ACID PRODUCTION IN SHED BLOOD.

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THE carbon dioxide capacity of blood is at its highest level in freshly drawn blood, and usually suffers considerable reduction when the blood is allowed to stand out of the body. Although this observation was made by Zuntz as long ago as 1868(1), and though recent work has enlarged our knowledge of the conditions under which the phenomenon occurs, no satisfactory explanation of it has yet been proposed. Separated plasma does not undergo this change, and the speed of the change in whole blood is accelerated by temperature elevation. All observers are agreed that the phenomenon is irregular in incidence and magnitude. Human venous blood usually shows it well, though occasionally there is no change even after several hours at room temperature. The results obtained by Christiansen, Douglas and Haldane(2) showed that the change may occur with considerable but progressively diminishing velocity while blood is being brought into equilibrium with gas mixtures at body temperature. Extrapolation and interpolation of their values indicates that during successive periods of 20 minutes the CO_2 capacity fell about 2·5 p.c., 1·8 p.c., 1·1 p.c., 0·8 p.c. and 0·7 p.c. This slowing-down of the speed of change I have also been able to confirm. As I have previously pointed out(3), this post-mortem diminution of the available base of the blood offers a serious obstacle to the interpretation of data obtained from blood which is not perfectly fresh. Peters, Barr and Rule(4) have also encountered, but partially circumvented, this same difficulty in their determinations of arterial CO_2 tensions from the CO_2 dissociation curve and the arterial CO_2 content of the blood. Henderson and Haggard(5), however, and Mellanby and Thomas(6) have shown independently of one another that the velocity and extent of the irreversible change are greatly exaggerated in blood which has been temporarily exposed to a lowered carbon dioxide pressure, and Mellanby and Thomas(6) further showed that the change so produced was associated with, and presumably caused by, an increase in the lactic acid content of the blood.

It is evident from the data that the cause of this autogenous acid production is to be sought in the formed elements of the blood, and is not due to bacterial action. In searching for its cause it occurred to me that it might well be associated with the phenomenon of glycolysis. This is likewise of irregular occurrence, is associated with the formed elements (chiefly the leucocytes, according to Maclean and Weir(7)), is accelerated by alkali (Cl. Bernard(8)) and, therefore, by removal of CO_2 (Rona and Döblin(9)). Moreover, the rate at which glycolysis occurs is most rapid in fresh blood and is subsequently retarded (Edelmann(10)). If we remember that glycolysis is associated with a conversion of glucose into lactic acid, as shown by Slosse(11), Kraske, Kondo and v. Noorden(12), such an explanation seems most probable. I have, therefore examined and, as I think, confirmed this hypothesis, by experiment.

Methods. Samples of human blood were drawn from suitable arm veins into serum syringes provided with appropriate anticoagulants. Dog's and goat's blood was drawn in a similar way from the external jugular vein. The blood of cats (decerebrated) was drawn from an arterial cannula and that of rabbits from the ear vein. As anticoagulants, oxalate 0·1 to 0·5 p.c., or hirudin, were employed; in some cases the blood was defibrinated. In order to retard the change in blood the following procedure was adopted in the earlier experiments. Two thermos flasks were filled, one with ice and salt and one with ice and water. In the first of these a boiling-tube filled to a depth of 1–2 cm. with liquid paraffin was cooled down to about − 12° C.; the fresh-drawn blood was run straight into this, beneath the surface of the paraffin, and stirred with a thermometer until its temperature had fallen to 0° when the tube with its contents was at once transferred to the second flask where it was kept at 0° till required. About five minutes is required thus to cool 10 or 20 c.c. of blood from body temperature to 0°. If loss of a little CO_2 is immaterial, the paraffin layer may be omitted, the boiling tube being then closed by a rubber bung. An advantage of this method of cooling is that the blood can, if necessary, be transported in the cold condition and the thermos flask kept on the laboratory bench while determinations are made. Keeping at 0°, however, does not always entirely prevent change if many hours elapse. A further difficulty is that, as soon as the cooled blood is warmed up again, as in the usual procedure for saturation with a known pressure of CO_2, the change again sets in with rapidity. In the earlier experiments this was minimised by carrying out the equilibration at a low temperature, 20–22°, in later ones by

addition of sodium fluoride, as will presently be described; the latter procedure enables equilibration with gas mixtures to be carried out safely at body temperature. For this purpose, a mixture of one part of sodium fluoride and four parts of potassium oxalate, both in fine powder, was used and 0·3 to 0·5 p.c. of the mixture added to the blood, which was at once cooled as described above.

Carbon dioxide contents were determined by Van Slyke's method. In some cases dissociation curves were plotted. More usually, economy in time and material was effected by the determination of one point only, viz., the CO_2 content at approximately alveolar CO_2 tension, the actual CO_2 pressure being in each case accurately determined by analysis of the gas after equilibration. A part of the CO_2 dissociation curve was then plotted for one sample of the blood, and the proper small corrections made from this for the reduction of all the various determinations to the same pressure of CO_2. Blood sugar determinations were made by Benedict's method. Although the question of vital importance concerns the extent of the autogenous acid production in blood kept for a *short* time at room temperature, or equilibrated at body temperatures for periods up to 20 minutes, I have in these experiments not infrequently warmed blood to 37° for periods of one to three hours or left it at room temperature for longer intervals, in order to get more extensive changes. Bacterial action, may of course, play some part in these, but has not been separately studied.

The extent of the change. Effect of temperature. As stated above, the appearance of the phenomenon is irregular, and its extent depends especially on temperature, CO_2 loss, etc. Rarely, in spite of apparently favourable conditions, the change is insignificant. There may even be a slight increase in CO_2 capacity; as this was seen most strikingly in blood to which urea had previously been added, I imagine it to be due to a measurable production of ammonia, the content of which is known to rise in blood on standing. But in the great majority of cases when blood is allowed to stand, even at room temperature, there is, in an hour or two, a loss of CO_2 capacity of one or two volumes per cent.; less frequently, the reduction may amount to 6 vols. p.c. or even more. Exp. 1 is an illustration of the magnitude of the change under favourable conditions, when the blood is partially depleted of CO_2 while still in the body.

Exp. 1. Decerebrate cat submitted to excessive artificial respiration. Arterial blood drawn and 0·2 p.c. oxalate added to it. A portion of this cooled at once to 0° was found from its dissociation curve to have a CO_2 content of 37·3 vols. p.c. at 40 mm. CO_2 and

37·5° C. A portion of the blood allowed to stand for 1½ hours at 20° C. and then similarly cooled to 0° till required, had under the same conditions a CO_2 content of 33·8 vols. p.c.—a loss of 3·5 vols. p.c. The content of CO_2 of the arterial blood, as drawn, was 32·5 p.c. From the dissociation curve for the fresh blood the CO_2 pressure in the arterial blood, as drawn, would, therefore, be about 27·5 mm.; that deduced from the stale blood however, would be about 36·5 mm.—a difference of 9 mm.

Fig. 1 shows an experiment with human blood.

Relation between fixed and free CO_2. If the change in shed blood is due, as Mellanby and Thomas's results would lead us to suppose, to a formation of fixed acid, then it should be possible to show that, if the blood, previously brought to a low CO_2 pressure, is kept out of contact

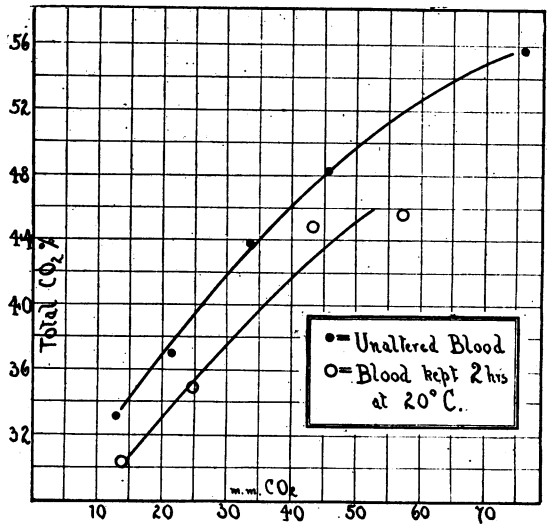

Fig. 1. CO_2 dissociation curves of fresh (fluoride) human blood, and of the same without fluoride after two hours' exposure to air at 20° C.

with the air, the free CO_2 rises in proportion as the fixed CO_2 falls. Experiments were made as follows: The CO_2 content of the blood was lowered, either *in vivo* in cats by the carrying out of excessive artificial respiration, or for human blood *in vitro* by rapid evacuation at a low temperature (18–20° C.). The blood was either oxalated (·15 p.c.) or hirudinised. A 20 c.c. all-glass syringe, containing a glass bead for mixing, was completely filled with the blood, sealed, and kept at the desired temperature. Samples were withdrawn at intervals for determinations of the CO_2 pressure by Krogh's micro-method (13). In Exp. 2 there was, even after 2¾ hours, no reduction in the fixed CO_2 content of the blood, and in agreement with this the only change in CO_2 tension

was a slight fall, due, no doubt, to a slight loss of CO_2. In Exps. 3 and 4 the fixed CO_2 content fell considerably and the free CO_2 rose.

Exp. 2. Cat's blood.

Time kept at 20° C. hrs.	Pressure of CO_2 at 38° C. mm.
0	20
1¾	17
2¼	16

Exp. 3. Human blood.

Temperature at which kept ° C.	Time kept hrs.	Pressure of CO_2 at 38° C. mm.	CO_2 capacity at 40 mm. and 38° C. p.c.
38	0	18	46·2
38	½	50	—
38	1	49	—
20	1½	—	41·0

Exp. 4. Rabbit's blood.

38	0	7·0 } (Mean 5·7 } =6·4)	49·0
38	3	16·1	34·0

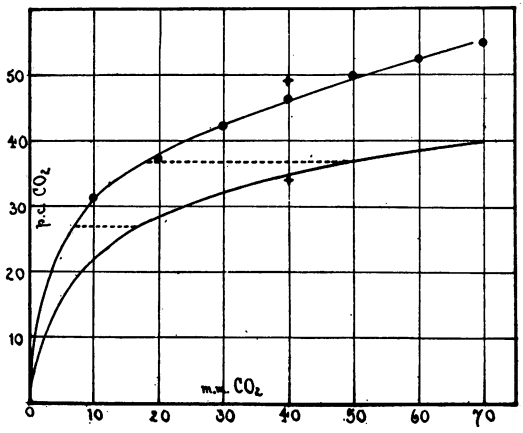

Fig. 2. Upper curve, with dots, the CO_2 dissociation curve for the fresh blood of Exp. 3. Lower curve, that for the same blood after 1 hour at 38° C., as deduced from the change of CO_2 tension of the blood (upper dotted line). Crosses indicate approximate position of curves for fresh and warm bloods of Exp. 4, and the lower dotted line shows the CO_2 tension change in this blood.

The CO_2 dissociation curves for the fresh bloods of Exps. 3 and 4 fell close together (Fig. 2), and though that for the warmed blood of Exp. 3 was not determined, it probably fell to about the same level as that of Exp. 4, shown by the lower curve of the figure. (Even at 20° C. the blood had lost 5·2 vols. p.c. of CO_2 in 1½ hours.) The horizontal dotted

lines illustrate the accompanying alterations of CO_2 pressure as actually determined in the two experiments, the upper line (Exp. 3) showing a greater change than the lower (Exp. 4) because the latter falls at steeper parts of the curves. That is to say, that the greater the initial CO_2 pressure of the blood, the greater the rise of CO_2 pressure produced by a given fall in the fixed CO_2. In practice, however, the actual extent of change in fixed CO_2 is likely to be greater the *lower* the CO_2 pressure initially, as shown by Henderson and Haggard. In using the dissociation curve as a means of measuring the arterial CO_2 pressure, the change can, therefore, as proved by Exp. 1, never be ignored; probably an initial CO_2 pressure of about 20 mm. is liable in such cases, to lead to the greatest errors.

The relation between glycolysis and acid production. Experiment soon convinced me that there was a close correspondence between glycolysis and acid production. According to the hypothesis which I have attempted to test, there might even be expected to be a quantitative relation between the observed loss of sugar and that of available base from the blood. If all the available base were present as bicarbonate, this relation would be a simple one, for then, a conversion of 1 mg. of sugar completely into lactic (or pyruvic) acid would lower the CO_2 capacity of the bicarbonate system about 2·5 vols. p.c. The curve relating sugar loss to fall of CO_2 capacity would then be a straight line. My experimental data (Fig. 3), however, showed that the curve was not a straight line. But we know that in blood the amount of available base present in the form of bicarbonate is a variable which depends on the hydrogen-ion concentration, and, therefore, on the $\frac{NaHCO_3}{CO_2}$ ratio of the plasma, the ionic balance being determined by the Hamburger interchange between corpuscles and plasma. It would be anticipated, therefore, that the effect on the blood, and especially on the true plasma, of the production of a

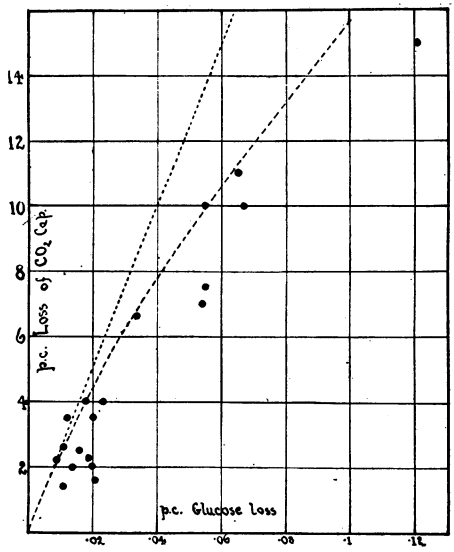

Fig. 3. Relation between glycolysis and change of CO_2 capacity of blood in different experiments.

given amount of fixed acid in the blood, would be considerably smaller than that calculated on the assumption that the acid was entirely used in replacing fixed CO_2. As a matter of fact, I have already shown(3) that when the CO_2 capacity of blood falls during its stay *in vitro*, this change chiefly affects the corpuscles by altering what Haggard and Henderson(5) call their "acid load," while leaving the true plasma but little changed.

Before a comparison between the change of sugar content and CO_2 capacity can be satisfactorily made, it is, therefore, necessary to know the effect on the CO_2 capacity, of the addition to blood of varying amounts of fixed acid. I have accordingly tested this point by adding to fluoride

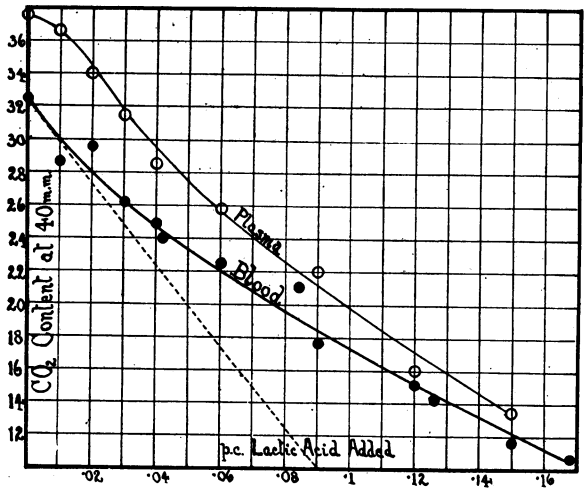

Fig. 4. Effect of lactic acid on the CO_2 content of blood and of true plasma, at 40 mm. CO_2 and 38° C.

blood amounts of lactic acid varying, in stages, from 0·01 to 0·17 p.c. The blood so treated was in each case equilibrated at 38° C. with CO_2 at 40 mm. and the CO_2 content of whole blood and of true plasma determined. The results, which are given in Fig. 4, clearly bear out the anticipations referred to above. The straight dotted line indicates how the blood should behave if the added acid only replaced fixed CO_2. The curves obtained show that the curve for whole blood departs a good deal, and that of true plasma even more, from the behaviour required by such a supposition. This is a further instance of the important secondary buffering, which the blood owes to its red corpuscles. If allowance is made for the fact that in the experiment which these curves represent, all the samples of blood were diluted 20 p.c. (with saline

solution and lactic acid in varying proportions), it is clear that the effect of this secondary buffering in undiluted blood, depending as it does on the corpuscular content (Evans(3)), would be still greater than that found here. This experiment bears out the results of Mellanby and Thomas(6), who also found that the effect of addition of lactic acid was smaller than might have been expected.

We may now consider the results of the experiments given graphically in Fig. 3 in which glycolysis and fixed acid production in blood were determined simultaneously. The straight dotted line is the hypothetical curve which would be expected if fixed CO_2 alone were displaced, while the curved dotted line gives the one that would be expected if the effect of the acid were that found from Fig. 4. It will be seen that the points fall a little below this curve, but appear to lie on a similar curve parallel to it. As these experiments were made with bloods from different sources, and all of them undiluted, I think the inference is clear. It is that the fall of CO_2 capacity is due to the almost quantitative conversion of each molecule of glucose lost by glycolysis, into two molecules of some monobasic acid, presumably lactic acid (or, less probably, one molecule of a dibasic acid).

The effect of mere lowering of free carbon dioxide on the change. It might be inferred from the experiments of Haggard and Henderson(5) and of Mellanby and Thomas(6) that it is the mere reduction of the free CO_2 which in some way at once leads to the irreversible fall of CO_2 capacity of the blood. According to my view, however, all that the removal of CO_2 does is to produce a hydrogen-ion concentration so low as greatly to accelerate the normal process of glycolysis. Exp. 5 shows that this is so.

Exp. 5. Fresh rabbit's blood (0·2 p.c. of potassium oxalate) was cooled down to 0°. As a control 0·05 p.c. of NaF was added to a portion of it. The remainder was evacuated at the low temperature of 18° C., as thoroughly as possible with a filter pump, and then divided into four equal parts: to No. 1 0·05 p.c. of NaF was added at once; No. 2 was warmed to 38° C. for 5 min.; No. 3 for 30 min.; and No. 4 for 3 hours; and then 0·05 p.c. of NaF was added to each. The CO_2 content at 40 mm. and the sugar content of each sample was then determined, with the results given in the table.

Treatment of blood	CO_2 content p.c.	Sugar content p.c.
Fresh	49·0	0·247
Evacuated at 18° C.	49·0	0·250
„ „ warmed 5 min.	46·5	0·234
„ „ „ 30 min.	45·0	0·232
„ „ „ 3 hrs.	34·0	0·129

This experiment shows that the change is not produced by mere removal of CO_2, but that it sets in very rapidly on warming *after* this has been done.

The results of a similar experiment are given graphically in Fig. 5, which shows even more clearly that the velocity of the change both in the sugar and in CO_2 capacity is most rapid at first.

Method of controlling the change. The change in CO_2 capacity can, like the glycolysis, be considerably retarded by cold, but best by the addition of small amounts of sodium fluoride and cooling as well. The

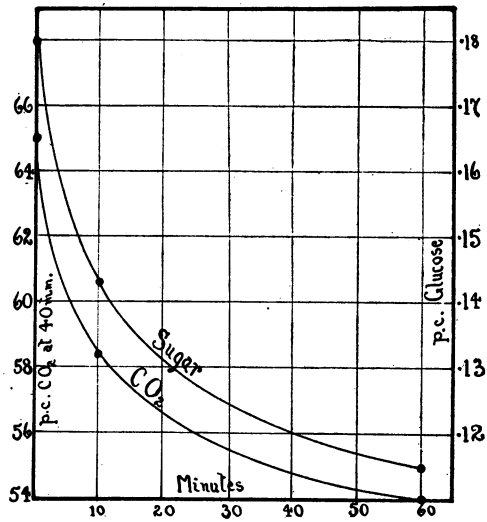

Fig. 5. Rate of reduction of CO_2 capacity (measured at 40 mm. and 22° C.) and glucose content in hirudinised human blood after removal of most of the CO_2 *in vacuo*.

facts are illustrated by the table, which shows the effect of adding varying amounts of sodium fluoride to defibrinated rabbit's blood.

	Fresh blood		Same blood allowed to stand 24 hrs. at room temperature	
NaF added p.c.	CO_2 at 45 mm. and 22° C. p.c.	Glucose p.c.	CO_2 at 45 mm. and 22° C. p.c.	Glucose p.c.
0	49·2	0·151	42·2	0·097
0·1	48·8	—	48·9	0·151
0·2	46·3	—	46·8	—
0·5	44·1	—	43·7	—
1·0	41·8	—	41·9	0·151

One drawback attending the use of the particular sample of fluoride which I employed is evident, namely that it causes an immediate reduction of CO_2 capacity amounting to about 0·9 vols. p.c. for each 0·1 p.c. of NaF added. As 0·1 p.c. in presence of 0·4 p.c. of oxalate is adequate to inhibit both the glycolysis and the acid production, unless

the blood is to be kept at body temperature for more than 30 minutes, I have avoided using more than that amount, and have applied the correction indicated by the above results. For most purposes even 0·05 p.c. fluoride is enough. It has been shown by Macleod(14) that oxalate considerably retards glycolysis and my own experience is that it also retards the acid formation. For this reason, I think that the use of the mixture of oxalate and fluoride is an advantage. Formalin is of no use as an inhibitant for the purpose of these experiments, because of its ready conversion into formic acid.

	CO_2 capacity of blood at 42·5 mm. and 22° C.		Sugar content of blood	
Oxalate added p.c.	Fresh p.c.	2 hrs. at 37° C. p.c.	Fresh p.c.	2 hrs. at 37° C. p.c.
0	45·8	41·8	0·348	0·325
0·2	45·4	43·2	0·365	0·356
1·0	46·0	45·4	0·365	0·365

Cause of the change. It might be supposed that, on account of the instability of glucose in alkaline solutions, the mere removal of CO_2 might, by rendering the blood more alkaline, lead to a destruction of sugar, and perhaps even to a formation of acid, such as has been described. This possibility is, I think, removed by two observations. (1) Michaelis and Rona(15) have shown that alkaline reactions up to *p*H 9·0 are inadequate to cause appreciable change; (2) the change does not occur in the plasma. There seems no reasonable doubt that the phenomenon is an expression of the glycolytic change, and, like the latter, is effected by intracellular enzymes. I have made one experiment to ascertain whether the white or red corpuscles were mainly responsible, and, like Maclean and Weir(7), found that the whites are decidedly the more active.

The significance of the acid change. The importance of these results is almost self-evident. Although in normal blood the change might sometimes be negligible, yet it is liable to affect the accuracy of all CO_2 dissociation curves, and, therefore, of all data based on them, such as determinations of arterial CO_2 tensions. It is more likely to be met with to a serious extent in blood from breathless patients, because in them there has been *in vivo* a lowering of the CO_2 pressure. The irreversible change in the blood of dogs submitted to artificial over-ventilation, which was described by Henderson(16) is very probably a change of this nature. After periods of hyperpnœa in man a rapid rate of change may be expected, more especially as Collip and Backus(17) have shown

that there is, under these conditions, both a leucocytosis and a hyperglycæmia. I have found that the addition of glucose to blood decidedly accelerates this change. Under such conditions, delay in the cooling of blood samples, and lack of proper precautions to prevent the change, may result in grave errors. I am not prepared to suggest to what extent these changes affect the oxygen-dissociation curve of blood, but that there is some effect seems inevitable.

SUMMARY.

1. The fall in CO_2 capacity of shed blood is due to a conversion of glucose into lactic acid as a result of glycolysis.

2. The change is greatly accelerated, though not actually produced, by a lowering of the CO_2 pressure of the blood, as shown by Mellanby and Thomas, and Henderson and Haggard.

3. These facts are of importance in all experiments on the CO_2 dissociation curve. The change can be retarded by adding 0·05–0·1 p.c. of sodium fluoride to the blood. Equilibrations at body temperature can then be performed with little change in the CO_2 capacity of the blood.

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