the surface of the fluid in the receiver.

The liquid in both flasks is heated to boiling and distillation continued for ten minutes into a receiver containing 5 cc. of 25 % caustic soda and a known volume (excess) of 0.01*N* iodine. 5 cc. of 25 % sulphuric acid are then added to the distillate and the excess iodine titrated with 0.01*N* sodium thiosulphate. 1 cc. of 0.01*N* iodine = 0.0967 mg. acetone. A blank determination is made on the reagents and this value deducted.

At the end of the ten minutes the first receiver is replaced by a second receiver containing 5 cc. of 40 % soda. The gas is lowered so that the liquid only just boils and 25 cc. of the solution of potassium dichromate¹ allowed to run in drop by drop during 30 minutes. The solution is then boiled rapidly for ten minutes. 0.5 cc. of 30 % hydrogen peroxide (Merck's Perhydrol) is added to the distillate, which is transferred to a 500 cc. flask, brought cautiously to the boil and distilled for ten minutes, the acetone being estimated exactly as before. A blank determination is again made on the reagents.

As in the original method of Shaffer there is a constant yield of acetone from β -hydroxybutyric acid of about 85 % of the theoretical. The cc. of 0.01*N* iodine are therefore multiplied by the factor 0.2 to give milligrams of β -hydroxybutyric acid.

Lactic acid has been estimated in urine by the method of Clausen [1922]. The ether extraction method of Clausen has been employed, the phenols being first removed by extracting with ether at p_H 7.0. The error due to β -hydroxybutyric acid has been eliminated, as he suggests, by the method of Shaffer, the distillate being redistilled after the addition of 0.5 cc. of 30 % hydrogen peroxide and 5 cc. of 40 % soda. In the experiments in which lactic acid determinations have been made, the urine has been collected in dry vessels, and a minimal quantity of water used in washing out the vessels and making up to a known volume.

The following are the diets used in the experiments reported:

Diet 1, a normal diet for experiments of short duration: casein 10, starch 50, sugar 17, lard 15, salts 2.

Diet 2, a diet free from carbohydrate but containing some protein: casein 10, lard 50, salts 2.

Diet 3, free from both protein and carbohydrate: filtered butter 100, salts 3.

The salts employed had the following composition: normal calcium phosphate 15, di-sodium hydrogen phosphate 10, potassium di-hydrogen phosphate 27.5, sodium chloride 10, potassium chloride 5.

Ketosis occurring in rats fed upon a diet deficient in carbohydrate.

When man is deprived of carbohydrate ketosis rapidly sets in; but this is not commonly the case with other animals [Baer, 1906]. The rat does not appear to have been studied in this connection.

¹ 20 g. potassium dichromate, 250 cc. concentrated sulphuric acid, water to 1 litre.

The urine of rats, particularly old individuals, gives a positive reaction to the nitro-prusside test, which is due at least in part to acetone, since the distillate from such urine also gives a positive test. The normal excretion of "total acetone" ranges from 0.0–0.30 mg. per diem, and of β -hydroxybutyric acid from 0.8–2.5 mg.; though, as will be shown later, the latter is subject to wide variations.

Table I represents a typical experiment showing the response of the rat to a diet of fat alone (diet 3).

Table I.

Figures in milligrams per diem.
Two young rats fed on diet 3 after 3. vi. 24.

Date	Weight g.	"Total acetone"	β -hydroxybutyric acid
3. vi. 24	133	0.00	0.70
	107		
4	124	0.06	1.42
	101		
5	122	1.45	4.56
	98		
6	120	6.34	21.20
	96		
7	120	1.86	5.08
	97		
9	121	0.31	1.44
	96		

It is clear that the rat shows a very definite ketosis on this diet. The excretion of "total acetone" and of β -hydroxybutyric acid rises for the first few days of the experiment reaching its maximum at the third or fourth day. At the height of ketosis the output of β -hydroxybutyric acid is usually between two and four times that of the "total acetone." In a few cases a secondary rise in ketosis has occurred at about the tenth day, but when this has been the case the excretion has soon returned to normal once more. The rats show a rapid fall in weight during the first two days but thereafter the loss in weight usually does not exceed 1 g. per diem and may often remain constant for several days during the experiment. The height to which the ketosis may rise is exceedingly variable, but as in the case of man it is much more marked in young animals, and in a few old individuals has been almost absent.

Rats fed on diet 2 (*i.e.* with some protein added to the fat) ordinarily show a much less intense ketosis, though the time relations of the adaptation are the same as before. Such an experiment is given in Table II.

Table II.

Figures in milligrams per diem.
Rat (about 200 g.) fed on diet 2 after 3. iii. 24.

Date	"Total acetone"	β -hydroxybutyric acid
3. iii. 24	0.13	1.95
4	0.19	1.31
5	0.83	1.92
6	1.76	3.18
7	1.31	3.50
8	0.41	1.78
9	0.29	1.22

If the animal is killed after about ten days on a fat diet, when the ketone excretion is within normal limits, the liver is seen to be small in size and laden with fat. Otherwise the organs appear perfectly normal.

It has long been known that the body possesses a considerable power of adaptation to a diet rich in fat. It is clear that in the rat this adjustment occurs with remarkable rapidity, even when the only antiketogenic element in the diet is the glycerol of the fat itself. Moreover it is worth noting in Table I that after the first three days on the fat diet there was practically no further loss of weight during the experiment. That is to say, at the time when the rat is showing the rapid adjustment to the fat diet (as judged by the ketosis) there is no evidence that it is drawing upon any endogenous source of antiketogenic substance. It is difficult to reconcile these results with the Shaffer [1921] theory of antiketogenesis.

The effect of alkalosis on the excretion of ketone bodies by the rat.

It has long been known that the administration of sodium bicarbonate to diabetics causes a marked increase in the excretion of ketone bodies. Similarly in experimental alkalosis in the normal subject a slight ketosis may occur. Most authors, in discussing the ketonuria of these conditions, have described the appearance of the organic acids in the urine as a regulatory mechanism for neutralising the alkali. Haldane, Wigglesworth and Woodrow [1924], however, obtained evidence that simultaneously with the ketosis there was a considerable upset in the metabolism of carbohydrate; and they were inclined to attribute the ketosis to a more profound disturbance of metabolism than would be implied by a mere "washing out" of these substances by an excess of alkali. The experiments here described were made upon the rat in an attempt to throw further light on this point.

In Table III is shown a typical experiment in which 1 g. of sodium bicarbonate per diem was added to the normal diet.

Table III.

Figures in milligrams per diem. Rat of about 200 g.			
Date	"Total acetone"	β -hydroxybutyric acid	Remarks
6. ii. 24	0.25	0.86	Diet 1
7	0.28	1.3	" + 1 g. NaHCO ₃
8	0.16	8.87	" "
9	0.28	10.32	"
10	0.19	4.59	"

Under these conditions there is a very marked rise in the excretion of β -hydroxybutyric acid but the output of acetoacetic acid is unaffected. In experiments in which the administration of bicarbonate has been continued for many days, there has been no sign of any drop in the β -hydroxybutyric acid excretion indicating an adjustment to the condition. If the animal be

killed after the β -hydroxybutyric acid excretion has been at a high level for several days and its liver examined, this shows no fatty infiltration as it does during carbohydrate inanition.

In an investigation of the mechanisms employed by the body in combating an excess of alkali, McLeod and Knapp [1919] found that administration of sodium carbonate caused an increase in the excretion of lactic acid. Since the method (the v. Fürth-Charnass permanganate oxidation) employed by these workers would also estimate considerable quantities of β -hydroxybutyric acid, it seemed desirable to repeat the experiments making simultaneous estimations of the acetone bodies and the lactic acid. Table IV illustrates such an experiment.

Table IV.

Two rats (each about 150 g.). The output of acids in milligrams per diem.

Date	"Total acetone"	β -hydroxybutyric acid	Lactic acid	Remarks
2. vi. 24	0.19	2.20	4.1	Diet 1
3	0.36	2.25	3.27	" + 2 g. NaHCO_3
4	0.34	15.30	10.4	"
5	0.20	23.7	11.6	" + 2 g. NH_4Cl
6	0.19	3.78	3.90	"

It will be seen that whereas again the total acetone excretion is not affected, the β -hydroxybutyric acid and lactic acid outputs rise. In this experiment the former is more affected than the latter, but this is not constantly the case. On the 5th, 1 g. of ammonium chloride was added to the normal diet bringing about a rapid reversal of the bicarbonate effect.

As in the experiments of McLeod and Knapp the part which these acids can play in neutralising the excess of alkali is quantitatively very small. For example on 5. vi. in Table IV, the titratable alkalinity of the urine was 89.5 cc. $N/10$ alkali, whereas the β -hydroxybutyric and lactic acids together will account only for about 3.4 cc. $N/10$ alkali. Moreover, the extent to which they rise is by no means directly proportional to the alkalinity of the urine. In fact any substance which during its metabolism will liberate an excess of base will apparently bring about this increased excretion of organic acids, while the urine may be only very slightly alkaline or even slightly acid.

For example, it was observed in the case of a rat fed on a diet containing calcium lactate in the salt mixture that the excretion of β -hydroxybutyric acid rose from 2.3 mg. per diem to 9.5 mg. with the urine neutral or slightly acid. After administration of ammonium chloride it fell to 0.90, and rose again to 5.64 mg. when this was stopped. It is noteworthy in this experiment that administration of acid reduced the β -hydroxybutyric acid excretion to considerably *below* the normal level of about 2 mg. for a rat of this size.

Similar results have been obtained after administration of sodium acetate. In one case the excretion of β -hydroxybutyric acid rose from 2.1 mg. to 23.92 mg. after sodium acetate had been added to the food for one day. Doubtless many organic acids would behave in the same way; hence it follows that experiments based on the increase in the excretion of lactic, β -hydroxy-

butyric and possibly other acids after the administration of the sodium salts of other substances must be interpreted with caution.

It was of interest to see whether "alkali-forming foods" would have the same effect upon the output of organic acids as feeding with alkalies. With this object rats were fed on diet 1 with the addition of carrots. Table V gives the results of such an experiment.

Table V.

Date	Output of acids in milligrams per diem. Two rats (each about 160 g.).			Remarks
	"Total acetone"	β -hydroxybutyric acid	Lactic acid	
20. vi. 24	0.10	1.4	3.1	Diet 1
21	0.14	1.35	2.9	" + carrots
22	—	—	—	" "
23	0.17	5.55	13.4	"
24	0.23	4.8	13.5	"
25	0.22	4.03	4.74	"
26	0.23	1.62	2.5	"

The excretion of organic acids is seen to rise as after the administration of sodium bicarbonate. It is noteworthy that the output had not returned to normal until three days after the carrots had been discontinued. This may perhaps be due to the gradual liberation of base in the tissues during metabolism. The effect of sodium bicarbonate administered as such seldom persists for more than one day. A similar "lag" may sometimes be seen in the onset of the increased excretion of organic acids, as compared with the changes in titratable acidity, after the administration of sodium bicarbonate, and suggests that the changes in their production occur in the tissues and not during the secretion of urine.

*The effect of glucose on the excretion of organic acids after
administration of alkali.*

Since carbohydrate has such a powerful influence in depressing the excretion of β -hydroxybutyric acid in ketosis it was interesting to see whether it had any influence on the excretion of this acid in alkalosis. Though the rats were on a diet rich in carbohydrate it seemed possible that massive doses of glucose might affect equilibria in the body in such a way that the excretion of the acids might be affected. Tables VI, VII, and VIII, show experiments on these lines.

Table VI.

Date	Rat of about 200 g. weight on diet 1.			Remarks
	"Total acetone"	β -hydroxybutyric acid		
25. iii. 24	—	—	5 cc. of 10 % NaHCO_3 injected at 12.15 p.m.	
26	0.14	10.79	NaHCO_3 as 25th at 12.15 + 5 cc. of 20 % glucose	
27	0.14	5.18	NaHCO_3 at 12.15 as before	
28	0.14	9.25		

Table VII.

Rat (about 200 g.) on diet 1 with addition of 2 % calcium lactate.

Date	"Total acetone"	β -hydroxybutyric acid	Remarks
26. v. 24	0.16	8.86	5 cc. of 20 % glucose injected
27	0.23	6.56	
28	0.23	11.44	

Table VIII.

Two rats (each about 160 g.) fed with diet 1 with the addition of 2 % sodium bicarbonate after 21. vi. 24.

Date	"Total acetone"	β -hydroxybutyric acid	Lactic acid	Remarks
21. vi. 24	0.28	2.25	3.37	5 cc. of 20 % glucose injected to each
23	0.00	44.5	10.4	
24	0.00	37.0	17.2	
25	0.20	49.6	8.6	Glucose given as on 23rd
26	0.00	39.0	15.1	
27	0.19	52.2	11.9	

In Table VI a marked depression of the β -hydroxybutyric acid excretion due to sodium bicarbonate is seen to occur after injection of 5 cc. of 20 % glucose. Table VII illustrates a similar effect when the high β -hydroxybutyric acid output is due to the calcium lactate in the diet. Table VIII shows an experiment in which simultaneous estimations of lactic acid were made. While the β -hydroxybutyric acid excretion shows the usual depression, the lactic acid output seems to be increased. The mode of action of the glucose in these experiments is not clear. It does not appear to be due to its evoking an increased secretion of "insulin" by the pancreas, since injections of insulin have not this effect.

A comparison of the effects of alkalosis with the ketosis due to a diet of fat.

The experiments described have shown that the administration of alkalies causes in the rat a rise in the excretion of β -hydroxybutyric acid and lactic acid, but little or no change in the excretion of acetoacetic acid; whereas during fat feeding both acetoacetic and β -hydroxybutyric acids increase in the urine. Table IX shows an experiment in which the two procedures were carried out successively on the same animal. There are the usual changes during the bicarbonate alkalosis: a rise in the excretion of β -hydroxybutyric and lactic acids. The rat was an old animal so that the ketosis on the fat diet was not very intense, but there was a definite rise in the acetoacetic acid and β -hydroxybutyric acid excretion while the output of lactic acid tended to fall. Bicarbonate was administered after adaptation to the fat diet was complete, at first in the food and then by injection. The β -hydroxybutyric acid rose greatly while the lactic acid, though definitely affected, was apparently less readily so than when the rat was on a mixed diet. The acetoacetic acid showed a slight rise. This point will be returned to later.

Table IX.

Figures in milligrams per diem.
 Rat (about 300 g.) previous to experiment fed on diet 1.

Date	"Total acetone"	β -hydroxybutyric acid	Lactic acid	Remarks
5. vi. 24	0.22	2.21	3.1	Diet 1 + 2 % NaHCO_3
6	—	—	—	"
7	0.08	20.50	19.0	" + 2 % NH_4Cl
8	—	—	—	"
9	0.08	3.06	2.87	Diet 3
10	0.10	2.40	2.7	"
12	3.92	10.95	2.63	"
13	2.12	3.99	2.16	"
15	0.54	1.23	1.77	"
16	—	—	—	" + 2 % NaHCO_3
17	0.35	19.0	2.63	" + 5 cc. 10 % NaHCO_3 injected
18	2.1	30.6	8.0	"

The differences between the two types of experiment are very clearly shown in Fig. 1.

Fig. 1. Abscissa: days. Ordinate: excretion in milligrams per diem. Curve A, "total acetone." Curve B, β -hydroxybutyric acid. Rat of about 250 g. weight, fed throughout on diet 3. The thickened portion of the base line indicates the days on which 5 cc. of 10 % sodium bicarbonate were injected.

The experiments so far described seemed to indicate that there is a very definite distinction between the "ketosis" due to alkalosis in the rat and the true ketosis of carbohydrate inanition. In the experiments of Haldane, Wigglesworth and Woodrow [1924], however, the occurrence of the ketosis synchronising with the upset in carbohydrate metabolism and with a very

low respiratory quotient, suggests very strongly that we have here to do with a real disturbance of fat metabolism—brought about either directly or indirectly as the result of a deranged carbohydrate metabolism. In this same work it was noted that the excretion of β -hydroxybutyric acid was much more affected than that of acetoacetic acid and acetone: analogous, that is, to the condition described in the rat. It was thought that the distinction might lie in the fact of the rat being more resistant to this treatment with alkalis than is man; and that more massive doses of sodium bicarbonate might reproduce this condition in the rat. Table X shows an experiment of this nature which has been confirmed several times.

Table X.

Rat of about 180 g.			
Date	"Total acetone"	β -hydroxybutyric acid	Remarks
11. ii. 24	0.20	1.30	Diet 1 + 4 % NaHCO_3
12	0.16	8.87	" + 5 cc. of 10 % NaHCO_3 injected
13	0.28	16.7	" + 10 cc. of 10 % NaHCO_3 injected
14	1.13	20.3	"
15	0.82	5.15	"

It will be seen that when the dose of sodium bicarbonate was increased to 10 cc. of 10 % solution, an undoubted rise in the total acetone excretion occurred, and this persisted until the next day.

These results do not necessarily prove that the normal metabolism of fat has been interfered with; but taken in conjunction with experiments to be described later on the effect of alkalosis during adaptation to a fat diet, they do suggest that in certain individuals, or when the intensity of the alkalosis is sufficient, some essential step in fat metabolism is obstructed. In any case it is not easy to picture the intimate differences between the two conditions. Perhaps in mild alkalosis we have to do with a direct effect of alkali on chemical equilibria in the body whereas when the alkalosis is intense we may be affecting factors which control the normal oxidation of fats. If this point of view is correct, then during alkalosis in man we encounter a twofold phenomenon: an excretion of organic acids analogous to the effect of mild alkalosis in the rat and, superimposed on this, a slight true ketosis.

The influence of sodium bicarbonate on adaptation to a diet of fat.

Since it appears that large doses of alkali may upset the metabolism of fats, it was of interest to see whether the adaptation to a diet of fat would be interfered with if bicarbonate were added to the food throughout the experiment.

A few experiments bearing on this point have already been mentioned. In that detailed in Fig. 1, administration of sodium bicarbonate after the adaptation was complete had no effect on the "acetone" excretion, but caused a great rise in the β -hydroxybutyric acid output. In Table IX the acetoacetic

acid excretion showed a tendency to rise also (though to a less extent), and in other rats it has behaved similarly. It seemed probable that variations in the intensity of the alkalosis and in the sensitivity of different rats were the causes of these differences. The question was now investigated in greater detail.

Table XI shows the first experiment of this series. The rats (B) with 6 % of sodium bicarbonate in the fat showed an immediate rise in the β -hydroxybutyric acid excretion, but the output of total acetone ran approximately

Table XI.

Four rats from the same litter (about 120 g. each).

Date	A. Fed on diet 3 after 12. v. 24		B. Fed on diet 3 with the addition of 6 % NaHCO_3	
	"Total acetone"	β -hydroxybutyric acid	"Total acetone"	β -hydroxybutyric acid
12. v. 24	0.06	2.25	0.07	2.0
13	0.53	2.0	0.10	20.25
14	6.3	15.16	8.4	53.9
15	19.2	48.5	14.2	86.0
16	4.3	12.44	1.5	36.9
17	—	—	0.55	28.3
18	1.37	3.86	0.82	25.5

parallel with that of the controls. After the third day there was a perfectly definite return of the acetoacetic acid excretion to normal, the β -hydroxybutyric acid remaining high in the bicarbonate rats.

From this experiment it appeared as though the two forms of organic acid excretion were simply behaving additively—the alkali having no influence on the course of the ketosis. This, however, has not been the invariable result. In fact in all of the eight cases in which precisely the same experiment has been repeated, both the intensity of the ketosis and the occurrence of adaptation have been definitely affected. Evidently we have here once more to do with differences in the sensitivity of the rats.

In all the latter experiments, though the ketosis has occasionally shown a slight subsidence towards the tenth day, the rapid adjustment invariably shown by the controls has been completely eliminated. Usually the ratio of β -hydroxybutyric acid to total acetone output has been higher in the bicarbonate experiments. The experiment detailed in Table XII is quite characteristic and will serve to illustrate the main points.

In the bicarbonate rat (C) the ketosis developed more rapidly than in the controls, rose to a higher maximum, and beyond a slight subsidence after the third day, showed no tendency to return to normal by the eighth day; whereas the controls were almost completely adapted by the fifth day.

In order to see whether the power of adaptation had been permanently affected the bicarbonate was discontinued in the diet of (C) on the 16th. It will be seen that though the bicarbonate had been got rid of by the next day (as evidenced by the acidity of the urine), the ketosis subsided only gradually.

On the 15th, 6 % sodium bicarbonate was added to the diet of (B); *i.e.* these rats were given the same diet as (C) had been having. The β -hydroxybutyric acid excretion rose, and so to a less extent did the total acetone; but the effect was very much less than that shown by the rats (C) which had been on this diet throughout. On the other hand the addition of double this quantity of sodium bicarbonate to the diet of (A) at the same time brought about a complete collapse of the adaptation which had been established. This is typical of the results obtained on other rats. Up to a certain dose of bicarbonate the β -hydroxybutyric acid alone is affected; but when the alkalosis reaches a certain intensity the adaptation seems to break down and both acetoacetic and β -hydroxybutyric acids rise to very high concentrations in the urine.

Table XII.

Six rats from the same litter (about 80 g.).						
Date	A. Fed on diet 3 after 8. vii. 24		B. Fed as for A		C. Fed on diet 3 with 6 % NaHCO_3	
	"Total Acetone"	β -hydroxybutyric acid	"Total Acetone"	β -hydroxybutyric acid	"Total Acetone"	β -hydroxybutyric acid
8. vii. 24	0.08	1.78	0.05	1.46	0.06	1.44
9	0.47	2.76	0.33	1.64	0.93	5.04
10	12.67	31.7	7.3	16.0	38.2	120.1
11	16.33	46.0	12.56	36.6	46.0	126.0
12	28.5	81.4	34.8	92.0	35.5	102.2
13	1.69	4.5	6.1	18.8	34.3	98.5
14	1.74	4.10	0.34	2.0	42.7	103.2
15	0.72	2.8 *	0.48	1.82†	30.3	97.6
16	13.82	46.2	0.72	3.2	32.6	101.4†
17	43.2	131.0	2.5	13.0	28.6	73.6§
18	—	—	4.4	20.0	12.57	30.0

* 12 % NaHCO_3 added.† 6 % NaHCO_3 added.‡ NaHCO_3 stopped.

§ Urine acid.

These experiments fully bear out the views put forward above: that when the alkalosis exceeds a critical intensity the ketosis is accentuated. They further suggest that the ketosis is more susceptible to the effect of alkali during the stage of adaptation than after this has been established. Moreover, the adjustment of young rats seems to be more easily broken down than does that of older animals, a fact which is probably correlated with the greater susceptibility of the former to ketosis.

The effect of acids on the ketosis due to a diet of fat.

The effect of acids in causing a depression of the ketonuria of diabetes has never been so definite as the converse effect of alkali. Recently, however, Adlersberg [1923] has shown that there is a marked diminution of ketosis in man, in diabetes and during carbohydrate inanition, after giving ammonium di-hydrogen phosphate.

It has been seen that ammonium di-hydrogen phosphate or ammonium chloride given to a rat on a normal diet leads to a depression in the excretion

of organic acids. These salts were now administered along with a diet of fat alone, to see what effect they would have on the adaptation to this diet.

When rats were fed on diet 3 with the addition of 3 % of ammonium dihydrogen phosphate, variable results were obtained. In some cases the intensity of the ketosis was considerably depressed but in others it was scarcely affected. When however the content of the phosphate was increased up to 10 %, a very marked suppression of the ketosis occurred, and in some cases this was almost completely eliminated. This effect is probably not due to the phosphate ion since addition of a neutral mixture of phosphates did not affect the ketosis. Moreover, the same change is shown when the ammonium phosphate is replaced by ammonium chloride, which would tend rather to rob the body of phosphate. Two experiments showing an average degree of suppression of the ketosis, one with ammonium phosphate and one with ammonium chloride, are given in Tables XIII and XIV. The former salt is usually the more effective. The time relations of the rise and fall in the ketosis do not seem to be constantly affected.

It would appear then that acids have an effect on ketosis just the converse to that of alkalis, and that they exert a favourable influence on the metabolism of fats.

Table XIII.

Date	Four rats from the same litter about 65 g.			
	A. Diet 3 after 19. vii. 24		B. Diet 3 + 10 % $\text{NH}_4\text{H}_2\text{PO}_4$	
	"Total acetone"	β -hydroxybutyric acid	"Total acetone"	β -hydroxybutyric acid
19. vii. 24	0.00	0.75	0.04	0.69
20	0.62	2.68	0.04	0.88
21	32.0	100.8	3.58	8.4
22	28.4	100.6	5.17	10.1
23	10.3	22.9	1.11	2.7
24	3.82	8.2	0.48	1.6

Table XIV.

Date	Four rats from same litter (each about 60 g.).			
	A. Diet 3 after 21. vii. 24		B. Diet 3 + 8 % NH_4Cl	
	"Total acetone"	β -hydroxybutyric acid	"Total acetone"	β -hydroxybutyric acid
21. vii. 24	0.00	0.88	0.06	0.76
22	0.27	1.6	0.15	0.80
23	25.3	65.2	16.4	35.3
24	17.6	42.4	6.0	14.8
25	13.7	30.6	1.5	4.2
26	5.46	12.2	0.29	2.2
27	1.35	3.8	0.24	1.8

A note on alkali therapy.

One practical point deserves mention. It has long been customary to treat the depletion of the alkali reserve of the diabetic by administration of sodium bicarbonate. So long as the increased excretion of acetone bodies which such

treatment evokes was regarded as a mere "washing out" of these substances, there was no evidence that the alkali was actually harmful to metabolism. Haldane, Wigglesworth and Woodrow pointed out that the disturbances in carbohydrate metabolism induced by large doses of bicarbonate were in many respects so like the metabolic disturbances of diabetes as to suggest that the alkali might well accentuate the disease. The present researches show that so far as concerns the metabolism of fat in the absence of carbohydrate—which is the problem always before the diabetic—large amounts of alkali have a definitely inhibitory influence.

SUMMARY.

A modification of existing methods for the estimation of acetone and β -hydroxybutyric acid in small amounts of urine is described.

The normal urine of the rat contains from 0.0–0.30 mg. of "total acetone," and from 0.8–2.5 mg. of β -hydroxybutyric acid per diem.

When deprived of carbohydrate in the diet (and particularly upon a diet of fat alone) the rat shows a marked ketosis. This ketosis increases during the first few days, reaches its maximum at about the third day, and thereafter subsides rapidly, adaptation being usually complete by the fifth day. The ketosis is much more marked in young animals. At the height of the ketosis the ratio of β -hydroxybutyric acid to total acetone is usually between 2 and 4.

Administration of sodium bicarbonate to the rat causes a rise in the output of lactic and β -hydroxybutyric acids but no change in the excretion of total acetone. Acids reverse this effect. There does not appear to be any adaptation to this condition.

Calcium lactate, sodium acetate (and probably the alkali salts of other oxidisable organic acids) have the same effect as alkali. Alkali-forming food (carrots) has the same effect. The bearing of these facts on the results of feeding experiments is pointed out.

The injection of large doses of glucose during the alkalosis causes a fall in the excretion of β -hydroxybutyric acid.

If very large amounts of sodium bicarbonate are injected into the rat the total acetone excretion shows a distinct rise. This is interpreted as indicating a real disturbance in the metabolism of fat.

With one exception the presence of 6 % of sodium bicarbonate in a diet of fat has eliminated the usual adaptation. The adaptation occurs when the alkali is discontinued. The adaptation seems to be less susceptible to alkali once it is established; but sufficiently large doses of sodium bicarbonate will lead to its being broken down completely.

Ammonium di-hydrogen phosphate and ammonium chloride will, in sufficient concentration, cause a suppression of the ketosis due to a diet of fat.

The bearing of these findings upon alkali therapy is pointed out.

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