XLIV. ACIDOSIS AND SOME OF THE FACTORS WHICH INFLUENCE IT.

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(Received October 20th, 1915.)

INTRODUCTION.

The excretion of acetone, aceto-acetic acid, and β -hydroxybutyric acid, to which the name of acidosis was applied by Naunyn, is of great interest to the physiologist, the pathologist and the clinician. Its importance lies in the fact that the output of these substances affords us practically the only clue to the faulty metabolism of fat. It is true that a certain amount of information regarding the quantitative metabolism of fat has been acquired by a study of the respiratory quotient, but by this method we gain little information concerning the qualitative or intermediary metabolism. All that has been ascertained with certainty is that fat is, under normal circumstances, completely oxidised to carbon dioxide and water.

It has long been known that acetone is a normal constituent of the body fluids. It can be detected in small quantities in the blood, in the saliva, in the breath, in the liquor amnii and in the urine. Of these channels of excretion, that by way of the urine is of the most importance and the estimation of the urinary acetone has been adopted here. The other acetone bodies, namely aceto-acetic acid and β -hydroxybutyric acid, are also mainly found in the urine and some account of them will be given in our experiments.

One of the great questions which has been long under discussion [*vide* Magnus Levy, 1908; v. Noorden, 1907; Geelmuyden, 1900; Forssner, 1909, etc.] is the source of the acetone bodies, some workers maintaining that they were derived from fats alone, others just as actively supporting the contention that proteins played a part in their production. Modern work has shown,

and the present communication will bear out the conclusion, that fats may be considered the main source, though, of course, it must be remembered that Dakin [1912] and others have clearly demonstrated that certain constituent amino-acids of the protein molecule can, on decomposition, yield aceto-acetic acid.

METHODS.

The total nitrogen was estimated by Kjeldahl's method and the ammonia by Folin's method. The preformed acetone was estimated by Folin's method, using an apparatus modified to suit the occasion. The total acetone (acetone + aceto-acetic acid) and β -hydroxybutyric acid were estimated by Shaffer's [1908] method. Several workers have investigated this method of Shaffer's and all agree that when estimating the amount of acid in the sodium salt 90-96 % of the theoretical value is found. Kennaway [1914] also finds a similar value but is not satisfied that such a small error is present when the method is applied to urine. He states that "it is of course much more difficult to find out whether the acetone obtained from urine by oxidation is derived wholly from β -hydroxybutyric acid and there seems no satisfactory way of doing this." Certain variations in the results obtained by this method in previous experiments aroused some suspicions as to the possibility of a fallacy, so that before the present experiments were proceeded with, a series of control analyses was made. It was found for instance, when estimating total acetone in a normal urine of a subject on constant normal diet, that the amount of acetone varied greatly from day to day, and, as different quantities of basic lead acetate solution were added to each urine, according to the variations in the quantity of urine, the influence of this salt was naturally investigated first. Accordingly, dry basic lead acetate was heated in a test tube which was connected with another tube containing a measured volume of an alkaline solution of N/10 iodine. After heating 2 g. of the salt for a couple of minutes and then titrating the iodine solution in the ordinary way it was found that some substance capable of combining with iodine to the extent of 3.6 cc. N/10 iodine had been distilled off. In another experiment 10 cc. basic lead acetate solution (U.S.P.) were put into a test tube, 1 cc. H_2SO_4 added, as in estimation of acetone (PbSO_4 was precipitated), and the tube connected up as before with another test tube containing a measured volume of N/10 iodine in alkaline solution. The lead acetate tube was boiled for about a minute and some substance was given off which caused a loss of 0.8 cc. N/10 iodine. In view of the fact

that one of the ordinary laboratory methods for the preparation of acetone is the dry distillation of calcium acetate, it may be assumed that in the present instance the iodine-combining substance distilled off was acetone. This assumption was confirmed by a positive result with the nitroprusside test.

Several other blank determinations were then carried out by the ordinary Shaffer method, but using water in place of urine.

40 cc. basic lead acetate solution (U.S.P.) were added to 250 cc. of water and the whole well shaken. 20 cc. ammonium hydrate were added, mixed, made up to 500 cc. and again well shaken. After standing for some minutes the liquid was filtered. 200 cc. of the filtrate were made up with water to 600 cc. and 15 cc. pure sulphuric acid added. A second 200 cc. were treated the same way. These were then distilled, using an ordinary condenser, until 200 cc. had collected in each receiving flask. The distillate was re-distilled, boiling for twenty minutes with 10 cc. 10 % sodium hydrate. Previous to each distillation the receiving flasks contained 300 cc. of cold water, and care was taken that the connecting tube dipped beneath the surface and that the water did not become hot. The acetone in the distillate was estimated by the iodine titration method. As the figures given show, a small quantity of acetone was evolved in each case.

N/50 iodine cc.	N/50 thiosulphate cc.	Difference cc.
5	3.2	1.8
5	3.3	1.7
5	3.05	1.95
5	3.35	1.65
4	2.25	1.75
4	2.4	1.6
2	0.5	1.5
2	0.7	1.3
4	2.2	1.8
4	2.05	1.95

The difference is seen to be fairly constant and to have an average of 1.7 cc. N/50 iodine.

When carrying out estimations in diabetic urine, or in urine containing a large quantity of acetone bodies, this error may be relatively small, but in normal urine a considerable error is always produced, in some cases more than 100 %.

When analysing normal urines—i.e. practically sugar free—10 cc. of the lead acetate solution were used if less than 100 cc. of urine were taken, 20 cc. solution for 100–150 cc. of urine and 40 cc. solution if more than 150 cc. of

urine. Accordingly after the use of 40 cc. solution, 1.7 cc. was subtracted from the iodine difference obtained, and after 20 cc. or 10 cc. one-half or one-quarter of this amount.

The method, thus corrected, was found to be an excellent one for estimating even very small quantities of acetone.

In the estimation of β -hydroxybutyric acid Shaffer himself admits that there is an error of about 10 %.

Several check experiments were carried out, but, as the results obtained were so discordant, and as the other methods for the estimation of this substance were too slow for our purpose, β -hydroxybutyric acid was estimated in only a few of the experiments recorded in the present paper.

EXCRETION OF ACETONE BY THE NORMAL INDIVIDUAL¹.

A comparatively large number of investigations have been carried out from time to time on the normal urinary acetone output. The results which have been obtained vary to a certain extent, the differences being in part due to the differences in the method of estimation. The results which workers like Hirschfeld [1895] and Geelmuyden [1900] have obtained show that during the twenty-four hours 10-30 milligrams of total acetone are excreted in the urine. The results which I have found in the case of several healthy individuals are in complete agreement with these. It was found also, that on a constant diet the total acetone excreted is practically constant in amount. The quantity found is influenced by the diet, being greater with a high protein intake than with a low one. In one experiment of four days duration, where 400 g. of protein (mainly consisting of caseinogen) per diem were taken, 53 mg. of acetone + aceto-acetic acid were found in the fourth day's urine. The total nitrogen in the same urine was 52.16 g., and in the faeces 2.56 g.

In another experiment where a constant "standard" diet was taken, containing 118 g. of protein, derived from milk, eggs and bread, the amount of total acetone excreted daily in the urine lay between 15.7 mg. and 18.2 mg. The lowest recorded output of acetone in my own case was 5.2 mg. found on the third day on a diet of 100 g. cane sugar per diem.

While carrying out some investigations on post-anaesthetic diaceturia some very low total acetone outputs were found. One patient, who had been operated on for appendicitis, under chloroform and ether, excreted only

¹ The subject employed in all the experiments detailed in this paper was the writer himself.

29.7 mg. total acetone during the four days following the anaesthetic, while another patient, who exhibited no post-anaesthetic diaceturia, excreted 5.1 mg. on the fourth day after a general anaesthetic.

Another point of some interest arises, namely whether, as in the case of the nitrogenous products of metabolism, there is a diurnal variation in the excretion of these acetone bodies. In order to elucidate this point the following experiment was carried out. The standard diet detailed below was taken for five consecutive days. On the first day, the food was taken in three equal parts, at intervals of eight hours. On the second day, two-thirds of the food was consumed in equal amounts two hourly from 7 a.m. till 11 p.m. At 11 p.m. the remaining third of the food was taken. On the third, fourth and fifth days one-twelfth of the food was taken two hourly, day and night. The urine was also collected two hourly and the two hourly output of total acetone and also of total nitrogen for the third, fourth and fifth days are given in the following table (Table I).

Diet.

Wheaten bread	...	...	360 g.	Protein	...	...	107 g.
Dried separated milk	...	...	252 "	Carbohydrate	...	...	480 "
Walnut butter	...	...	72 "	Fat	...	...	73 "
Cane sugar	...	...	211.2 "	Energy value	...	...	3081 cal.

It is seen that the rate of excretion of the acetone bodies is not absolutely constant from hour to hour, but that any variation detected cannot be related to any particular period of the twenty-four hours. Forssner [1909] obtained a very similar result.

Influence of the Nature of the Diet.

Another factor of very considerable importance is the actual nature of the diet. It has long been recognised that, when the external supply of carbohydrate is greatly diminished or entirely cut off, as in starvation, the condition known as acidosis rapidly ensues. The less carbohydrate there is metabolised, relative to the fat metabolised, the greater will be the degree of acidosis and the more acute its onset.

In the following experiments, the influence of fat and protein on the output of preformed acetone and of total acetone on a standard diet was investigated. The standard diet (see previous experiment) was taken for four days. The food was taken every four hours throughout that time. The urine was collected four hourly during the last three days. At 8 a.m. on the third day 300 g. of pure olive oil were taken in addition to the other

TABLE I.

Day of diet	Hour	Total acetone mg.	Total nitrogen g.
3	7-9 a.m.	1.76	1.43
	9-11 "	1.68	1.43
	11-1 p.m.	1.35	1.25
	1-3 "	1.66	1.34
	3-5 "	1.82	1.29
	5-7 "	1.95	1.26
	7-9 "	1.73	1.20
	9-11 "	2.53	1.14
	11-1 a.m.	1.95	1.23
	1-3 "	1.98	1.32
	3-5 "	2.18	1.31
	5-7 "	1.40	1.51
4	7-9 "	1.37	1.62
	9-11 "	1.64	1.62
	11-1 p.m.	1.37	1.34
	1-3 "	1.85	1.41
	3-5 "	1.73	1.13
	5-7 "	1.98	1.50
	7-9 "	1.56	1.39
	9-11 "	1.58	1.43
	11-1 a.m.	1.72	1.28
	1-3 "	1.45	1.52
	3-5 "	1.79	1.55
	5-7 "	1.66	1.58
5	7-9 "	1.63	1.51
	9-11 "	1.81	1.65
	11-1 p.m.	1.43	1.63
	1-3 "	1.24	1.53
	3-5 "	1.31	1.57
	5-7 "	1.24	1.49
	7-9 "	1.47	1.39
	9-11 "	1.40	1.44
	11-1 a.m.	1.19	1.35
	1-3 "	1.23	1.29
	3-5 "	1.16	1.64
	5-7 "	1.63	1.79

food. The oil was well emulsified with water containing 3 g. anhydrous potassium carbonate. The urine collected at midnight and at 4 a.m. was examined in the morning and the remainder examined as collected.

The experimental data given below (Table II) show that the catabolism of fat in the presence of an abundance of carbohydrate does not produce any increase in the output of the acetone bodies in the urine, but rather a slight temporary decrease. This is probably due to the protein-sparing action of the fat, as indicated by the fall in the total nitrogen. As protein is responsible for some of the acetone present, it might be assumed that

a diminished protein metabolism would result in less total acetone being formed. It will be noted that the decrease in the output of the acetone bodies was most marked in the case of the preformed acetone.

TABLE II.

Day	Hour	Total acetone mg.	Preformed acetone mg.	Preformed acetone %	Total nitrogen g.
2	4-8 a.m.	2.15	1.63	75.6	2.59
	8-12 noon	2.88	1.63	56.4	2.90
	12-4 p.m.	2.64	1.97	74.8	2.55
	4-8 „	2.25	0.81	36.1	2.59
	8-12 m.n.	2.78	—	—	2.76
	12-4 a.m.	2.39	1.16	48.5	2.58
3	4-8 „	2.39	0.99	41.3	2.60
	8-12 noon*	2.44	0.99	40.4	2.70
	12-4 p.m.	1.93	1.39	72.0	2.52
	4-8 „	2.20	0.28	12.7	2.16
	8-12 m.n.	1.96	0.52	26.6	1.88
	12-4 a.m.	2.30	0.87	37.8	2.32
4	4-8 „	2.35	0.52	22.3	2.43
	8-12 noon	2.30	0.58	25.2	2.65
	12-4 p.m.	1.79	0.46	26.5	2.35

* 300 g. olive oil at 8 a.m.

When protein is added to the standard diet in place of the oil a definite increase of the total acetone results (Table III).

TABLE III.

Day	Hour	Total acetone mg.	Total nitrogen g.
3	7-11 a.m.	3.72	2.56
	11-3 p.m.	3.48	2.37
	3-7 „	3.60	2.32
	7-11 „	3.99	2.32
	11-3 a.m.	3.14	2.16
	3-7 „	4.08	2.25
4	7-11* „	3.96	3.54
	11-3 p.m.	4.93	4.65
	3-7 „	4.62	3.88
	7-11 „	3.29	3.34
	11-3 a.m.	3.75	3.22
	3-7 „	4.20	3.08

* 100 g. caseinogen at 7 a.m.

The standard diet was taken for four days, during the first two days eight hourly, and during the next two days four hourly, the food being equally partitioned as in previous experiments. At 7 a.m. on the fourth day 100 g. of caseinogen were taken along with the usual food. A sample of the caseinogen boiled with dilute hydrochloric acid and filtered did not reduce Fehling's solution.

Unfortunately, on this occasion the preformed acetone was not estimated, and it is therefore impossible to say which of the acetone bodies increased, or whether both were raised in amount. The figures are in keeping with those of other experiments, and all indicate that the greater the protein break-down, the higher is the amount of total acetone excreted.

EFFECT OF PRECEDING DIET ON THE ACIDOSIS OF STARVATION.

The next series of experiments was carried out chiefly to observe the effect of the preceding diet on the acidosis of starvation. This acidosis is almost certainly due to the great deficiency in the supply of carbohydrate available for immediate use. The sudden increase in the urinary acetone bodies probably occurs when the stored carbohydrate has all been utilised. Almost every experiment points to the fact that the animal organism prefers to derive its energy from carbohydrate rather than from fat, or even from protein. Carbohydrate is the most easily oxidised food-stuff and is a better protein sparer than fat, and for these reasons, when the body is starving and requiring the maximum amount of energy with the minimum of protein destruction, carbohydrate will be used so long as it is available. When this has been exhausted, most of the energy would appear to be derived from fat. The ratio of the fat oxidised to the carbohydrate oxidised then becomes much higher than it was previously, and, when this ratio rises above a certain definite value, abnormal quantities of acetone bodies appear in the urine. The results of experiments done on this subject point to the existence of a quantitative relationship, which has a very definite minimal value, between the fat and the carbohydrate utilised to prevent acidosis. In the light of these observations the initial rise in the excreted acetone bodies is readily understood.

Starvation following a high Carbohydrate Diet.

Second day before the fast—normal diet to which was added cane sugar and other carbohydrate-rich food.

Pre-day's diet consisted of:

Cane sugar	...	450 g.	Cornflour	...	40 g.
Tapioca	...	115 g.	Milk	...	50 g.
Bread	...	300 g.	Butter	...	15 g.
Potatoes	...	150 g.	Lean meat	...	50 g.

Some jam.

This gives a carbohydrate intake of some 757 g. The urine was collected from the time at which the last meal was taken. None of the urine collected reduced Fehling's solution, though that on the pre-day did so slightly. The period of fasting was three days. The results are shown in Table IV.

TABLE IV.

Day of fast	Total acetone mg.	Total nitrogen g.
1	20.51	4.17
2	28.79	4.41
3	138.96	—
	{First 12 hrs 30.22 Second „ 108.74	

Starvation after high Protein Diet.

Second day before—high protein diet

Pre-day: Caseinogen ... 460 g. Bread ... 150 g.
Lean meat ... 100 g. Butter ... q.s.
2 eggs.

The last meal consisted of 120 g. caseinogen and the urine was collected from that time. The period of fast was three days (see Table V).

TABLE V.

Day of fast	Total acetone mg.	Total nitrogen g.
1	26.75	34.72
2	77.84	15.47
3	235.85	18.92
	{First 12 hrs 57.58 Second „ 178.27	

Starvation after high Fat Diet.

Pre-day: 300 g. pure olive oil well emulsified with water and 3 g. K_2CO_3 .
1 egg and 30 g. oatmeal at 10 a.m.
90 g. lean meat at 1 p.m.

Last meal, consisting of 150 g. olive oil, at 6 p.m. and the urine collected from that time. The period of fast was two days (see Table VI).

TABLE VI.

Day of fast	Total acetone mg	Total nitrogen g.
1	61.70	7.21
2	259.01	10.53
	{First 12 hrs 98.30 Second „ 160.71	

These results show conclusively that the acetone output in the early days of starvation is governed by the nature of the preceding diet.

In the first experiment there was sufficient stored carbohydrate to prevent acidosis for some forty-eight hours. The output of total acetone during that period was not above the normal. During the third day, however, a marked increase of the bodies in question occurred, 30.2 mg. being excreted in the first twelve hours and 108.7 mg. in the second. It will be noticed that the onset of acidosis is very rapid. This is found to be the case in almost every experiment, and, as will be shown later, its disappearance is even more rapid. This rise in the total acetone may occur at any part of the twenty-four hours, neither day nor night having any influence over it.

The second experiment shows that a large intake of protein previous to starvation also delays the onset of acidosis. The delay is not due to the presence of protein itself, for this is rapidly deaminised after absorption, the nitrogen-free part of the molecule being catabolised or stored, probably as carbohydrate. In all probability it is the carbohydrate derived from protein which postpones acidosis, and, as there would be less carbohydrate stored up at the beginning of this fast than at the beginning of the previous one, acidosis would here be apparent at an earlier period. On the first day's starvation 26.75 mg. of total acetone were found in the urine; but as the total nitrogen was 34.72 g. a considerable part of this acetone was almost certainly derived from the protein. On the second day the total acetone found was relatively much greater than the normal. On the third day, especially in its latter half, a further and more pronounced increase took place.

As one would expect, when a high fat diet precedes starvation, there is an increased output of acetone bodies almost from the beginning, due to the fact that this fat during its combustion would draw on the stored carbohydrate. The sudden rise in the total acetone excreted is here observed on the second day.

ACIDOSIS IN STARVATION.

The amount of acetone excreted in the urine during starvation has been determined by Brugsch [1905], Bönniger and Mohr [1906] and others. On the first day the quantity is generally small. On the second day variable amounts are found, but by the third day a marked rise is always observed and the increase continues till about the fifth day. A diminution in the amount of the acetone bodies then occurs, and a more or less constant output of 0.4–0.8 g. is recorded, this being about half or less of the quantity found when the output is at its maximum.

Brugsch for example found the following results for the twenty-third to the thirtieth day of a thirty days fast by Succi.

Day	Total acetone mg.	Total nitrogen g.
23	569	5.837
24	410	6.410
25	463	6.272
26	569	6.182
27	525	6.302
28	339	4.437
29	242	4.193
30	115*	8.421

* Lemonade with sugar taken.

To what is this fall in the total acetone output due? Acidosis can be diminished by the catabolism of carbohydrate or of protein, and if only for economic reasons, the former is the more likely cause of the change, unless some altogether unknown factor in the animal metabolism plays a part. We have next to find the source of this carbohydrate, assuming that it has brought about the decreased acetone excretion.

Landergren [1903] stated that the sole source of carbohydrate in starvation was protein. Now there is less protein metabolised per diem in the second week of starvation than in a like period during the first few days, and consequently at the later period less carbohydrate would be produced, leading to an increased fat : carbohydrate ratio. This would inevitably be accompanied by an accentuated acidosis. On the contrary, however, the reverse is found, so that another cause must be sought.

Although the conversion of fat or fatty acids into carbohydrate has not been proved, the possibility of such a change has been put forward by several physiologists of repute, and a certain amount of evidence in favour of it has accumulated. It does seem possible that, under special circumstances, when, for example, the body is in great need of carbohydrate, such a change may occur. That sugar can be derived from glycerol is already known, but whether this occurs in the body remains to be proved. A certain amount of evidence in favour of the view that in diabetes an increased output of sugar follows the administration of glycerol does certainly exist. If fat yields carbohydrate the diminished acidosis might readily be explained.

FACTORS INFLUENCING ACIDOSIS.

The results which have been dealt with up to the present deal with the factors which influence the onset of acidosis, and it is now proposed to consider some of the factors which influence the acidosis after it is definitely present.

1. *Effect of feeding Carbohydrate.*

The subject had been on a fat diet (320 g. pure olive oil per diem) for three days. At 7 a.m. on the fourth day 100 g. of cane sugar were taken and the same again at 2 p.m. At 6.30 p.m. and 11 p.m. eggs and bread and butter were taken.

Table VII gives the results for the third (the last) day of the oil diet and for the fourth day when carbohydrate was given.

TABLE VII.

Day	Hour	Total acetone mg.	Preformed acetone mg.	Preformed acetone %	Total nitrogen g.
3	7-10 a.m.	89.83	11.84	13.1	1.94
	10-1 p.m.	85.72	11.60	13.5	1.70
	1-4 "	91.53	10.73	11.7	1.53
	4-7 "	88.87	11.84	13.3	1.49
	7-10 "	104.10	13.23	12.7	1.52
	10-1 a.m.	91.28	16.59	18.2	1.30
	1-4 "	114.49	18.22	15.9	1.76
	4-7 "	141.81	22.34	15.7	1.67
4	7-10* "	50.43	14.16	28.1	1.29
	10-1 p.m.	12.23	6.27	51.0	1.24
	1-4* "	9.96	5.57	55.9	1.19
	4-7† "	4.50	—	—	0.97
	7-10 "	2.27	1.86	81.8	1.21
	10-7† a.m.	4.98	3.48	69.9	3.60

Day β -Hydroxybutyric acid

3 1273 mg.

4 206 "

* 100 g. of cane sugar at 7 a.m. and 2 p.m.

† Eggs, bread and butter at 6.30 p.m. and 11 p.m.

2. *Effect of feeding Protein.*

The subject had been fasting for two days, the fast being preceded by a high fat diet. At 6 p.m. on the second day 100 g. of caseinogen were taken, and no more food for twelve hours afterwards (see Table VIII).

TABLE VIII.

Day	Hour	Total acetone mg.	Preformed acetone mg.	Preformed acetone %	Total nitrogen g.
2	6-10 a.m.	50.19	7.35	14.64	1.88
	10-2 p.m.	53.33	7.54	14.15	2.20
	2-6* "	57.20	8.12	14.21	1.83
3	6-8 "	15.38	3.87	25.15	1.74
	8-10 "	6.91	3.19	46.15	1.42
	10-2 a.m.	10.54	2.71	25.68	4.28
	2-6 "	15.38	2.71	17.61	3.46

* 100 g. caseinogen at 6 p.m.

As will be seen from the preceding tables both carbohydrate and protein cause a rapid diminution in the amount of acetone bodies found in the urine.

(1) The sudden disappearance of acidosis after feeding sugar is very striking, the output of total acetone dropping from 141.8 mg. in one period of three hours to 50.4 mg. in the subsequent three hours. This change actually occurred even more rapidly than the figures indicate, since some thirty minutes would elapse before the sugar was available in the body, and during this time acetone bodies would be excreted at the same rate as before the sugar was taken. Evidently the presence of carbohydrate causes a profound alteration in the course of fat catabolism. The suddenness of the change suggests that it is the latest stages in the oxidation of the fatty acids which are affected. It is not improbable that the first stages in the oxidation of fat are accomplished independently of carbohydrate, but that the later stages of the catabolism are diverted from the normal in the absence of this material. At least the glyceryl radicle of fat can be oxidised in absence of carbohydrate.

The diminished acetone output was accompanied by an immediate fall in the total nitrogen, so that presumably the same moiety of carbohydrate which is available for the prevention of acidosis acts also as a protein sparer.

(2) As both absorption and combustion of protein take place more slowly than they do in the case of carbohydrate, the effect of protein on the metabolism is longer in becoming apparent. The acidosis was quickly checked, but not reduced to normal, probably because the caseinogen did not yield sufficient carbohydrate or did not yield it quickly enough.

The marked rise in the total nitrogen (about 100 %) in the first two hours after feeding showed that deamination was already going on.

3. *Effect of feeding Fat.*

The subject had been fasting for three days. 200 g. pure olive oil, well emulsified, were taken immediately at the close of the fasting period. No further food was taken for twelve hours.

The effect of fat will be seen from the following table (Table IX).

TABLE IX.

Day	Hour	Total acetone mg.	Preformed acetone mg.	Preformed acetone %	Total nitrogen g.
3	8-12 noon	27.68	3.68	13.28	1.89
	12-4 p.m.	38.12	4.64	12.18	2.02
	4-8 „	42.95	4.64	10.74	1.74
4	8-10* „	10.31	3.09	29.97	0.90
	10-12 m.n.	13.20	3.35	25.38	0.74
	12-8 a.m.	91.81	19.34	21.07	—

* 200 g. olive oil taken at 8 p.m.

The figures show that after feeding fat, there was an immediate drop in the total acetone output during the first four hours and perhaps longer. This was succeeded by a very definite rise. A change was apparent during the first two hours so that the effect of the oil on the metabolism must soon have been felt. It must be remembered that the oil, being well emulsified, would be quickly absorbed.

More than one explanation is suggested for this unexpected decrease. The fat supplied certain energy needs which in the starving organism were probably covered by carbohydrate thus liberating carbohydrate for metabolic purposes. Again the oil might be more readily utilised than the body fat and consequently a sparing of the latter would occur, while the former would be broken down. If the first stage of this oxidation do not require the presence of carbohydrate in order to be normally carried out—and this is probably the case—then there will be a relatively greater amount of carbohydrate available to bring about the complete oxidation of the already partially broken down body fat, carbon dioxide and water being excreted in place of acetone bodies.

This decrease in the degree of acidosis, following immediately on taking fat, seems to be an argument in favour of the first stages in the oxidation of fat occurring without the aid of carbohydrate. A similar result will be alluded to in a future experiment. The subsequent rise in the total acetone output is again due to the disturbed quantitative relationship between the carbohydrate and the fat catabolised.

4. *Effect of feeding Glycerol.*

In another experiment 100 g. of glycerol were fed at 8 p.m., after fasting for three days. The results obtained are given in Table X.

TABLE X.

Day	Hour	Total acetone mg.	Total nitrogen g.
3	8-12 noon	31.23	4.00
	12-4 p.m.	60.10	2.95
	4-8 "	86.93	1.88
4	8-10* "	37.86	1.15
	10-12 m.n.	12.47	0.90
	12-8 a.m.	5.42	5.04

* 100 g. glycerol were taken at 8 p.m.

Glycerol also causes a disappearance of acidosis. During the first two hours the decrease in the total acetone was slight, but by the end of four hours a marked diminution was observed. A normal amount of total acetone was excreted between the fifth and twelfth hours inclusive after taking glycerol.

How does glycerol act? It may possibly be converted into sugar. The greatly diminished acidosis which followed its ingestion suggests that such a conversion has taken place. This change may not occur under normal circumstances, but when carbohydrate is scarce such a conversion may be postulated. Again glycerol, as an energy yielding substance, will cause the body fat to be spared, thereby reducing the fat : carbohydrate ratio and bringing about a diminished acidosis. Such a marked change as was recorded could not have occurred, however, had glycerol acted only in this way.

5. *Effect of feeding Alcohol.*

The pre-day diet contained approximately 80 g. protein, 400 g. carbohydrate and 80 g. fat.

On each of the two following days 40 g. of absolute alcohol were taken and no other food. The alcohol was well diluted with water and taken at regular intervals from morning till night. Table XI shows the results obtained.

TABLE XI.

Day	Total acetone mg.	Total nitrogen g.	NH ₃ g.
1	132.2	11.49	0.416
2	192.0	14.83	0.588

The results confirm Hirschfeld's [1895, 1897] statement that alcohol does not prevent acidosis.

Being a source of energy it will probably cause a slight diminution in the total acetone by sparing fat, but it exerts no such influence as does carbohydrate, protein or glycerol on the later stages of the oxidation of fat.

INFLUENCE OF HIGH FAT DIET ON ACIDOSIS.

A series of experiments was next carried out to find the effect of protein-free fat-rich diets on the urinary output of acetone bodies. The results of two of these experiments are compared with results obtained in experiments where the fat was omitted from the diet and carbohydrate alone fed. Unfortunately, owing to the disagreeable nature of the diet and to the discomfort it produced, the experiments could not be carried out for longer periods than three days. At the beginning of each period, during which oil was being taken, 2-3 g. calcium carbonate were taken. Diarrhoea was altogether absent and, in fact, the bowels rarely moved. This is in marked contrast with the extremely severe diarrhoea met with on a fat diet, when butter is the fat ingested.

Pure Fat Diet.

The pre-day diet contained very little carbohydrate and consisted of 6 eggs, 320 g. filleted cod, 280 g. fresh haddock, 170 g. potatoes, 50 g. bread, butter, q.s.

Total carbohydrate about 84 g., the last of which was taken seventeen hours before the oil diet began.

Diet days. 80 g. pure olive oil, emulsified as before, using 1.5 g. potassium carbonate, were taken four times a day at equal intervals, viz. 7 a.m., 1 p.m., 7 p.m., 1 a.m. This was continued for three days. The urine was collected three hourly from 7 a.m. on the first day. The first table (Table XII) is a daily chart, the second (Table XII A) a three hourly one.

TABLE XII.

Day	Total acetone mg.	Preformed acetone mg.	Preformed acetone %	β -Hydroxybutyric acid mg.	Total nitrogen g.
1	147.9	37.4	25.3	411	14.78
2	389.2	61.7	15.8	616	14.96
3	817.6	116.4	14.3	1273	13.50
Day after	84.4	32.1	38.1	206	9.51
					31—2

TABLE XII A.

Day	Hour	Total acetone mg.	Preformed acetone mg.	Preformed acetone %	Total nitrogen g.
1	7-10 a.m.	2.25	—	—	2.57
	10-1 p.m.	4.01	0.81	20.2	1.94
	1-4 "	8.66	2.21	25.5	1.83
	4-7 "	22.35	6.50	29.0	1.43
	7-10 "	11.72	4.13	35.3	1.31
	10-1 a.m.	35.90	8.82	24.6	1.27
	1-4 "	35.32	8.12	22.9	1.88
	4-7 "	27.71	6.85	24.7	2.00
2	7-10 "	53.33	7.25	13.6	1.92
	10-1 p.m.	49.70	8.47	17.0	2.05
	1-4 "	52.85	7.89	15.0	1.82
	4-7 "	48.98	6.61	13.5	1.45
	7-10 "	41.73	6.15	14.9	1.32
	10-1 a.m.	34.72	8.47	24.4	1.59
	1-4 "	42.94	8.34	19.4	1.66
	4-7 "	64.94	8.53	13.1	1.87
3	7-10 "	89.83	11.84	13.1	1.94
	10-1 p.m.	85.72	11.60	13.5	1.70
	1-4 "	91.53	10.73	11.7	1.50
	4-7 "	88.87	11.84	13.3	1.49
	7-10 "	104.10	13.23	12.7	1.52
	10-1 a.m.	91.29	16.59	18.2	1.30
	1-4 "	114.49	18.22	15.9	1.76
	4-7 "	141.81	22.34	15.7	1.67

A gradual increase in the urinary acetone bodies is observed from beginning to end. It will be noticed, however, during the second and third days of the diet, that on most occasions there was less total acetone in the urine passed during the second three hours after taking oil, than during the first three hours. A similar occurrence was observed and commented on in a previous experiment where oil was fed during starvation.

How long the increasing acidosis would have continued it is difficult to say. Would it cease earlier or later when fat is being fed than when the body is living on its own fat? Would the acidosis become so great as to produce serious symptoms? These questions must remain unanswered till a more prolonged series of experiments is carried out. Is there a more pronounced acidosis when the body is living on food fat than when living on its own fat? Forssner [1910] found that the addition of fat to a diet of fat and protein caused a rise in the total acetone excreted. In the experiment at present under consideration there is a much greater acetone output than in any of the starvation experiments. Moreover the highest acetone output during starvation was found after a high fat diet, when the body was using

external fat as well as its own fat, so that as far as these results go this question may be answered in the affirmative. Forssner gave as a reason for this either that food fat is broken down in a different manner from body fat or that part of the former is converted into acetone bodies after ingestion.

The preformed Acetone.

The figures in this experiment, as well as those of other experiments communicated in this paper, show that the greater part of the total acetone is present in the form of aceto-acetic acid. Where the amount of total acetone is small the percentage of preformed acetone is high, perhaps 50–60 or more, but where the total acetone output is high only about 15 % of it is in the form of preformed acetone. A similar result was obtained by Piper [1913] and led him to use the term diaceturia in preference to acetonuria.

It is not to be supposed that the small quantity of acetone found in the urine is all that is produced in the body. This substance is very volatile and is excreted in larger quantities in the breath than in the urine. In order to test this point the following experiments were carried out.

On the evening of the third day on this diet about half of the air expired during twenty minutes was passed through a measured quantity of alkaline N/50 iodine with the result that 1.5 mg. of acetone were detected. Owing to the lack of a proper respiratory apparatus it was impossible to carry out an absolute quantitative examination of the expired air, and the result obtained must be regarded as representing a mere fraction of the acetone present. The breath had a marked odour of acetone.

EFFECT OF ADDING CARBOHYDRATE TO A FAT DIET.

The addition of sugar to the diet causes a great reduction in the amount of acetone bodies excreted. The greater the weight of sugar added the less is the amount of acetone bodies found.

In order that the results might be uniform the quantities of protein and fat taken on the day preceding each of this series of diets were practically the same in every case, the former amounting to some 180 g. and the latter to about 80 g. The same quantity of carbohydrate was taken on each pre-day as was taken on the succeeding diet days. The olive oil was always emulsified as already described.

The addition of 60 g. cane sugar reduced the daily amount of urinary acetone from decigrams to centigrams, but the well-marked increase in the total acetone from day to day showed that a greater amount of sugar was

required to prevent acidosis. With the addition of 100 g. of cane sugar there is still more acetone than normal after the first day. When 150 g. of sugar were added to the fat more acetone was excreted on the third day than on the first, but the increase was slight. The amount of cane sugar required to prevent acidosis when 320 g. of fat are fed is then about 150 g. If all the oil is utilised the fat : carbohydrate ratio will be a little more than 2 : 1.

TABLE XIII.

Diet : 60 g. cane sugar—320 g. olive oil.

Day	Total acetone mg.	β -Hydroxybutyric acid mg.	Total nitrogen g.
1	40.13	256	10.97
2	60.56	210	9.18
3	81.11	499	7.55

TABLE XIV.

Diet : 100 g. cane sugar—320 g. olive oil.

Day	Total acetone mg.	β -Hydroxybutyric acid mg.	Total nitrogen g.
1	15.37	173	11.54
2	35.01	268	10.85
3	69.72	234	4.83

TABLE XV.

Diet : 150 g. cane sugar—320 g. olive oil.

Day	Total acetone mg.	Total nitrogen g.
1	10.12	6.94
2	21.47	4.73
3	24.05	2.24*

* Repeated analysis showed this result to be correct.

EFFECT OF FEEDING CARBOHYDRATE ALONE.

When 30 g. of sugar per diem are taken the amount of acetone found is only one-sixth to one-eighth of that found when the organism is totally deprived of food (Table XVI).

TABLE XVI.

Diet : 30 g. cane sugar per diem.

Day	Total acetone mg.	β -Hydroxybutyric acid mg.	Total nitrogen g.	NH ₃ g.
1	27.86	174	9.12	0.366
2	25.47	254	10.35	0.337
3	34.91	601	10.34	0.396

With 60 g. sugar more acetone was found than in the previous experiment. This must be due to the effect of the preceding diet, though the pre-day diet was almost the same in each case (Table XVII).

TABLE XVII..

Diet : 60 g. cane sugar per diem.

Day	Total acetone mg.	β -Hydroxybutyric acid mg.	Total nitrogen g.
1	21.64	186	8.51
2	45.51	224	8.01
3	79.05	214	9.70

TABLE XVIII.

Diet : 100 g. cane sugar per diem.

Day	Total acetone mg.	Total nitrogen g.
1	6.6	6.94
2	9.7	11.28
3	5.2	9.81

When 100 g. of cane sugar are taken (Table XVIII) in the course of the twenty-four hours, there is less total acetone found in the urine than is found when one is taking a normal diet. This quantity of sugar is sufficient then to prevent acidosis when no fat is taken in the food. As there was not much work done, about 2500 cal. would supply sufficient energy for one day. The sources of energy would be roughly as follows:

100 g. cane sugar ...	...	...	...	380 cal.
9 g. N = 57 g. protein ...	...	...	...	235 cal.
				<hr/> 615 cal.

The remaining 1885 cal. would be mainly derived from 203 g. body fat. Here again the fat : carbohydrate ratio is a little more than 2 : 1. While it is freely admitted that these two experiments giving a similar fat : carbohydrate ratio are not absolutely conclusive, the assumption may be made that there is a definite minimum fat : carbohydrate ratio required to prevent acidosis, and that this ratio is a little more than 2 : 1.

EFFECT OF THE VARIOUS DIETS ON THE TOTAL NITROGEN.

It was also noticed that the more sugar there was added to the diet the less was the amount of total nitrogen found in the urine, and that on the diets containing fat + carbohydrate less total nitrogen was eliminated than on the diets of carbohydrate alone. In each experiment where the diet consisted of olive oil a feeling of intense lassitude and dullness was present,

accompanied by abdominal discomfort. The former symptom was never absent, while the latter persisted for about two hours after taking oil. No vomiting occurred in any of the experiments recorded although a feeling of intense nausea was frequently present. A few experiments had to be discontinued owing to rejection of the oil even when taken through a stomach tube. These symptoms were less marked when sugar was added to the diet. If carbohydrate (100 g. cane sugar) was taken at the conclusion of the oil period all feelings of discomfort disappeared in less than two hours, although the limbs remained "tired" for some five hours longer. During these diets thirst was rarely experienced and little water was taken. Immediately after taking food an intense thirst developed and remained for about a day.

THE SIGNIFICANCE OF THE ACETONE BODIES.

It is known that in the body the fatty acids are oxidised in the β -position, and that eventually butyric acid is always formed [Ringer, 1913].

The prevailing opinion is that the oxidation of this acid in the β -position gives rise to β -hydroxybutyric acid as a normal intermediary product of fat metabolism. In the absence of carbohydrate this acid is only partially oxidised, aceto-acetic acid and acetone being formed.

Blum [1910] however maintains that normally butyric acid is first converted into aceto-acetic acid and that this is reduced to β -hydroxybutyric acid. Marriot [1914] also favours this view. In any case when carbohydrate is available the fatty acids are completely oxidised, carbon dioxide and water being the end products.

How does this carbohydrate act?

Ringer [1913] concludes from a series of very interesting experiments that it is not the whole carbohydrate molecule, but only the CHO radicle, that is necessary to prevent acidosis. He found that gluconic acid, differing only from glucose in having the CHO radicle replaced by CO.OH, did not prevent acidosis, while $\text{CH}_3 \cdot \text{CHO}$ and $\text{C}_2\text{H}_5 \cdot \text{CHO}$, when fed to phloridzinised dogs, brought about a great decrease in the total acetone output.

Ringer [Ringer, Frankel and Jonas, 1913] has also shown that isobutyric acid, when fed to phloridzinised dogs, gives rise to extra glucose and

diminished acidosis; whereas normal butyric acid does not give rise to glucose but to an increased acidosis. He suggests that a glucosidic union is accomplished between β -hydroxybutyric acid and glucose by means of the CHO radicle, and that the derivatives of normal butyric acid are in this way converted into iso-compounds. This would explain the seeming necessity of a constant fat : carbohydrate ratio.

But more than a mere chemical union between β -hydroxybutyric acid and glucose seems to be involved. This is suggested by the fact that the feeding of 30 g. of sugar is sufficient to reduce the total acetone of complete starvation from 200–300 mg. to 35 mg. (see Table XVI), while a further addition of 70 g. of sugar is necessary to reduce this to less than 10 mg. This reduction could not be due to a diminution of the quantity of acetone derived from protein, as the total nitrogen in the latter case was quite as high as in the former. Again, the addition of 60 g. of sugar to a fat diet reduced the total acetone from 818 mg. to 81 mg. (Tables XII and XIII) while with a further addition of 90 g. of sugar 24 mg. of total acetone were excreted.

Ringer, in his experiments already mentioned, administered acetaldehyde and propyl aldehyde to phloridzinised dogs on a constant diet. The result was that not only was the acetone in the urine greatly diminished, but there was an increase in the output of sugar. This increase was more than could be accounted for even if all the carbon in these aldehydes had been converted into glucose carbon. He concluded that this increase in glucose was due to some non-glucogenetic substance in the animal metabolism becoming glucogenetic owing to the influence of the CHO radicle. Perhaps the same change can take place when sugar is given in starvation. A small quantity of carbohydrate may be sufficient to bring about the necessary change, while a large amount may be unable to increase its extent, or, when the non-glucogenetic substance has become glucogenetic it may only remain in that condition so long as acidosis is present in a marked degree. It is very probable that this potential glucogenetic substance is either fat itself or one of the products formed during the early stages of fat catabolism.

This work was carried out under the direction of Dr E. P. Cathcart, to whom I offer my thanks.

The expenses in connection with this research were in part defrayed by a grant made to Dr E. P. Cathcart by the Carnegie Trust.

CONCLUSIONS.

1. The quantity of acetone bodies excreted by the normal individual, on an ordinary diet containing a sufficiency of carbohydrate, is influenced chiefly by the protein intake. On an ordinary diet 10–30 mg. are excreted daily.
2. The administration of quite small amounts of carbohydrate to the starving organism brings about a great reduction in the acidosis.
3. The administration of protein to the fasting organism causes a similar decrease, but this is neither so marked nor so rapid as in the case of carbohydrate.
4. The administration of glycerol under similar conditions also causes a very definite reduction in the acidosis.
5. The administration of fat to the starving organism increases the acidosis.
6. Administration of alcohol is without effect on the degree of acidosis.
7. The amount of acetone bodies in the urine during the first few days of starvation depends on the initial carbohydrate storage. These substances appear in abnormal amounts immediately the ratio of the fat to the carbohydrate burnt becomes greater than about 2:1. Immediately the ratio becomes less than this the acetone output is reduced to normal.
8. While a relatively large amount of carbohydrate is required to prevent acidosis, quite a small amount suffices to check it very markedly.
9. When acetone bodies are excreted in excessive amounts they are for the most part derived from fat.
10. Some evidence is put forward in favour of the possibility of fat being converted into carbohydrate in the body.

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