XXVIII. THE ACTION OF WHOLE BLOOD UPON ACIDS.

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ALTHOUGH very many investigations have been made upon the alkali reserve of serum and plasma, and upon the movements of ions which take place when red corpuscles are exposed to different tensions of CO_2 , it seems that no quantitative study of the acid-neutralising power of whole blood has been made. It is commonly assumed and stated that whole blood is more perfectly buffered than plasma. There is no difficulty in investigating this matter if the blood is placed in an isotonic medium.

METHOD.

Whole blood was shaken with 0.01N H_2SO_4 containing 0.9 % NaCl. The mixture was then centrifuged and the supernatant fluid titrated to ascertain how much of the acid had been neutralised, and examined by the electrode for H-ion concentration. The number of volumes of 0.01N acid added to one volume of blood varied from 2.28 to 9.66; the actual amounts used are given in the tables. The mixture of blood and acid was in all cases made up with 0.9 % NaCl to 8.25 cc. as this volume gave a convenient amount for duplicate titrations and H-ion determination. It was then shaken vigorously for five minutes in a wide centrifuge tube in a strong current of CO_2 -free air to remove CO_2 , and centrifuged in corked tubes. Of the supernatant fluid duplicate portions of 2.5 cc. were taken, mixed in test tubes with 5 cc. freshly boiled distilled water and nine drops of 0.1 % phenol red in alcohol, and titrated with 0.01N NaOH in a comparator, a phosphate solution of p_{H} 7.4 being used as colour standard. The remainder of the supernatant fluid was used for H-ion determination. Plasma was treated in exactly the same way as the whole blood except that the centrifuge was not used. Two series of observations were made, namely upon human blood derived from one healthy subject over a period of about three months, and upon guinea-pig's blood (Tables I and II, and Figs. 1 and 2).

It is at once evident in Fig. 1 that the behaviour of whole blood is quite different from that of plasma. However much acid is added to the human

plasma, roughly the same amount is neutralised; in fact plasma behaves, though with much wider variations, like a solution of caustic soda. With whole blood on the other hand the amount of acid neutralised increases with the amount added in such a way that round about 80 % of the acid is neutralised in every case. The exact percentages can be seen in Table I; they range from 89 to 78 %, and tend to diminish as the amount of acid increases.

Fig. 1. NEUTRALISATION OF ACID BY HUMAN WHOLE BLOOD AND PLASMA. The points of intersection of the diagonal line with the ordinates represent amounts of acid added to 1 cc. blood or plasma; the points marked ● and □ on these ordinates represent the fractions of these amounts which are neutralised by the blood (●) or plasma (□). A second oblique line has been drawn at a height which is 80 % of the height of the diagonal.

The results obtained with the whole blood of guinea-pigs (Fig. 2) are confirmatory; the amounts neutralised keep rather closer to the 80 % line. With the plasma, however, extremely wide variations were found; in some cases the plasma had scarcely any neutralising power. This is probably due to the disturbances of circulation and respiration brought about in the taking of the blood, which are quite absent when blood is drawn from the human subject. It is remarkable that there is no indication in the results obtained from whole blood of these variations in the plasma. The maximum amount of 0.01N acid neutralised by 1 cc. plasma is about the same (2.3 to 2.4 cc.) in both cases (man and guinea-pig) and indicates that the "alkali reserve" of the plasma is equivalent to that of a 0.024N solution.

The addition of increasing amounts of acid to whole blood cannot of course be carried on indefinitely, because haemolysis supervenes. This occurs in human blood when more than about 11 cc. 0.01N acid is added to 1 cc. blood, whereas 1 cc. guinea-pig's blood is lysed by 7 or 8 cc. 0.01N acid. The amounts

of acid used in these experiments are not so large as to be altogether beyond the range of what may occur in the body; thus in diabetic coma the concentration of compounds of aceto-acetic and β -hydroxybutyric acids in the blood may be that of a 0.03N solution [Kennaway, 1918]. This would be in some degree comparable with an experiment in which 3 cc. 0.01N acid was added to 1 cc. blood (see Figs. 1 and 2). No methhaemoglobin was detected with the spectroscope in these mixtures even when more than enough acid to produce lysis was added.

Fig. 2. NEUTRALISATION OF ACID BY GUINEA-PIG'S WHOLE BLOOD AND PLASMA.
For description, see Fig. 1.

Fig. 3. p_H OF MIXTURES OF ACID AND HUMAN BLOOD (●) OR PLASMA (□).

Table I (see Figs. 1 and 2). *Neutralisation of acid by whole blood.*

All mixtures of blood and acid were made up to 8.25 cc. with 0.9 % NaCl.

Mixture cc.			Human blood				Guinea-pig's blood		
Blood	+	Acid	Ratio cc. 0.01N acid to 1 cc. blood	1 cc. blood neutralised cc. 0.01N acid	Percentage of acid neutralised	Final p_H	1 cc. blood neutralised cc. 0.01N acid	Percentage of acid neutralised	Final p_H
		0.01N							
1.75	4.0	—	2.286	1.83	80.2	7.05	1.906	83.4	—
"	"	—	"	1.83	80.2	—	—	—	—
"	"	—	"	1.94	85.0	7.1	—	—	—
"	"	—	"	2.03	88.8	6.6	—	—	—
1.0	—	5.0	2.5	2.20	88.0	—	—	—	—
1.50	4.0	—	2.66	2.22	83.4	—	2.20	82.7	—
1.50	5.0	—	3.33	2.90	87.1	6.5	2.76	82.9	6.15
"	"	—	"	2.84	85.3	—	2.84	85.4	—
"	"	—	"	2.87	86.0	6.35	—	—	—
0.75	—	5.0	"	2.83	85.0	—	—	—	—
1.0	—	7.25	3.6	3.04	84.4	—	—	—	—
0.75	—	6.0	4.0	3.39	84.8	—	—	—	—
0.75	—	7.25	4.8	4.05	84.3	—	—	—	—
1.0	5.0	—	5.0	4.15	83.0	5.2	4.01	80.2	5.3
"	"	—	"	4.08	81.6	5.65	4.13	82.6	5.8
0.75	—	7.5	"	4.25	85.0	—	—	—	—
0.8	5.0	—	6.25	—	—	—	5.14	82.2	5.55
0.75	5.0	—	6.66	5.35	80.3	5.4	5.52	82.9	5.05
"	"	—	"	5.53	83.0	5.3	5.34	80.2	5.8
1.0	7.25	—	7.25	5.93	81.8	5.05	lysis	—	—
"	"	—	"	6.01	82.5	4.8	—	—	—
0.5	—	7.75	7.75	6.16	79.5	—	—	—	—
0.75	6.0	—	8.0	6.28	78.5	5.65	—	—	—
0.75	6.5	—	8.66	6.79	78.4	4.95	—	—	—
0.75	7.0	—	9.33	7.48	80.1	—	—	—	—
0.75	7.25	—	9.66	7.55	78.2	4.5	—	—	—
"	"	—	"	7.58	78.5	—	—	—	—
0.5	5.5	—	11.0	lysis	—	—	—	—	—

Table II (see Figs. 1 and 2). *Neutralisation of acid by plasma.*

Acid and plasma were mixed in the same amounts as those given in the first column of Table I for acid and whole blood.

Ratio cc. 0.01N acid to 1 cc. plasma	Human plasma			Guinea-pig's plasma		
	1 cc. plasma neutralised cc. 0.01N acid	Percentage of acid neutralised	Final p_H	1 cc. plasma neutralised cc. 0.01N acid	Percentage of acid neutralised	Final p_H
2.286	—	—	—	0.579	25.3	5.05
"	—	—	—	1.797	78.6	6.4
"	—	—	—	1.825	80.0	6.7
2.66	1.93	72.5	6.5	1.308	50.0	5.55
"	2.25	84.6	—	1.59	60.0	6.6
3.33	2.41	72.4	6.15	1.595	47.7	5.8
5.0	2.38	47.6	—	0.347	7.0	4.2
"	2.40	48.0	—	2.18	43.6	4.5
"	—	—	—	2.31	46.2	4.35
6.66	1.915	28.8	4.0	0.84	12.6	4.0
"	2.22	33.3	4.25	2.18	32.7	3.95
"	—	—	—	1.10	16.5	4.1
8.0	2.06	25.8	—	—	—	—
8.33	1.88	22.6	—	0.96	11.6	—
9.66	2.09	21.6	3.65	0.73	7.6	3.45
"	2.06	21.3	—	1.86	19.3	3.05
"	2.11	21.8	—	2.16	22.4	3.4

The measurements of H-ion concentration (Fig. 3 and Tables I and II) show that the whole blood does not bring this concentration to any constant level when different amounts of acid are added. With the addition of increasing amounts the H-ion concentration increases at a diminishing rate, roughly from p_H 7 to 4. In striking contrast to their effects upon titratable acidity, the whole blood and the plasma have very much the same effect upon p_H ; the plasma shows a rather greater acidity, as one would expect. The rather wide variations in p_H are no doubt due to the errors of pipette measurement, and they would be wider still if the mixtures were not buffered; thus an error of only 0.01 cc. in the pipetting of 0.01*N* acid in these experiments would, in an unbuffered solution, be nearly sufficient to produce the difference between p_H 5 and 6.

This quite unexpected behaviour of whole blood towards acids requires further investigation. The word "neutralisation" has been used above, for want of any less committal term, to denote simply a disappearance of titratable acid, which is all that is actually observed; this disappearance might be due to combination with inorganic or organic bases, or to some form of adsorption, or to both of these. The inorganic bases of the red corpuscles are insufficient to account for the neutralisation of these amounts of acid. 1 cc. of blood can neutralise 7.5 cc. 0.01*N* acid, of which 1.2 cc. is to be assigned to the plasma and 6.3 cc. to the corpuscles (see Tables I and II). The amounts of K and Na present, according to Schmidt's analyses, in 0.5 cc. of red corpuscles, are equivalent to about 5 cc. 0.01*N*. Hence these bases would be unable to neutralise such an amount of acid even if they were wholly combined with carbonic acid, whereas the analyses show that they must be combined chiefly as chlorides, phosphates, and sulphates.

The following points suggested themselves for investigation:

(a) Is the amount of acid neutralised dependent upon its dilution?

Fig. 4 and Table III show the amounts of acid neutralised when constant amounts of human blood (0.75 cc.) from the same subject and of 0.01*N* acid (5 cc.) were allowed to react in the presence of varying amounts of saline, the acid being diluted before the blood was added, and the total volume ranging from 6.25 to 45.75 cc.; the concentration of acid thus varied seven-fold. The differences observed in the case of any one of the four samples of blood examined thus are mostly within the range of experimental error. Hence, under the conditions studied, dilution does not have any distinct influence upon this neutralisation of acid.

(b) Is the acid taken up by the corpuscles liberated if they are re-suspended in a fresh neutral medium? This was found not to be the case. 1 cc. blood was shaken with from 5 to 7.5 cc. 0.01*N* acid in saline in the manner described under "Method" above, and centrifuged, and the supernatant fluid drawn off as completely as possible; the corpuscles were then re-suspended in neutral saline, shaken for five minutes, let stand for a time, the mixture then centrifuged and the supernatant fluid titrated. Only traces of acid (0.05 cc. 0.01*N*)

were recovered; such an amount might well have remained enclosed between the packed-down corpuscles when the first supernatant fluid was removed.

(c) Is the neutralisation of acid by whole blood dependent upon the structure of the red corpuscles, or does lysed blood act in the same way?

Fig. 4. NEUTRALISATION BY WHOLE BLOOD OF ACID IN DIFFERENT CONCENTRATIONS. 0.75 cc. blood was mixed with 5 cc. 0.01N acid diluted with saline. Points plotted represent the amount of acid neutralised. The numbers 1, 2, 3, 4 refer to four different samples of blood.

Table III (see Fig. 4). *Neutralisation by whole blood of acid in different concentrations.*

0.75 cc. blood + 5 cc. 0.01N acid.					
Sample of blood	Total volume cc.	cc. 0.01N acid neutralised	Sample of blood	Total volume cc.	cc. 0.01N acid neutralised
1	6.25	4.17	3	11.25	4.37
	25.75	4.31		16.25	4.40
2	15.75	4.32	4	20.75	4.37
	30.75	4.24		6.75	4.41
	35.75	4.25		18.25	4.43
	45.75	4.05		30.75	4.37
				45.75	4.27

The difficulty in settling this question is due to the very deep colour of lysed blood; this necessitates considerable dilution before titration can be carried out. The small amount of acid to be titrated is in very dilute form and it is difficult to prevent the presence of appreciable quantities of CO_2 in the large volume of water required. It might be possible to employ dialysis, but it seemed that this might introduce various other sources of error. It may be best to describe one of the experiments in detail. The lysed blood was prepared by freezing and thawing four times. The following mixtures were made:

Blood + { 0.01N H_2SO_4 in } normal saline }		Normal saline	Total volume	Burette reading cc. 0.01N NaOH
Unlysed	{ 0.5	4	8.5	0.20, 0.21
	{ 0.5	0	„	0.11, 0.12
Lysed	{ 0.5	4	„	0.50, 0.50
	{ 0.5	0	„	0.115, 0.11

The mixtures were shaken for five minutes and centrifuged, in the ordinary way; the two with 8 cc. saline only and no acid served as "blanks." Duplicate portions of 1 cc. of each supernatant fluid were taken, diluted with 36 cc.

freshly boiled water and 2 cc. 0.1 % phenol red and titrated in the comparator; the colour in the lysed samples was very slight at this dilution (1 of blood in 663) but was matched by diluted blood placed in front of the colour standard. The results of three such experiments were as follows:

						% neutralised
I.	{ 1 cc. unlysed blood neutralises	6.21 cc. out of	8.0 cc.	0.01N		77.7
	{ 1 cc. lysed " "	1.54 "	" "			19.2
II.	{ 1 cc. unlysed " "	6.28 "	" "			78.5
	{ 1 cc. lysed " "	1.37 "	" "			17.1
III.	{ 1 cc. unlysed " "	6.93 "	" "			86.9
	{ 1 cc. lysed " "	2.31 "	" "			29.0

As has been explained above, this form of experiment is not adapted to a high degree of accuracy; but it does show beyond doubt that lysed blood has only from one-quarter to one-third of the acid-neutralising power of unlysed blood. The actual difference measured in this comparison of lysed and unlysed blood is that between two burette readings; with the burette employed the difference of level was about $2\frac{1}{2}$ inches, a sufficiently obvious amount. The acid-neutralising power of lysed blood is not greater than that of plasma (see Table II). The remarkable property of whole blood which is illustrated in Figs. 1 and 2 seems then to be due to the structure or physical condition of the red corpuscles. Probably there are many such forms of adsorption or pseudo-adsorption which have not yet been described.

SUMMARY.

The action of whole blood upon acids is quite different from that of plasma. If a series of amounts of sulphuric acid is added to plasma, and to whole blood, so as to produce p_H between 7 and 4, it is found that the amount of titratable acid removed by whole blood increases with the amount added in such a way that a roughly constant percentage, namely about 80 %, of the acid disappears; whereas the amount neutralised by plasma does not vary with the amount added.

It was not found possible to wash out the acid taken up by the red corpuscles. Within the range investigated, the amount of acid removed was independent of the dilution. The inorganic bases of the corpuscles are insufficient to neutralise the acid. Since lysed blood does not take up acid in this way it seems that the action of whole blood upon acids must be due to some form of adsorption which is dependent upon the structure or physical condition of the red corpuscles.

REFERENCE.

Kennaway (1918). *Biochem. J.* **12**, 120.