X. THE ACETONE BODIES OF THE BLOOD IN DIABETES.

By ERNEST LAURENCE KENNAWAY.

From the Physiological Laboratory, Guy's Hospital, and the Bland-Sutton Institute of Pathology, Middlesex Hospital.

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THE analyses of blood given below were made some years ago, but were not published as the method of estimation of aceto-acetic acid employed was found to be unsatisfactory. When known amounts of sodium aceto-acetate are added to urine, a yield of from 97 to 100 % of the theoretical amount of acetone can be obtained by the method published previously [Kennaway, 1914, 1]. But when the same method is applied to blood, a yield of no more than 88 % is obtained, and no means have been found of overcoming this defect. β -Hydroxybutyric acid shows no such difference in behaviour in blood and in urine; possibly the greater chemical activity of aceto-acetic acid, which is indicated by its constitution [Hurtley and Trevan, 1916], causes it to combine with some constituent of the blood which is not present in urine.

However, no results have been found in the literature which have been proved to be more accurate than those here brought forward; the analytical methods employed previously have been tested not with aceto-acetic acid, but with acetone, and the recovery of acetone from blood is a comparatively easy matter. Moreover, in a recent paper Hurtley [1916] has drawn attention to the great lack of any information on the amounts of acetone bodies present in the blood in diabetes; the analyses of Geelmuyden and of Marriot appear to be the only ones to be found in the literature. Geelmuyden [1909] estimated the acetone bodies in the blood obtained post-mortem from two cases of diabetic coma; in his earlier series [1904] the β -hydroxybutyric acid was not estimated. Sassa [1914] estimated the β -hydroxybutyric acid alone in the organs of three diabetics, but did not examine the blood. Marriot [1914] gives the amounts of acetone bodies obtained by his nephelometric method in the blood, withdrawn during life, from eight cases of diabetes; only one of these showed any signs of impending coma. The results from this case, together with those of Geelmuyden, are appended to Table II below for comparison.

METHOD OF ESTIMATION.

The estimations were made by the combination of the Schaffer and Scott-Wilson method described previously [Kennaway, 1914, 1] for the estimation of acetone bodies in urine. As a rule the addition of basic lead acetate solution (U.S.P.) and ammonia (sp. gr. 0.88) in the proportion of 60 cc. of the former and 25 cc. of the latter for 50 cc. blood produces a perfectly clear colourless filtrate; if too little ammonia be added the filtrate is coloured with reduced alkaline haematin and froths on distillation. The first distillates are redistilled with caustic soda and hydrogen peroxide as described by Schaffer [1908]. This method gives satisfactory agreement between duplicate analyses; the final Scott-Wilson estimations are carried out invariably in duplicate, as ample material is available at this stage.

TESTS OF METHOD.

(a) *With sodium β -hydroxybutyrate.*

The salt used gave practically theoretical figures on combustion [see Kennaway, 1914, 1]. The loss in the estimations on blood given below is no greater than that observed in estimations on pure solutions of the substance (i.e. yield of acetone about 95 % of that calculated [Schaffer and Marriot, 1913; Kennaway, 1914, 1]).

(1)	50 cc. ox blood ¹ + 0.1616 g. sodium β -hydroxybutyrate = 74.4 mg. acetone
	found 70.3 mg. = 94.5 %.
(2)	50 cc. ox blood + 0.1519 g. sodium β -hydroxybutyrate = 69.9 mg. acetone
	found 67.3 mg. = 96.3 %.

(b) *With sodium aceto-acetate.*

Aceto-acetic ester was saponified in the cold with the theoretical amount of caustic soda, the solution diluted, and the aceto-acetic acid estimated by Scott-Wilson's method.

		mg. acetone in 25 cc. distillate
Calculated from weight of ester		0.8490
Found		0.8485

When such a solution is added to urine a practically quantitative yield of acetone can be obtained, whereas in the presence of blood no more than about 88 % of this amount was recovered by the same method. This is not due to any products of decomposition in the blood used, since freshly drawn blood

¹ The amounts of acetone obtained from the blood alone in a blank estimation were of course subtracted from the result in all these experiments.

(rabbit) does not constantly give better results, although in one such experiment 93.7 % was obtained. Serum causes as much loss as does the whole blood. No relation was found between the percentage lost and the absolute amount present. The following are examples from among a considerable number of experiments.

- (1) 50 cc. ox blood + sodium aceto-acetate solution = 70.7 mg. acetone
found 63.1 mg. = 89.1 %.
- (2) 50 cc. ox blood + sodium aceto-acetate solution = 30.08 mg. acetone
found 26.07 mg. = 86.7 %.
- (3) 50 cc. ox blood + sodium aceto-acetate solution = 30.94 mg. acetone
found 27.16 mg. = 87.8 %.
- (4) Sodium aceto-acetate solution treated in same
way with basic lead acetate and ammonia,
but without blood
calculated 31.20 mg. acetone
found 31.22 mg. = 100 %.

(c) *With acetone.*

20 cc. acetone solution titrated by iodoform method 31.3 mg. acetone
20 cc. added to 50 cc. ox blood 30.5 mg. = 97.4 %.

Control analyses of non-diabetic bloods.

These results (Table I) serve merely as controls upon the much larger amounts of acetone bodies obtained in fatal cases of diabetes; they are not to be regarded as showing the actual amount of acetone bodies present in normal blood. It is probable that a large part of the substance obtainable from normal blood which is precipitated by Scott-Wilson's reagent is not acetone at all, for the ratio between the amounts resulting from the operations for the estimation of aceto-acetic acid and β -hydroxybutyric acid respectively is very irregular in the series. For this reason the total amounts only are given in the Table.

TABLE I. *Control analyses of non-diabetic bloods.*

				Total acetone bodies as mg. aceto-acetic acid in 100 cc. blood
Human cases, at autopsy	{	Heart failure	...	16.8
		Head injury; death 3 hours later	...	17.3
		Head injury; death 1 hour later	...	6.6
		Head injury; death 1 hour later	...	7.6
		Septicaemia	...	11.3
		Carcinoma of liver	...	4.7
		Eclampsia	...	3.0
		Sheep	...	5.0
		Sheep	...	8.7
		Horse (in life)	...	1.7
		Ox	...	6.2
		Ox	...	4.3
		Ox	...	4.5

TABLE II. Amounts of acetone bodies are expressed as mg. of aceto-acetic acid in 100 cc.

	Urine				Blood				NaHCO ₃ given by mouth on last days of life	Remarks		
	Acetone + aceto- acetic acid		Total acetone bodies	β - ratio	Acetone + aceto- acetic acid		β -Hydroxy- butyric acid	Total acetone bodies			β - ratio	
	β - ratio	β -Hydroxy- butyric acid										
Case 5	177	306	483	63	109	170	279	61	81	1	7. vii. 13, not in coma; alveolar CO ₂ 17.6 mm. 8. vii. 13, in coma; alveolar CO ₂ 8.8 mm. 9. vii. 13, death in coma	
"	6	114	396	510	78	53	132	185	71	39	3	Coma; boy, aged 11
"	7	149	522	671	78	96	165	251	66	31	3	10. iii. 13, not in coma; alveolar CO ₂ 13.8 mm. 12. iii. 13, coma. 13. iii. 13, death in coma
"	(1) 370	880	1250	70	91	152	243	62	70	5	5	Blood and urine (1) obtained on 30. i. 14; alveolar CO ₂ then 17.8 mm. Conscious till death on 31. i. 14.
"	(2) 185	422	607	69	96	117	213	55				Blood and urine (2) at autopsy
"	10	229	528	757	70	106	181	287	63	62	1	6. i. 14, not in coma; alveolar CO ₂ 15.0 mm. 7. i. 14, 5 p.m. in coma; alveolar CO ₂ 7.2 mm. Death at 10 p.m.
"	11	311	621	932	67	98	169	267	63	?	—	Coma
"	12	98	192	290	66	83	125	208	60	40	2	Coma
"	26	628	1682	2310	73	38	95	133	71	?	—	Well-nourished boy, aged 22 months. Diarrhoea and vomiting began 18. vi. 14. Hyperpnoea, drowsiness. Death on 22. vi. 14. At autopsy, "status lymphaticus."
"	29	—	—	—	59	40	99	40	40	?	—	Very drowsy for some days, but not in coma till three hours before death
"	30	—	—	—	63	215	278	77	?	?	—	Coma
"	31	—	—	—	60	101	161	63	?	?	—	Coma
"	32	—	—	—	76	lost	—	—	—	?	—	Very drowsy but never comatose. Persistent vomiting. At autopsy, twisted ovarian dermoid
"	33	—	—	—	36	18	54	33	?	?	—	Blood taken just before discharge from hospital; no signs of approaching coma. Blood sugar 0.44 %; average daily sugar in urine 340 g.
Geelmuyden												
A.Z.	241	646	887	73	89	119	208	57	—	—	—	Coma. At autopsy
P.F.	491	498	989	50	94	244	338	72	—	—	—	"
L.H.	—	—	—	—	119	—	—	—	—	—	—	"
K.O.	190	—	—	—	95	—	—	—	—	—	—	"
P.K.	81	—	—	—	28	—	—	—	—	—	—	"
Marriot												
Case 6	—	—	—	82	49	79	128	62	—	—	—	"Showed signs of impending coma"

Marriot [1914] states that normal blood (of man, dog, pig and ox) contains less than 9.7 mg. total acetone bodies, reckoned as aceto-acetic acid, in 100 g. This result is in agreement with those given in Table II; in some of the pathological cases which give a higher figure an abnormal formation of acetone bodies may well have been in progress, since acetonuria may occur in almost any condition of disease.

Analyses of blood from cases of diabetes.

These are given in Table II. The cases up to No. 26 are numbered as in a previous paper [Kennaway, 1914, 2], where analyses of the urine on several days preceding death are recorded for some of them. All the cases in the table were adult diabetics, except No. 6, a diabetic boy, and No. 26, a non-diabetic infant; all the diabetics died in coma except Nos. 9, 32 and 33. All the samples of urine and blood were obtained at autopsy, except the first in case 9 and that in case 33. The estimations of alveolar CO_2 were made by Dr Poulton, to whom I am indebted for permission to include them here.

The term " β -ratio" was adopted in the previous paper to denote briefly the percentage of the total acetone bodies which is made up by β -hydroxybutyric acid, each substance being expressed in the form of acetone. If, in the absence of separate estimations of the acetone present, one regards this substance as aceto-acetic acid¹, the above percentage gives with sufficient accuracy the molecular proportions of the two acids, since their molecular weights differ by less than 2 %.

DISCUSSION OF RESULTS.

(1) Hurlley [1916] and Hurlley and Trevan [1916] have brought forward evidence to show that aceto-acetic acid has a specific toxic action, apart from its acid character, and that β -hydroxybutyric acid is a much less toxic substance both because it is a weaker acid (by eight times), and because it lacks the $\text{C.OH} = \text{CH}$ group, which is held to be the cause of the special toxicity of aceto-acetates and of other compounds having this group (sodium salicylate, acetylacetone). One may examine the figures given in Table II to see if they have any application to this view. If one excludes the non-fatal

¹ The quantity of acetone may be by no means unimportant in this connection; in Marriot's Case 6 (see Table II) it was found to be 63 % of the acetone + aceto-acetic acid. In such estimations, however, there must always be uncertainty as to the amount of decomposition of the acid to acetone which has occurred in vitro.

case No. 33 and the non-diabetic case No. 26 the amounts of acetone bodies show the following range (see also Fig. 1):

	Minimum	Maximum	Ratio
Aceto-acetic acid, mg.	53 (Case 6)	109 (Case 5)	1 : 2
β -Hydroxybutyric acid, mg.	40 (Case 29)	215 (Case 30)	1 : 5.5

Marriot's case (in life) gave a slightly lower figure (49 mg.) for aceto-acetic acid; two out of Geelmuyden's seven results are above these limits (119 mg. aceto-acetic acid in L.H. and 244 mg. β -hydroxybutyric acid in P. F.), and one is considerably lower (28 mg. aceto-acetic acid in P.K.).

In cases 29, 30 and 31 one has three instances in which the amounts of aceto-acetic acid are almost the same (59, 63, and 60 mg.) while those of β -hydroxybutyric acid (40, 215 and 101 mg.) include the maximum and minimum given in the summary above. Fig. 1 illustrates the variations which occur in the relative amounts of the two acids.

Fig. 1. Cases of Diabetes, at autopsy.

Ordinates represent acetone bodies, expressed as mg. aceto-acetic acid, in 100 cc. blood.

Upper series, β -hydroxybutyric acid.

Lower series, acetone + aceto-acetic acid; these are placed in order of magnitude.

The horizontal lines represent the respective means of the two series.

If one is to derive from these results any evidence as to the relative toxicity of the two acids, it is certainly suggestive that the range of the amounts present at death is much smaller in the case of the aceto-acetic acid; differences in individual susceptibility might perhaps cause variations of 100 % in the lethal dose of a substance, but would be unlikely to explain the range of 550 % seen in the amounts of β -hydroxybutyric acid. It might be said

that the large differences in the amounts of the latter acid tolerated before the fatal issue were due to differences in the amounts of alkali administered, but the data on this point given in Table II do not show any such influence; and furthermore, this factor should lead one to look for as large variations in the amounts of aceto-acetic acid, since this is an acid eight times as strong as the other.

It is noteworthy that in the 24 hours preceding death in case 9, the β -hydroxybutyric acid underwent a distinct fall, and the aceto-acetic acid a slight increase, in amount; this might be attributed [Hurtley, 1916] to failure of the protective activity of the liver in converting the latter acid into the former. The recent experiments of Wilder [1917] show the conversion of aceto-acetic acid to β -hydroxybutyric acid (in the dog).

These results are then compatible with the view that aceto-acetic acid has a specific toxic action and are perhaps as favourable to it as can be expected from their nature; single observations cannot throw much light upon the course of a progressive intoxication. A much larger series of observations, made in each case before and during coma, and after death, together with measurements of the hydrogen ion concentration in the blood and full daily analyses of the urine, and records of the administration of alkali, are required to give any definite information on this question.

(2) Although, as was pointed out above, there is no constant relationship between the amounts of β -hydroxybutyric acid and aceto-acetic acid in the blood, the most frequently occurring proportion in this series is certainly such that there are between 1.5 and 2 molecules of the former acid to one molecule of the latter, for the β -ratio in seven of the ten fatal diabetic cases lies between 60 and 66. Case 33, in which there were no signs of approaching coma, shows a much smaller amount of acetone bodies, together with a much lower β -ratio (33), than do any of the other cases. The high proportion of aceto-acetic acid in such a case is to be expected from the results obtained with a considerable number of urines from cases of diabetes and of other conditions of acidosis [Kennaway, 1914, 2]. When the amount of the two acids is low, the amount of aceto-acetic acid is relatively high; it falls as the severity of the case increases, and tends to rise again on the approach of coma [Hurtley, 1916]. If one adopts the view that aceto-acetic acid has a special toxicity the explanation of this series of changes would seem to be as follows: if the total amount of acids is sufficiently small, the presence of a high proportion of aceto-acetic acid is of no great importance, and the β -ratio remains low: as the absolute amount rises, a protective mechanism

for the conversion of aceto-acetic into β -hydroxybutyric acid is called into increased activity, and the β -ratio rises; while the final increase in the proportion of aceto-acetic acid would indicate the failure of this mechanism (see Table II, first and second samples in case 9).

The figures for the β -ratio given in Table II show that the proportion of β -hydroxybutyric to aceto-acetic acid is always higher in the urine than in the blood¹. One might be inclined to attribute this difference to a final accumulation of aceto-acetic acid in the blood before death, after the bulk of the urine found in the bladder at autopsy had been secreted; but the samples of blood and urine obtained from case 9 in life show the same difference. The explanation may be that the kidney receives the blood within a few seconds of its passage through the lung, where acetone has escaped; whereas the venous blood, after its slower passage through the systemic capillaries and veins, will have developed a fresh quantity of this substance.

(3) The data given in Table II enable one to form some quantitative idea of the strain which the production of these quantities of acids must place upon the maintenance of neutrality in the body.

(a) Suppose that a patient such as No. 5, with acetone bodies equivalent to 280 mg. aceto-acetic acid in 100 cc. blood, weighed 70 kilos and contained 3.5 litres of blood; the amount of acid in the whole blood would be 9.8 g. According to Schmidt's [1850]² analyses, the plasma of 1000 g. of human blood contains 1.66 g. sodium. 9.8 g. aceto-acetic acid is equivalent to 2.21 g. sodium, which would be contained in the plasma of 1330 g. or 1250 cc. of blood, which is more than one-third of the whole blood in this case. Of course other bases, especially ammonia and calcium, are available as well; but some calculation of this sort may serve to illustrate the difficulties under which neutrality is maintained, or nearly maintained [Poulton, 1915] in severe diabetes.

The concentration of acetone bodies in this case (No. 5) if expressed as aceto-acetic acid, and regarded as an aqueous solution of the free acid, is nearly that of a 0.03 *N* solution.

(b) In case 9, the blood in life contained 243 mg. acetone bodies as aceto-acetic acid in 100 cc.; this is equivalent to 52 cc. CO₂ from NaHCO₃ at 0° and 760 mm., i.e. to the whole amount of CO₂ obtainable from this

¹ Geelmuyden's case P.F. is an exception.

² These seem to be the most modern full analyses of human blood, and are those which appear in most text-books. Schmidt's tables give 1.66 g. sodium in the 487 g. plasma of 1000 g. of human blood; Arronet and Warnach [1887, 1888] found 0.344 g. sodium in 100 g. of human serum. The results on plasma and serum therefore agree very closely ($0.344 \times 4.87 = 1.67$).

volume of normal blood. The alveolar CO_2 tension at the time when this blood was withdrawn was found by Dr Poulton to be 17.8 mm.; if the curve given by Christiansen, Douglas, and Haldane [1914] showing the CO_2 content of blood in relation to CO_2 tension were applicable to abnormal bloods, one could obtain from these data the amount of CO_2 displaced from the blood by this known amount of abnormal acid, which would be of considerable interest.

(c) 243 mg. aceto-acetic acid (see case 9) is equivalent in molecular proportions to 214 mg. lactic acid; this amount of lactic acid, when added to 100 cc. blood, is sufficient to produce the same effect upon the dissociation constant of haemoglobin, as expressed by K, as is produced by the whole normal tension of CO_2 [see Barcroft, 1914, Fig. 111].

(d) The data given for the first eight cases in the table illustrate the part played by the kidney in the maintenance of neutrality. In this series, the mean concentration of acetone bodies in the urine (868 mg. %) is nearly four times that in the blood (230 mg. %)¹. In case 9, the complete 24 hours' urine could not be obtained; but if its volume had been 3 litres, a common amount in such cases, and the patient's blood-volume 3.5 litres, the protective action of the kidney would have been equivalent to freeing the whole blood of acetone bodies between four and five times in the 24 hours.

In the non-diabetic case 26, the very high concentration of acetone bodies in the urine (more than seventeen times that in the blood) is noteworthy; it is no doubt due to excessive loss of water from the alimentary canal. The figures emphasise the need for a very abundant supply of fluid, given by any available method, in such cases.

(e) Hurtley [1916] points out that if the intoxication in severe diabetes were no more than a simple acid poisoning, such as can be produced experimentally in animals, treatment by alkalis should be more successful than it is found to be in practice. As much as 3 ounces (93 g.) of sodium bicarbonate²

¹ Dr Poulton has pointed out to me that the data given in the previous paper [Kennaway, 1914, 2] for case 5, in which the percentage of acetone bodies in the urine at death (483 mg.) is only 1.9 times that in the blood (279 mg.), show a fall in the concentration of acetone bodies removed by the kidney, as well as in the alveolar CO_2 tension, the latter change being no doubt due to the retention of increasing amounts of acid.

	July 2	3	4	5	7	8	9 (day of death)	at autopsy
Total acetone bodies of urine, as gms. }	1.53	1.83	1.42	1.60	—	0.857	1.04	0.483
aceto-acetic acid per cent.								
Alveolar CO_2 , mms. Hg.	27.0	—	26.9	24.5	17.6	8.8	—	—

² Such large amounts of sodium, when given without counteracting ions, may not be altogether harmless. Locke's fluid for perfusion of the mammalian heart contains Na. K, and Ca approximately in the proportions Na : K : Ca/100 : 6 : 2.5. These proportions of the three kations are

is sometimes given in a day; this is sufficient to neutralise 113 g. of the acids, which is almost equal to the amount which might be present in the whole body of a case such as No. 9, supposing the acids to be distributed uniformly throughout the blood and other body fluids (about 50 litres in a person weighing 70 kilos). As a matter of fact, this case (No. 9) did receive on each of the five days preceding death an amount of NaHCO_3 (70 g.) sufficient to neutralise the abnormal acids in 35 litres of blood having the composition found at this time; this would be quite 10 times the blood volume of the patient. Moreover, it is exceptional for a diabetic to produce even as much as 50 g. of the acids, as indicated by the output in the urine, in a day. There can, of course, be no doubt that the body in such cases has urgent need of the assistance of alkaline remedies, but the disappointing nature of the results certainly indicates that some additional factor, other than the simple neutralisation of acid, is involved.

The intravenous or subcutaneous injections of alkali given when coma is impending or has supervened have as a rule only a transient beneficial effect. 350 cc. of the 2 % solution of sodium bicarbonate commonly employed would suffice to neutralise an amount of the acids equal to that (8 or 9 g.) present in the whole blood-volume of a patient such as No. 9; but since, as was pointed out above, the blood may be as it were refilled with acid four or five times daily, no persistent effect is to be expected without some method of continuous injection.

SUMMARY.

The concentration of acetone bodies in the blood in diabetic coma may be equivalent, when reckoned as aceto-acetic acid, to that of a 0.03 *N* solution; this amount is sufficient to combine with more than one-third of the sodium of the plasma. The amounts of aceto-acetic acid show less variation in the series of cases than do those of β -hydroxybutyric acid.

Methods for the estimation of acetone and aceto-acetic acid are as a rule tested with acetone only; this is quite inadequate, as the recovery of aceto-

contained, with sufficient accuracy, in a mixture of 50 parts NaHCO_3 , 2 parts KHCO_3 , and 1 part CaCl_2 ; the official KHCO_3 is here substituted for the KCl of Locke's solution. This simple ratio could be applied in the prescription of any desired amount of NaHCO_3 for administration by the mouth, *e.g.*:

Sodium Bicarbonate	150 grains
Potassium Bicarbonate	6 "
Calcium Chloride	3 "

This solution is of course turbid from the precipitation of CaCO_3 , but the CaCl_2 might be given at a different time from the rest.

acetic acid is liable to an error which does not occur in the case of either acetone or β -hydroxybutyric acid.

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