XLI. THE RELATIVE AMOUNTS OF β -HYDROXY-BUTYRIC ACID AND ACETO-ACETIC ACID EXCRETED IN ACETONURIA.

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Various workers have shown that a reversible reaction occurs in the body between aceto-acetic acid and β -hydroxybutyric acid [for references see Dakin, 1912]

Neubauer [1910] showed that when aceto-acetic acid was administered to a diabetic, not only this acid, but also β -hydroxybutyric acid was excreted in increased amounts, and he confirmed the older statement that the administration of β -hydroxybutyric acid in diabetes increased the output of both acids. He pointed out further that the relative amounts of the two acids in the urine in cases of acetonuria should indicate the equilibrium point of the reversible reaction within the body. He found that the amount of β -hydroxybutyric acid, expressed as a percentage of the total acetone bodies, was remarkably constant, this percentage being from 60 to 80 in well-marked cases of acetonuria, and somewhat lower than this when the total output of acetone bodies was small. In spite of the enormous numbers of estimations of acetone bodies which have been made, no other writers appear to have paid much attention to the relative amounts of these substances. The cases given below serve to provide some illustrations of Neubauer's statement.

It is necessary to have some short term by which to express the relative amounts of β -hydroxybutyric acid and aceto-acetic acid in urine. The term

" β -ratio" is used below to denote the percentage of the total acetone bodies which is made up by β -hydroxybutyric acid, each substance being calculated in the form of acetone.

No attempt is made here to establish with any great accuracy the proportions in which the different acetone bodies are produced in the body. To do this in a satisfactory manner, one would require firstly an accurate method for the estimation of β -hydroxybutyric acid. The method used in nearly all these observations gives results which are from 4 to 10 % too low [Shaffer and Marriot, 1913 ; Kennaway, 1914]. If the β -ratio in a urine were actually 75, and the result of the β -hydroxybutyric acid estimation were 10 % too low, the β -ratio found would be 72.96. Secondly, one would require estimations of the amount of acetone in the expired air. Since there is a considerable fall in molecular weight in the formation of acetone (from 102 to 58), the comparatively small amounts of this substance in the breath are of importance in ascertaining the total production of acetone bodies.

The estimations on Case 1 below were made by the Messinger-Huppert and polarimetric methods, and all the others by a combination of the Shaffer and Scott-Wilson methods [Kennaway, 1914]. The pathological urines were obtained with two exceptions from the wards of Guy's Hospital, and I wish to express my thanks for the material to the physicians in charge of these cases. I am greatly indebted also to Drs Pembrey, Poulton, and Graham for permission to include in the tables their estimations of alveolar carbon dioxide, and to Drs Poulton and Graham for the opportunity to make observations in the course of their numerous experiments upon themselves [Graham and Poulton, 1913, 1914, 1, 2].

CASES OF DIABETIC ACIDOSIS.

If a person is excreting two molecules of β -hydroxybutyric to one of aceto-acetic acid, the β -ratio will be 66.6 ; if five molecules to one, it will be 83.3, and so on. In Fig. 1, all the β -ratios obtained from Cases 1-14 of diabetes are arranged in order of magnitude ; Case 15, which showed a much smaller output of acetone bodies and lower β -ratio than any of the others, is omitted to save space (see Table IV).

It is hardly necessary to point out that this figure shows merely the range observed in these cases ; it does not of course show the frequency of occurrence of any particular ratio, the number of points near the level of 75 being due to the number of estimations made on Case 4.

The results given in the tables below indicate that Case 1 was excreting chiefly two molecules of β -hydroxybutyric acid to one of aceto-acetic acid; in Case 4, the proportion was about three to one, and in Case 7 probably five to one.

Fig. 1. β -ratios of Cases 1-14 (Diabetes), in order of magnitude. $\times$ indicates urine at autopsy.

TABLE I.

Cases 1-3. *Diabetes.*

	Date	Urine c.c.	G. acetone per day			β -ratio
			Aceto-acetic acid + acetone	β -hydroxy- butyric acid	Total	
Case 1.	27 vii.	1650	1.29	3.22	4.51	71.4
	29 vii.	2460	1.01	2.056	3.066	67.1
	30 vii.	1700	0.51	1.037	1.547	67.0
	1 viii.	1500	0.472	0.975	1.447	67.4
	2 viii.	1210	0.454	0.970	1.424	68.0
Case 2.	28 i.	3380	5.24	14.13	19.37	73.0
	6 ii.	5000	6.41	20.85	27.26	76.5
Case 3.	1 v.	4145	5.43	20.67	26.10	79.2
	7 v.	4125	6.04	21.55	27.59	78.1
	9 v.	3030	4.85	19.37	24.22	80.0

Case 4 (Table II and Fig. 2) had received before coming under observation large doses of sodium bicarbonate, on account of signs of incipient coma. The alveolar carbon dioxide pressure was found by Dr Pembrey to be abnormally high (49.3 mm.), no doubt as a result of the ingestion of alkali. In the table, the doses of bicarbonate are calculated as acetone derived from the

amounts of β -hydroxybutyric acid which could be neutralised by the bicarbonate, in order to show the assistance given to the neutralising powers of the body. On two occasions (Aug. 9th and 18th) the dose of alkali was doubled, and the alveolar carbon dioxide pressure showed a slight increase.

TABLE II.

Case 4. Diabetes.

Date	Urine c.c.	Alveolar CO ₂ mms. Hg.	NaHCO ₃ as g. acetone per day	G. acetone per day			β -ratio
				Aceto-acetic acid + acetone	β -hydroxy- butyric acid	Total	
18 vii. ..	—	—	62	—	—	—	—
19 ..	—	—	92	—	—	—	—
20 ..	—	—	32	—	—	—	—
21 ..	4640	49.3	16	6.39	14.72	21.11	69.8
22 ..	4000	46.4	„	5.37	13.19	18.56	71.1
23 ..	4040	39.1	„	3.99	10.68	14.67	72.8
25 ..	4250	39.4	„	3.59	12.01	15.60	76.9
28 ..	5830	35.7	„	4.54	13.71	18.25	75.1
30 ..	6670	34.2	„	6.57	19.21	25.78	74.5
31 ..	6510	—	„	7.82	21.24	29.06	73.1
1 viii. ..	4560	33.3	„	4.96	13.28	18.24	72.8
3 ..	2710	31.4	„	3.96	11.86	15.82	75.4
6 ..	4270	33.9	„	4.00	12.26	16.26	75.4
7 ..	4600	—	„	4.15	13.96	18.11	77.1
8 ..	3130	36.0	„	1.94	5.99	7.93	75.6
9 ..	7390	40.3	32	5.36	16.84	22.20	75.9
10 ..	4810	—	32	3.56	9.58	13.14	72.9
11 ..	9290	37.8	16	4.85	12.20	17.05	71.5
12 ..	7790	38.1	„	6.87	21.96	28.83	76.2
13 ..	6770	33.3	„	5.82	18.25	24.07	75.8
17 ..	5820	—	„	7.03	19.95	26.98	73.8
18 ..	7640	34.3	32	7.83	21.49	29.32	73.3
19 ..	9670	36.6	32	7.89	21.80	29.69	73.4
20 ..	7260	35.2	16	4.21	12.21	16.42	74.3
						mean	mean
						20.34	74.1

In Cases 5–13, the urine present in the bladder at autopsy was examined; the results obtained are enclosed in brackets in the tables. The β -ratio in this urine is in most cases low, and in those in which the comparison is possible (Cases 5, 7, 8, 9)¹, it is lower than it was on the previous days; a similar change was observed in a non-diabetic condition (Case 26). The explanation of this is not clear. One would not expect the oxidative change of the

¹ In case 6 the comparison cannot be made owing to the week's interval after the last estimation before death.

reversible reaction to be favoured in the hours preceding death. Possibly a diminished output of acetone from the lung may be the cause of the difference.

TABLE III.

Cases 5-13. Diabetes.

		Date	Urine c.c.	Alveolar CO ₂ mm. Hg.	G. acetone per day		Total	β -ratio
					Aceto-acetic acid + acetone	β -hydroxy- butyric acid		
Case 5.	..	25 vi.	1860	27.4	3.47	15.77	19.24	81.9
		2 vii.	2200	27.0	4.42	14.72	19.14	76.9
		3 vii.	2000	—	5.06	15.74	20.80	75.7
		4 vii.	1860	26.9	3.92	11.11	15.03	73.9
		5 vii.	3600	24.5	8.21	24.49	32.70	74.9
		7 vii.	—	17.6	—	—	—	—
		8 vii.	—	8.8	—	—	—	71.0
Not 24 hours urine	{ Coma ,,	9 vii.	—	—	—	—	70.0	
Death on	..	9 vii.	—	—	—	—	—	
Urine at autopsy		..	—	—	—	—	[63.4]	
Case 6.		3 iii.	3820	—	3.33	10.30	13.63	75.5
		4 iii.	3860	—	4.12	12.31	16.43	74.9
		5 iii.	3090	—	3.94	12.60	16.54	76.2
		10 iii.	5305	—	5.99	18.18	24.17	75.2
		11 iii.	4830	—	4.58	11.97	16.56	72.3
		12 iii.	6050	—	5.98	15.67	21.65	72.4
		Death in coma on	20 iii.	—	—	—	—	—
Urine at autopsy	..	—	—	—	—	[77.6]		
Case 7.		10 iii.	5580	13.8	4.30	20.33	24.63	82.5
		Coma on	.. 12 iii.	—	—	—	—	83.6
		Death on	.. 13 iii.	—	—	—	—	—
		Urine at autopsy	..	—	—	—	—	[77.7]
Case 8.		30 v.	4800	29.7	10.56	23.66	34.22	69.1
		Coma. Death on	2 vi.	—	—	—	—	—
		Urine at autopsy	..	—	—	—	—	[68.4]
Case 9.		30 i.	—	17.8	—	—	—	70.3
		No coma, death on	31 i.	—	—	—	—	—
Urine at autopsy		..	—	—	—	—	[68.1]	
At autopsy	{ females	Case 10	..	—	—	—	—	[69.7]
		Case 11	..	—	—	—	—	[66.6]
		Case 12	..	—	—	—	—	[66.2]
		Case 13	..	—	—	—	—	[67.4]

Cases 14 and 15 showed a much smaller output of acetone bodies, and the β -ratios are low. In Case 15, the urine was almost free from sugar; the amount of acetone from aceto-acetic acid decreased to 11 mg. per day, while normal urine yields from 2 to 4 mg. [Scott-Wilson, 1911].

TABLE IV.

Cases 14 and 15. Diabetes.

	Date	Urine c.c.	Alveolar CO ₂ mm. Hg.	G. acetone per day			β -ratio
				Aceto-acetic acid + acetone	β -hydroxy- butyric acid	Total	
Case 14.	13 v.	1240	36.1	0.347	0.627	0.974	64.4
	20 v.	1350	35.2	0.226	0.278	0.504	55.1
Case 15.	7 v.	1425	—	0.103	0.087	0.190	45.8
	20 v.	1580	38.7	0.0114	0.0077	0.0191	40.2

The largest change in the β -ratio within three days observed in this series of diabetics is that from 71.5 to 76.2 in Case 4.

Fig. 2. Case 4 (Diabetes). Continuous line represents β -ratio. Upper dotted line represents alveolar CO₂ pressure in mm. Hg. Lower dotted line represents daily output of total acetone bodies as grams of acetone. The days on which the dose of sodium bicarbonate was doubled are enclosed in brackets.

CASES OF NON-DIABETIC ACIDOSIS.

In the first days of carbohydrate starvation in a healthy person no constancy of the β -ratio, such as is seen in cases of diabetes, is to be expected, since the degree of acidosis and the whole state of metabolism are changing

rapidly. The ratio rises with the increasing formation of acids, and may reach the value seen in cases of severe diabetes (Table V and Fig. 3).

TABLE V.

Acidosis due to lack of carbohydrate in healthy persons.

				Urine c.c.	Alveolar CO ₂ mm. Hg	G. acetone per day			β -ratio	
						Aceto-acetic acid + acetone	β -hydroxy- butyric acid	Total		
<i>Case 16.</i>										
Diet of protein and fat (Graham)										
2nd day	..	..		700	33.4	0.280	0.303	0.583	52.0	
3rd day	..	..		1310	30.8	0.754	1.539	2.293	67.0	
<i>Case 17.</i>										
Diet of protein and fat (Poulton)										
2nd day	..	..		670	38.3	0.402	0.521	0.923	56.4	
3rd day	..	..		1070	37.0	0.446	0.556	1.002	55.4	
<i>Case 18.</i>										
Diet of protein and fat (Donaldson)										
2nd day	..	..		758	36.5	0.437	0.299	0.736	40.7	
<i>Case 19.</i>										
Starvation (Poulton)										
1st day	..	..		410	—	0.087	0.101	0.188	53.8	
2nd day	..	..		1230	36.1	0.584	1.007	1.591	63.3	
3rd day	..	..		730	35.3	0.539	1.383	1.922	72.0	
<i>Case 20.</i>										
Starvation (Graham)										
2nd day	..	..		1240	36.1	0.477	0.692	1.169	59.2	
3rd day	..	..		1680	33.0	0.527	0.905	1.432	63.2	
Diet of fat										
4th day	..	..		1300	32.5	0.462	1.126	1.588	70.9	
5th day	..	..		1390	—	0.882	2.638	3.520	74.9	

Cases 21, 22, and 23 (Table VI) show low ratios with the excretion of very small amounts of acetone bodies. Some of the samples obtained from cases of vomiting (24 to 28) in young children were very similar as regards percentage and proportions of acetone bodies to the urine in severe forms of diabetes. Case 24 was subject to attacks of cyclical vomiting, in which condition well-marked acid intoxication occurs, but whether the illness on this occasion was of that nature was uncertain. Case 26 showed a condition of hyperpnoea and drowsiness such as is seen before the onset of coma in diabetes; the administration of dextrose and sodium bicarbonate had no beneficial effect, and the child died on the fifth day of the illness. The volume of urine was of course small as a result of the vomiting, and the percentages of acetone

bodies in this case are the highest in the whole series. Unfortunately the 24 hours output could not be ascertained. Cases 27 and 28 (Table VII) show

Fig. 3. Cases of non-diabetic acidosis. Continuous line represents β -ratio. Lower dotted line represents day's output of β -hydroxybutyric acid in decigrams. Upper dotted line represents day's output of β -hydroxybutyric acid + aceto-acetic acid in decigrams.

TABLE VI.

				G. acetone in 100 c.c. urine			
				Aceto-acetic acid + acetone	β -hydroxy-butyric acid	Total	β -ratio
Case 21. Carcinoma of Oesophagus							
after about 36 hours fasting				0.016	0.0094	0.0254	37.0
4 hours later				0.0147	0.0123	0.027	45.6
Case 22. After anaesthesia				0.0181	0.0083	0.0264	31.6
Case 23. " "				0.054	0.077	0.131	58.8
Case 24 (age 2 years). Vomiting				0.058	0.149	0.207	71.5
Case 25 (age 5 years). "				0.139	0.468	0.607	77.1
Case 26 (age 22 months). " ..19 vi.				0.193	0.766	0.959	80.0
21 vi.				0.316	0.925	1.241	74.6
Death on 22 vi.				—	—	—	—
Urine at autopsy				0.357	0.956	1.313	[72.8]
				Amounts in 24 hours			
				Urine c.c.			
Case 27 (age 7 years). Vomiting 22 v.				520	0.393	2.033	2.42
Recovery25 v.				550	0.086	0.069	0.155
27 v.				550	trace	trace	—
Case 28 (age 6 years). Vomiting 24 vi.				575	0.917	3.76	4.67
Recovery27 vi.				1210	0.062	0.042	0.104

the fall in the β -ratio which occurs when the total amount of acetone bodies decreases (Fig. 3). The high proportion of β -hydroxybutyric acid in these cases is noteworthy. Case 28 excreted on one day 0.3 g. acetone bodies per kilo. body-weight (15.5 kilos.); in a patient with severe diabetes, excreting 35 g. acetone bodies and weighing 70 kilos., the output would be 0.5 g. per kilo.

DISCUSSION OF RESULTS.

The cases of non-diabetic acidosis show much the same range of β -ratio (from 31.6 to 83.8) as do the cases of diabetes (from 40.2 to 83.6). The value 31.6 indicates about one molecule of β -hydroxybutyric acid to two of aceto-acetic acid, while a ratio of 83.6 corresponds to a proportion of about five molecules of the former to one of the latter acid. These are then the extreme limits of composition of the mixtures observed in this series.

The results given in the tables above show that the higher β -ratios occur in the cases excreting the larger total amounts of acetone bodies, but there is no approach to any close parallelism between the two sets of figures. The data given here are of course much too few for the establishment of any rules, but they indicate that a person excreting daily acetone bodies yielding more than about one and a half grams of acetone produces two or more than two molecules of β -hydroxybutyric acid to one of aceto-acetic acid (Table VII).

TABLE VII.

					β -ratio	Daily output of acetone bodies as grams acetone	Alveolar CO ₂ mm. Hg.
Diabetes.	Case	3	..	..	mean 79.1	mean 26.0	—
"	"	5	..	..	" 76.7	" 21.2	mean 26.4
"	"	2	..	..	" 74.7	" 23.3	—
"	"	6	..	..	" 74.4	" 18.2	—
"	"	4	..	..	" 74.1	" 20.3	" 37.3
"	"	1	..	..	" 68.2	" 2.4	—
"	"	14	..	..	" 59.7	" 0.74	" 35.6
"	"	15	..	..	" 43.0	" 0.104	" 38.7

Results of all estimations on 24 hours urine.

Diabetic	..	..	..	{	over 75	" 20.5	" 32.4
					75 to 66	" 17.8	" 35.6
					below 66	" 0.42	" 36.7
Non-diabetic	..	..	..	{	over 66	" 2.73	" 32.9
					below 66	" 0.78	" 35.8
All cases	..	..	..	{	over 66	maximum 34.2	—
						minimum 1.42	—
					below 66	maximum 1.59	—
						minimum 0.019	—

A shifting of the equilibrium point in the direction of lessened oxidation along with increase in the total production of acetone bodies is evident in Fig. 3, which shows the rapidly developing acidosis in the cases of carbohydrate starvation, while the reverse change is seen in recovery from attacks of vomiting.

The cases of severe diabetes behave somewhat differently in this respect. The results tabulated under Cases 4 and 6 show that fluctuations of 100 % or more in the amounts of acetone bodies produced leave the β -ratio almost unaffected¹. In these conditions of persistent acidosis, the two acids seem to be produced in very constant proportions which are unaffected by large variations in their total amounts.

One might suppose that the higher proportion of β -hydroxybutyric acid in the urine in the cases of more severe acidosis was due to the washing-out of acetone from the blood into the expired air by the increased lung-ventilation which occurs in such cases [Beddard, Pembrey, and Spriggs, 1908]. If this were the whole explanation, the β -ratio should show an inverse relationship to the alveolar CO_2 pressure, the former rising as the latter falls. The cases given in the tables show that on the whole the higher β -ratios do accompany the lower CO_2 pressures (Table VII), but in detail the relation between the two quantities is very irregular, and there are some quite definite exceptions. Thus Case 4 (diabetes) showed an alveolar CO_2 pressure within or even above normal limits at a time when the β -ratio was above 70, and in Case 19 (starvation) the ratio rose on the third day to about this level, while the alveolar CO_2 was but slightly lowered. Changes in lung-ventilation do not then provide a sufficient explanation of the variations in the proportions of acetone bodies in the urine.

The rise in the β -ratio with increasing acidosis can hardly be due to a shortage of oxygen. If one atom of oxygen were required to convert one molecule of β -hydroxybutyric acid into aceto-acetic acid, the amount of oxygen required to lower the β -ratio for the fifth day in Case 20 (Table V) from the actual value of 75 to 33, would be about 280 c.c., an amount which would be absorbed in less than a minute. Moreover the production of the acid itself increases the supply of oxygen to the lung, by action on the respiratory centre.

The increase in the proportion of β -hydroxybutyric acid when the total amount of the two acids rises suggests that it is the hydroxy-acid which is

¹ Magnus-Levy [1908] has drawn attention to the large variations in the output of acetone bodies which occur even when all the conditions of diet and treatment are kept as constant as possible

the primary product ; the evidence upon this point obtained in other ways is not sufficient to decide this question [Dakin, 1912].

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SUMMARY.

Estimations are given of the relative amounts of β -hydroxybutyric acid and aceto-acetic acid in the urine in conditions of acidosis. The cases of diabetes show the constancy in the proportions of the two acids to which Neubauer drew attention. In carbohydrate starvation the proportion of β -hydroxybutyric acid rises with the increasing formation of acetone bodies. The estimations on cases excreting more than about two and a half grams of the two acids daily indicate the production of one molecule of aceto-acetic acid to from two to five molecules of β -hydroxybutyric acid.

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