J. Physiol. (1964), **175**, *pp.* 161–171
With 3 text-figures
Printed in Great Britain

DETERMINATION OF THE OXYHAEMOGLOBIN DISSOCIATION CURVES FOR MOUSE AND RAT BLOOD

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(*Received* 8 *August* 1963)

Schmidt-Nielsen & Larimer (1958) made a comparative study of the oxyhaemoglobin dissociation curves of mammalian blood in relation to body size. Previous to this very few measurements seem ever to have been made of the relation between the oxygen tension and the degree of saturation of the unhaemolysed blood of mice or rats. This is strikingly illustrated by the fact that the figure for the oxygen tension at 50 % saturation quoted by Bartels & Opitz (1958) is taken from Prosser (1950), who in turn quotes from the observations made in 1912 by Douglas, Haldane & Haldane (1912) on blood taken from animals asphyxiated by drowning, which was almost certainly heavily loaded with lactic acid and not characteristic of the animal at rest or in moderate activity. Our object was to compare the oxygen-dissociation characteristics of the blood of the mouse, the rat and man, at identical P_{CO_2} and pH.

Two methods of determining the dissociation curves are described below, preceded by reference to the procedures employed for the micro-assay of oxygen and an account of the processes of taking and equilibrating the blood, which are common to both.

METHODS

Taking and equilibrating the blood

The blood was drawn from the heart (in the case of human blood, from a vein in the arm) into a heparinized syringe. That from about a dozen animals was pooled and one drop of 1 % sodium fluoride added for every 3 ml. of blood. The blood was kept on ice until needed. Any adjustment of pH was carried out at this stage. Two millilitres of the agitated blood were drawn into a 30 ml. graduated syringe followed by successive amounts of nitrogen, oxygen and carbon dioxide appropriate to the required partial pressures. The P_{CO_2} was approximately 40 mm Hg throughout the experiments (individual values noted in each table). The gas mixture occupied a volume of 28 ml. at room temperature. The needle of the syringe was closed with a short blind length of small-bore rubber tubing, and the syringe rotated for 15 min at 37° C in a water-bath. Care was taken to ensure that the plunger of the syringe was quite free to move inside the barrel, so that the gas was at atmospheric pressure. After 15 min the syringe was removed from the water-bath and the gas expelled. A fresh gas mixture of the same composition was introduced into the syringe and the blood re-equili-

brated for 20 min. The use of a two-stage equilibration made it easier to control the final partial pressures of the various gases, with the gas-to-blood volume ratio employed (14:1).

After a certain number of measurements had been made on blood drawn from the orbital sinus of anaesthetized mice, it was noted that this blood was invariably acid (pH ~ 7·15) and therefore not comparable with human blood at pH ~ 7·45. In all subsequent work blood was drawn from mice and lightly anaesthetized rats by cardiac puncture. In the case of the mouse blood, the pH of the blood as drawn was often lower than 7·45. In such cases a 2 ml. sample was equilibrated as described below, but for only one period of 10 min, with a mixture of air and carbon dioxide such that the P_{CO_2} was 40 mm Hg. The pH of the blood was measured, and enough N-NaOH added from a microsyringe to the bulk of the blood (on the basis of previous determinations) to ensure that in subsequent equilibrations the pH would be approximately 7·45. The amount of alkali needed was about 1·5 % of the volume of the blood. The pH of the blood actually used for dissociation measurements was always determined. Consistency of duplicates was ~ 0·05 pH unit.

We added sodium fluoride to reduce the rate of glycolysis of the blood, and thereby to stabilize the pH. We found that if sodium fluoride were not added the pH fell during the equilibration of the blood at the rate of about 0·6 u/hr. With the addition of one drop of 1 % NaF/3 ml. blood, the fall was reduced to 0·1 u/hr. All samples of human blood were drawn from one person (J.M.S.), fresh 10 ml. samples being drawn for each day's measurement. Between two and four correlated measurements of P_{O_2} and saturation were made at each of a range of values of P_{O_2} on any one day. The ranges of P_{O_2} covered on different days were designed to overlap. The same general procedure, including multiple measurements at any one value of P_{O_2}, was followed for measurements on mouse and rat blood, except that any one day's collection comprised a pool derived from a number of animals (about a dozen in the case of mice).

Oxygen assay

Oxygen was measured electrochemically. When the oxygen had been liberated from oxyhaemoglobin by ferricyanide (method 1), it was collected by a stream of purified hydrogen which bubbled through the reaction mixture. When the oxygen was in a gas bubble (method 2), the bubble was introduced into a hydrogen stream through a seal of static oxygen-free water. Convenient techniques have been described by Deschner & Gray (1959) and Dewey & Gray (1961). The stream of hydrogen flowed through a Hersch analyser (Hersch, 1957) where a constant fraction of the oxygen reacted at a moist silver surface to generate a proportionate electric charge. This charge was measured by means of a fluxmeter, as described by Gray (1956) and Dewey & Gray (1961). The ultimate sensitivity and stability of the Hersch analyser was such that the standard error of an individual measurement was about $\pm 0{\cdot}001$ μl. oxygen at s.t.p. when quantities less than 0·1 μl. were measured, and rather better than 1 % for larger samples. Each measurement took about 3 min.

Determination of percentage saturation and oxygen tension

First method

The syringe was removed from the water-bath and about one half of the blood expelled, under paraffin over mercury, into a small tube so that it floated on the mercury and was covered by the paraffin oil. It was stored in this manner at room temperature. It was found necessary to equilibrate this paraffin oil with gas of approximately the same P_{O_2} as that used for equilibration of the blood, otherwise a significant exchange of oxygen would have occurred during the sampling procedure; for example, blood at a P_{O_2} of 4 mm Hg gained 1·3 % saturation in 30 min when stored under paraffin oil which had not been equilibrated in this way.

Blood pH. The blood remaining in the syringe was used for a pH measurement with a glass electrode at room temperature (vide infra). The pH meter (Electronic Instruments Ltd. Model 23A) readings were automatically compensated in such a way that the pH of a

solution under investigation would be correctly registered irrespective of the temperature at which the reading was made. In the case of blood, however, a complication has been pointed out (Brewin, Gould, Nashat & Neil, 1955; Rosenthal, 1948). The first-named authors have shown that (over the range 26–37° C) there is no change in the pH of blood with temperature when the P_{CO_2} is held constant. Since the solubility of the CO_2 varies with temperature, it is assumed that the consequential changes in pH which are to be expected are exactly compensated by other changes in the ionic equilibrium concerned. Rosenthal gives a formula (determined experimentally) for the difference in pH between two samples of blood from the same pool of equilibrated material, the one measured at 38° C and the other at a variety of temperatures. According to this formula, the difference in the temperature at which samples of blood were equilibrated and that at which the pH was measured, would result in our recorded pH values being too high by about 0·2 pH units. In our experiment the blood was transferred from a syringe, in which it had been equilibrated at 37° C, to the pH meter at room temperature without re-equilibration. The pH was measured within 1 min and remained constant for the next 5 min. If changes in ionic equilibria of the kind referred to by Brewin *et al.* were taking place under our experimental conditions, they must have been complete within 1 min, and it seems safe to assume that the errors in our estimation of blood pH, if any, were consistent for all blood samples. The possibility that the pH values of all blood samples were consistently 0·2 unit lower than those recorded in this paper must therefore be borne in mind. The agreement with Bock, Field & Adair (1924) speaks against the existence of such a systematic error.

Gas, P_{O_2} and P_{CO_2}. The gas with which the blood had been brought into equilibrium was transferred from the syringe to a sample tube, where it was stored over mercury until its oxygen and carbon dioxide contents were determined with a conventional Haldane gas analysis apparatus.

Blood oxygen contents. A micro-gas pipette (Dewey & Gray, 1961) was flushed out several times with oxygen-free distilled water (water bubbled with nitrogen) to remove air bubbles. All but a known volume (about 2 μl.) of water was expelled. Immediately, the tip of the pipette was lowered into the blood which had been stored under paraffin, of which 2–10 μl. were sucked up so as to fill an exactly known length of the pipette, followed by a small pellet of mercury to seal the tip. The small quantity of water retained in the pipette after flushing out only partly mingled with the blood and served to separate the concentrated blood from the mercury of the pipette; when this precaution was neglected the mercury meniscus was irregular and the total volume of the blood sample was somewhat ill-defined. The volume of the blood was exactly known by the length of the pipette which it occupied. The blood was finally expelled from the pipette into a test compartment of the Hersch cell (model H in Dewey & Gray, 1961) which contained alkaline (borate-buffered) ferricyanide at pH 10. The blood was visibly haemolysed during the period of reaction with ferricyanide. The oxygen released from the oxyhaemoglobin was measured in the manner described above. The observation was repeated. Two samples were sufficient for the accurate measurement of the oxygen released by the ferricyanide.

To determine the oxygen capacity of the blood the whole procedure was repeated with another 2 ml. sample of the blood which, in this case, was equilibrated with pure oxygen. The amount of oxygen calculated to be in simple solution in the blood at various partial pressures of oxygen (αO_2 at 37° C = 23·7 μl. O_2/ml. blood; Bartels & Opitz, 1958) was subtracted from the observed values in order to find the volume of oxygen in combination with the haemoglobin. In Fig. 1 our own observations for human blood are compared with two curves (for different individuals) published by Bock, Field & Adair (1924). It will be seen (Figs. 1 and 2 inset) that our points scatter about the curves of Bock *et al.* right down to values of P_{O_2} as low as 4 mm Hg, at which the adequacy of the ferricyanide medium at pH 10 for releasing the oxygen from the haemoglobin was presumably most severely tested. In the method we have used, the concentration of oxygen dissolved in the test compartment is at

all times very low, since the solution is continuously bubbled by oxygen-free nitrogen. This no doubt favours the removal of oxygen from the haemoglobin. We regard the agreement between our observations with human blood and those of Bock *et al.* (1924) over the whole range from 2·5 % to nearly 100 % saturation as strong evidence of the adequacy of our technique for the level of accuracy which is claimed in the text.

Second method

This technique enables the drop in P_{O_2} of a blood sample due to removal of a known amount of oxygen to be measured directly. Combined with a knowledge of the oxygen content of the blood at the high P_{O_2}, it therefore yields an estimate of the average slope of the dissociation curve between any two values of the P_{O_2}. In the experiments described here the starting P_{O_2} was of the order of 670 mm Hg.

About 2 ml. of blood, containing sodium fluoride and adjusted for pH if necessary, were equilibrated in the 30 ml. syringe with oxygen in the presence of 40 mm Hg of carbon dioxide, by the technique described earlier. The syringe was removed from the water-bath and about 0·5 ml. of the equilibrated blood transferred without contact with air to a modified 'Agla' micro-syringe (Dewey & Gray, 1961). The modification consisted of a 4 cm length of uniform-bore glass capillary tubing, drawn to a point, substituted for the normal needle. The excess blood was expelled from the micro-syringe, leaving a known volume. A bubble of oxygen-free nitrogen and carbon dioxide ($P_{CO_2} = 40 \pm 2$ mm Hg) was taken into the barrel of the syringe, followed by mercury to seal the tip. Great care was taken not to include any air. The syringe was rotated in the water-bath at 37° C for 15 min to allow the bubble to come into equilibrium with the blood. The size of the bubble varied between 50 and 100 μl. Meanwhile, the pH of the blood remaining in the 30 ml. syringe which held the stock of equilibrated blood was determined.

The test compartment of the Hersch cell was arranged for the introduction of gas samples, i.e. with a water seal (Dewey & Gray, 1961). A portion (about 10 μl.) of the bubble in the micro-syringe was expelled into the capillary and its length measured at 37° C with a travelling microscope. Knowing the diameter of the bore (previously calibrated with mercury) the volume of the sample could be calculated. The oxygen in the sample was measured by injecting the bubble into the test compartment, so that the bubble rose to the surface of the water, broke, and was carried away in the hydrogen stream, while the traces of blood fell on to the surface of the mercury. As a check, a second portion of the bubble was assayed for oxygen in the same way. Finally, the volume of the remainder of the gas was determined. If a bubble carried a small amount of blood up with it the observation was discarded. The next point on the dissociation curve was obtained by repeating the procedure with a fresh bubble of pure nitrogen and carbon dioxide.

Each bubble removed some of the oxygen from the blood and lowered the P_{O_2}. From the measurement of the volume and oxygen content of any given (10 μl.) sample, the P_{O_2} and the oxygen content of the whole bubble was calculated. Oxygen in the complete bubble comprised the difference between the amounts of oxygen (dissolved and associated with haemoglobin), at the original and at the reduced (final) P_{O_2} of the blood. By subtracting from the total that amount of oxygen lost from solution at each reduction in P_{O_2}, the amount given up by the haemoglobin was obtained. To express this as a percentage of the amount of oxygen carried by the haemoglobin of the blood when fully saturated, the oxygen capacity of the haemoglobin in the blood was determined by method 1 (p. 162) on a fresh 2 ml. sample.

The following figures are taken from a typical protocol:

Volume of blood = 0·363 ml.

Volume of bubble at 37° C and 759 mm Hg total pressure = 100·5 μl.

Allowing for water vapour pressure at 37° C = 47 mm Hg and

P_{CO_2} = 40 mm Hg.

Initial partial pressure of oxygen, P_{O_2} = (759 − 47 − 40) = 672 mm Hg.

Sample	Volume (μl. at 37° C)	Oxygen in sample (μl. at s.t.p.)	P_{O_2} (mm Hg)	Total oxygen in bubble (μl. at s.t.p.)
(i)	11·8	1·17	85·5 } mean 84·5	9·96 } mean 9·84
(ii)	11·9	1·15	83·5 }	9·72 }

Total dissolved oxygen in blood before introduction of bubble, at 37° C and 760 mm Hg external pressure

= 23·7 μl. O_2 at s.t.p. per ml. of blood,

therefore dissolved oxygen released during equilibration

$$= \frac{23{\cdot}7 \times 0{\cdot}363 \times (672 - 84{\cdot}5)}{760} = 6{\cdot}64\ \mu\text{l. at s.t.p.},$$

therefore oxygen released from oxyhaemoglobin

= 9·84 − 6·64 = 3·20 μl. at s.t.p.

Oxygen capacity = 212 μl. O_2 at s.t.p./ml. of blood.

Percentage drop in saturation $= \frac{3{\cdot}20}{0{\cdot}363 \times 212} \times 100 = 4{\cdot}14$,

therefore at P_{O_2} = 84·5 mm Hg percentage saturation of blood = 100 − 4·14

= 95·86 %.

RESULTS

Human blood

Data obtained by method 1 for human blood appear in Table 1 and are shown in Fig. 1 as filled circles. The four filled circles shown in Fig. 1 for oxygen tensions greater than 30 mm Hg record the first observations of the entire series, and were made when a Haldane gas analysis apparatus was not available for the P_{CO_2} and P_{O_2} estimations. On these occasions the values of P_{O_2} and P_{CO_2} were computed from the amounts of those gases taken into the syringe for the purpose of equilibration. On this account these points are to be regarded as less reliable than the rest and they are not plotted in the later figures.

Data obtained by applying method 2 to human blood pH 7·4 are shown in Table 1 and Fig. 1 (open circles) and Fig. 2 (filled triangles). Observations by this method were confined to oxygen tensions greater than about 40 mm Hg. In this range our observations on human blood seem to define a curve which lies systematically a little below that of Bock *et al.* (1924).

We consider that all our observations on human blood made by method 1 agree satisfactorily with the curves of Bock *et al.* (1924), shown in Fig. 1, and justify the conclusion that our technique (including that for the chemical liberation of the oxygen from the haemoglobin) was not subject to systematic errors significant in magnitude relative to the differences

TABLE 1. Percentage saturation of the haemoglobin of human whole blood with oxygen at 37–37·5° C in relation to oxygen tension at P_{CO_2} approximately 40 mm Hg

Method 1 at pH 7·4–7·45			Method 2 at pH 7·3–7·5		
P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	Saturation (%)	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg) (±2 mm)	Saturation (%)
3·0	42·2	2·5	47·8	40	80·0
4·3	42·6	3·3	53·6	40	87·8
5·7	41·3	3·7	56·2	40	87·6
6·9	42·3	5·5	69·1	40	93·0
10·0	43·3	10·4	84·5	40	95·9
17·7	37·8	28·8	111·2	40	98·5
32·6	37·6	59·5	121·3	40	97·9
32·8	37·8	71·5			
70·0	37·6	95·4			
70·6	37·8	97·9			

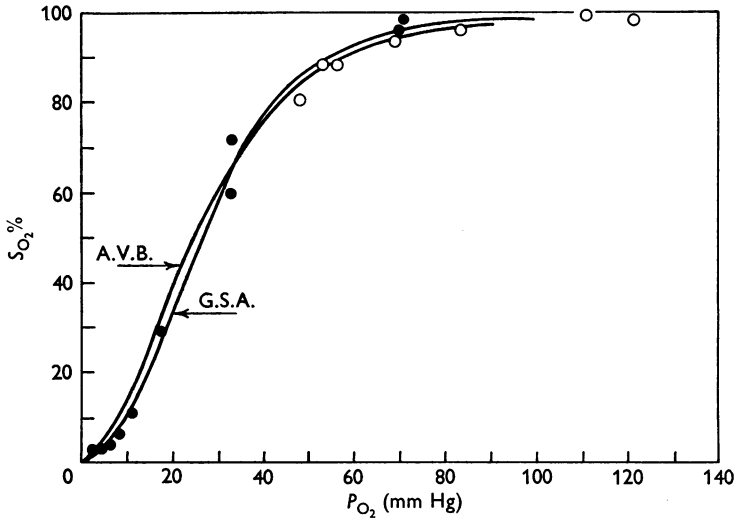

Fig. 1. Oxyhaemoglobin dissociation of human blood. Abscissae, partial pressure of oxygen (P_{O_2}, mm Hg); ordinates, percentage saturation of haemoglobin with oxygen (S_{O_2} %). The two solid lines reproduce the observations of Bock *et al.* (1924) for the blood of two individuals, A.V.B. and G.S.A. Experimental observations on blood of J.M.S. (see Table 1). ●, method 1 at pH 7·4–7·45; ○, method 2 at pH 7·3–7·5.

between human blood on the one hand and mouse and rat blood on the other, which it was our principal object to examine.

Mouse blood

Blood at pH ~ 7·4. Results obtained by methods 1 and 2, with blood taken on a variety of occasions by direct cardiac puncture appear in Table 2 and in Fig. 2.

Blood at pH ~ 7·2. The blood drawn from the orbital sinus of the anaesthetized animals was somewhat acid. The data appear in Table 3 and Fig. 2.

TABLE 2. Percentage saturation of the haemoglobin of mouse whole blood with oxygen at 37–37·5° C in relation to oxygen tension at P_{CO_2} approximately 40 mm Hg

Method 1 at pH 7·4–7·56			Method 2 at pH 7·35–7·4		
P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	Saturation (%)	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg) (±2 mm)	Saturation (%)
34·6	39·6	34·6	50·1	40	72·6
39·1	37·5	44·3	66·2	40	82·2
47·1	39·8	63·4	71·1	40	91·3
53·0	39·8	72·8	78·4	40	91·9
62·2	43·2	79·8	89·6	40	93·8
71·1	41·9	88·4	94·0	40	95·9
84·3	39·8	92·6	104·5	40	97·4
95·9	42·0	95·9	166·0	40	100·3
100·7	39·4	92·2			

TABLE 3. Percentage saturation of the haemoglobin of mouse whole blood with oxygen at 37–37·5° C in relation to oxygen tension at pH 7·15–7·2 and P_{CO_2} approximately 41–48 mm Hg

Determinations made by method 1

P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	Saturation (%)
4·0	47·8	0·9
6·1	48·3	1·5
10·5	40·6	2·8
14·9	45·2	5·0
106·0	47·1	87·1
107·0	48·2	83·8
132·3	46·7	93·7
146·0	45·4	94·1
164·7	44·6	95·2

TABLE 4. Percentage saturation of the haemoglobin of rat whole blood with oxygen at 37–37·5° C in relation to oxygen tension at pH 7·1 and 7·3–7·45 and P_{CO_2} from 45 to 51 mm Hg

Determinations made by method 1

P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	Saturation (%)
8·4	45·4	4·0
34·2	50·8	41·1
58·8	46·2	79·4
84·6	46·0	77·8*
85·0	50·0	88·2
112·9	49·7	90·0*
116·2	49·2	96·1

* Observations made at pH 7·1.

Rat blood

Blood at pH ~ 7·4. This blood was obtained by cardiac puncture on lightly anaesthetized animals. The results appear in Table 4 and Fig. 2.

Blood at pH 7·1. On one occasion the animal died under anaesthesia before the blood was taken from the heart, and the blood was rather acid. The two determinations appear in Table 4 and Fig. 2.

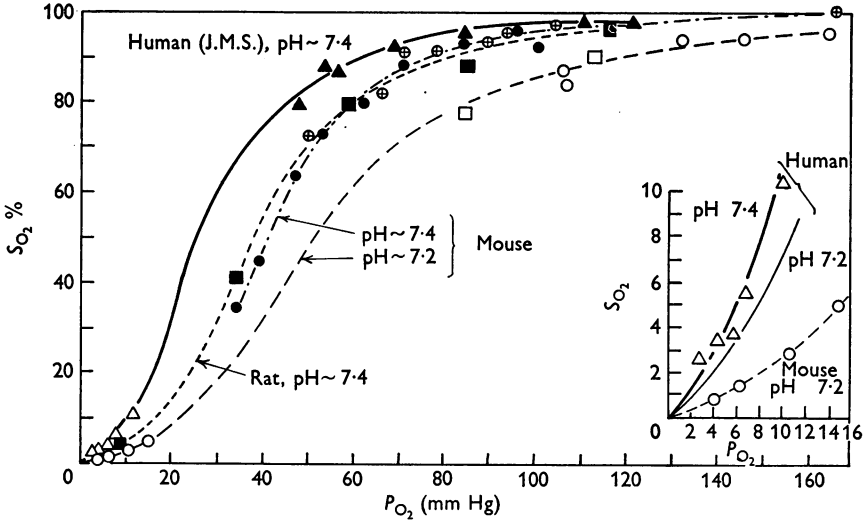

Fig. 2. Oxyhaemoglobin dissociation of human, mouse and rat blood. Abscissae, partial pressure of oxygen (P_{O_2}, mm Hg); ordinates, percentage saturation of haemoglobin with oxygen (S_{O_2}, %). △ human blood (J.M.S.) at pH ~ 7·4, method 1; ▲ human blood (J.M.S.) pH ~ 7·4, method 2; ■ rat blood pH ~ 7·4, method 1; □ rat blood pH ~ 7·1, method 1; ● mouse blood pH ~ 7·5, method 1; ○ mouse blood pH ~ 7·2, method 1; ⊕ mouse blood pH ~ 7·4, method 2. For ranges of pH values see Tables.

Inset: Enlarged scale for the same relations at low oxygen tensions. Symbols as in large figure; thin full line, curve for human blood adjusted to pH 7·2 (see text).

The lines are drawn through the experimental points except where specified.

The relative oxygen affinities of the blood of the three species

From Fig. 2 it will be seen that our best estimates of the partial pressure of oxygen at which blood from the three species at pH ~ 7·4 and in equilibrium with 40 mm Hg carbon dioxide is 50 % saturated are: man, 25 mm Hg; rat, 38 mm Hg; mouse, 41·5 mm Hg.

These figures indicate relative oxygen affinities of blood which is half saturated in the ratio man:rat:mouse = 1:0·66:0·60. At other degrees of saturation other ratios would be found. It may be seen from Fig. 3 that

when examined at pH ~ 7·45 samples of blood from the three species which are between 60 and 100 % saturated define approximately the same dissociation curve if for the abscissae we use scaled values $(P_{O_2})_S$ of the partial pressure of oxygen, where for man $(P_{O_2})_S = 1{\cdot}0(P_{O_2})_{observed}$, for the rat $(P_{O_2})_S = 0{\cdot}68(P_{O_2})_{observed}$, and for the mouse $(P_{O_2})_S = 0{\cdot}71(P_{O_2})_{observed}$.

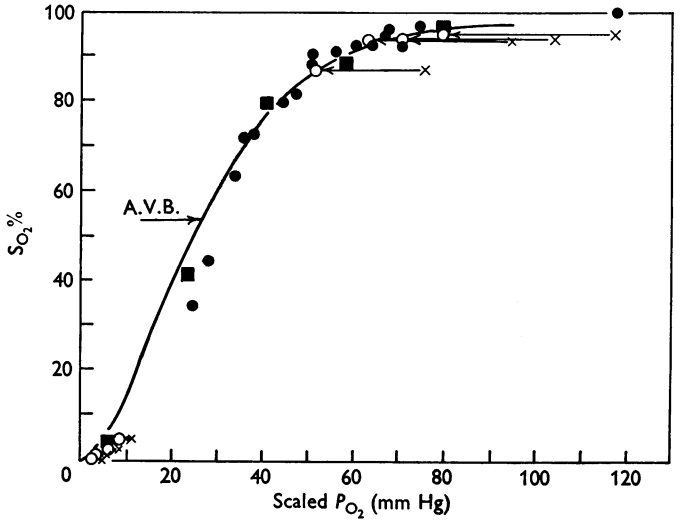

Fig. 3. Oxyhaemoglobin dissociation of human, mouse and rat blood. Abscissae, scaled partial pressure of oxygen (P_{O_2}, mm Hg) (see text); ordinates, percentage saturation of haemoglobin with oxygen (S_{O_2}, %). Curve of Bock *et al.* (1924). ■ rat blood at pH ~ 7·4; ● mouse blood pH ~ 7·4; × mouse blood pH ~ 7·2; ○ mouse blood pH ~ 7·2: scaled $P_{O_2} \times 0{\cdot}67$ (see text). For ranges of pH values see tables.

The influence of pH on the oxygen affinity of mouse and rat blood

As will also be seen from Fig. 3, the observations made with mouse blood at pH 7·2 (shown as cross) may be brought into approximate agreement with the values for samples measured at pH ~ 7·45 if, in addition to the scaling factor of 0·71 referred to above, we apply a factor of 0·67. This factor is a little less than the figure 0·72 for the Bohr factor for human blood which we derived from data given in the *Handbook of Respiration* (data of Severinghaus, 1958). The difference may be within our limits of accuracy, although such a difference between the magnitude of the Bohr effect for haemoglobin from mouse and human blood has been reported previously (Foreman, 1954; Riggs & Tyler, 1958).

In Fig. 2 (inset), observations made on mouse blood at pH 7·15–7·2 are compared on a larger scale with the curve of Bock *et al.* (1924) for human blood, measured at pH 7·45, but corrected by the appropriate

Bohr factor (Severinghaus, 1958) for human blood to pH 7·2. It is evident that for blood which is less than 5 % saturated the difference between the oxygen affinities of the human and mouse blood are much greater than when each is ~ 75 % saturated, unless the Severinghaus values of the Bohr factor are very greatly in error at low values of P_{O_2}. Although our data hinge upon the measurement of low values of P_{O_2} and small amounts of oxygen released from the blood, we believe that the data are reliable since the quantities of oxygen involved fall in a range where the accuracy of the Hersch cell is fully maintained.

DISCUSSION

As stated at the outset, this investigation was undertaken in the first instance in order to improve our understanding of some of the factors which control tissue oxygen tension in mice and rats under normal conditions and under conditions employed in radiobiological experiments with these animals–experiments which are used as a guide to the levels of damage likely to be sustained by different organs in man exposed to radiation either occupationally or for the radiotherapy of cancer. Our observations reveal species differences between the shapes of the curves relating oxygen tension and the degree of saturation of the blood, which may be important from this point of view. These differences seem to us to favour the small animals, compared with man, with respect to an elevation of tissue oxygen tension when oxygen at atmospheric pressure is breathed instead of air. This would accord with the observation that oxygen-breathing during irradiation substantially improves the cure rate of tumours in small animals but does not appear to have a comparable effect in man.

SUMMARY

1. The paper describes the application of a micro-procedure for assaying oxygen to the study of the oxyhaemoglobin of mouse and rat blood.

2. When the blood is about 70 % saturated with oxygen and at a P_{CO_2} of about 40 mm Hg, the oxygen affinity of the mouse and the rat blood is found to be only about two-thirds that of human blood.

3. At pH ~ 7·4 and P_{CO_2} = 40 mm Hg, we found the 50 % saturation partial pressure of oxygen to be: man, 25 mm Hg; rat, 38 mm Hg; mouse, 41·5 mm Hg.

4. Over the range of P_{O_2} 0–10 mm Hg, we found the difference between the oxygen affinity of mouse blood and human to be much greater than when the blood was approximately 50 % saturated.

We should like to thank Professor A. F. Huxley for the loan of the Haldane apparatus, Dr R. H. Thomlinson for assisting us in preparing samples of rat and mouse blood, and Professor Q. H. Gibson for critical comments on the text.

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