OBSERVATIONS ON THE TAKING UP OF CARBON MONOXIDE BY THE HÆMOGLOBIN OF THE SPLEEN.

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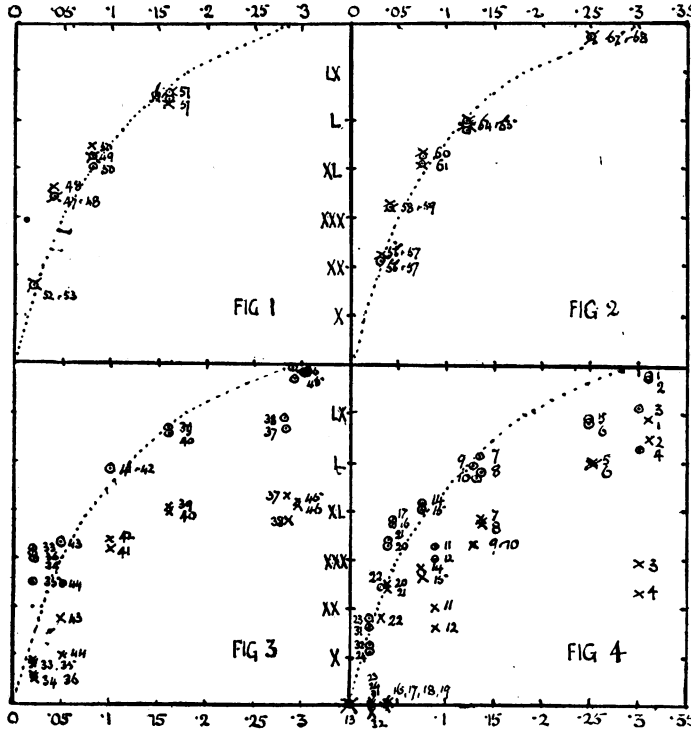
The present research is a continuation of that published by J. and H. Barcroft(1) who showed that on exposure of rats to an atmosphere which contained carbon monoxide the gas was rapidly taken up by the blood of the general circulation, but only slowly penetrated into the hæmoglobin of the spleen. They further showed that when in the spleen pulp the CO tended to remain there after it had disappeared from the general circulation. In their experiments the rats were in carbon monoxide at most for an hour and this time did not prove long enough for an equilibrium to be attained between the CO in the spleen pulp and that in the general circulation. Our first object was to ascertain the length of time taken for the attainment of this equilibrium. The method used was generally speaking the same as that described in the paper quoted and therefore need not be gone into, except where some special point arises. It became clear that our experiments would have to stretch over much longer time than those of our predecessors and therefore that much greater care would be necessary to maintain a constant concentration of CO in the chamber. The reason is as follows. Suppose the percentage of CO in the chamber were dropping, a false and premature equilibrium between the CO in the spleen and that in the blood would be established, not because the hæmoglobin of the spleen was gradually acquiring the gas, but because that of the blood was losing it.

Rigorous tests were therefore made of the capacity of the chamber to retain a given concentration of gas for a considerable time. The following is a sample—calculated concentration of CO put into chamber 0·165—hourly analyses made between the 11th and 18th hours after the concentration was set up gave results which varied between 0·160 and 0·166, the final one 0·163, thus the chamber retained all the CO so far as the experimental method of Haldane's apparatus could be expected to indicate.

Figs. 1 and 2 show the result of eighteen experiments in each of which

two rats were exposed to the concentration of carbon monoxide stated as the abscissæ for nine and six hours respectively, this series shows (*a*) that the hæmoglobin in the spleen is as fully saturated as that in the blood



Figs. 1–4. Abscissa = %CO in air, Ordinate = %CO hæmoglobin in ⊙ general circulation × spleen pulp. Guinea pigs, times of exposure in chamber Fig. 1, 9 hours; Fig. 2, 6 hours; Fig. 3, 4 hours; Fig. 4, 2 hours.

after six hours' exposure and (*b*) that no difference can be determined between the saturation either of the blood or of the spleen pulp by extending the experiment to nine hours.

If on the other hand the time be shortened to four hours (Fig. 3) a chart is obtained of a different character. The saturation of the hæmoglobin in the spleen is in every case less than that in blood by quite a considerable amount. Speaking generally the deficit is about the same at all parts of the curve; this is only a very rough generalisation but it is sufficiently accurate to point the contrast with a probable condition namely that the deficit would be proportional to the absolute saturation. Any scheme which one might visualise, of dilution of the spleen pulp

with the circulating blood, and actuated by regular rhythmic contractions of the spleen, however slow and shallow, would tend towards the deficit being proportional to the concentration.

The comparison of Figs. 2 and 3 shows that the time taken for the spleen pulp to attain equilibrium with the blood is between four and six hours in resting guinea pigs.

By far the most exhaustive set of experiments was the series carried out on guinea pigs at two hours' exposure. Here two new features appear: (*a*) the blood saturation at the higher points falls below the line obtained in the other experiments, this point will be referred to later, and (*b*) the spleen saturations at CO pressures of below ·05 p.c. of an atmosphere are in seven cases (Exps. 16–19, 23, 24, 31) nil and in one case (32) a minus quantity presumably due to an error in reading the reversion spectroscope. There are other experiments in the same region in which positive saturations are obtained in the spleen (20, 21 and 22) but the following statement covers the facts here presented:—in no case in which the blood saturation is below 24 p.c. is there any evidence of carbon monoxide having reached the spleen pulp in two hours in guinea pigs.

Exp. 13 is a control in which the animal was not put in the chamber and it appears on the chart as an isolated experiment. In point of fact a control of this kind was carried out for every pair of animals, thus there are over thirty experiments in guinea pigs alone to prove that the reading obtained from the hæmoglobin of spleen pulp in the ungassed animal is the same as that obtained from the hæmoglobin blood and therefore that the apparent absence of CO in the spleens of the animals noted, was not due to some systematic error which would produce a similar discrepancy in an animal whose blood contained no CO.

These facts seem to show that in guinea pigs at rest the spleen is practically shut off from the general circulation, and that the rhythmic contractions which are so constant a feature of plethysmographic tracings of the dogs' and cats' spleens are practically non-existent in the normal living guinea pig, when at rest, and possibly are due to experimental irritation of the spleens even in the animals on whose organs they have been observed.

A series of experiments was performed on rats very similar to the two hour series on guinea pigs and with much the same result, on the whole the figures both for spleen and blood tended to show a higher proportion of carboxyhæmoglobin at any given pressure of carbon monoxide but the deficit between the spleen and the blood was, as in the guinea pigs, usually about 15–20 p.c. There were two experiments in

which the CO hæmoglobin in the blood amounted to 28 and 24 p.c. respectively and in which no CO was found in the spleen. Twenty-four rats were used in this series.

In a few cases rats were kept some time after removal from the chamber and their bloods and spleens compared with those of animals killed in the chamber: the readings in this as in other experiments were made in duplicate and it will serve to show the sort of concordance which was obtained.

Rats 1, 2 and 3 were placed in the chamber for two hours, the concentration of CO was 0·145 p.c. 1 and 2 were then killed and 3 allowed to survive for another hour in air free from CO, the following are the saturations with CO:

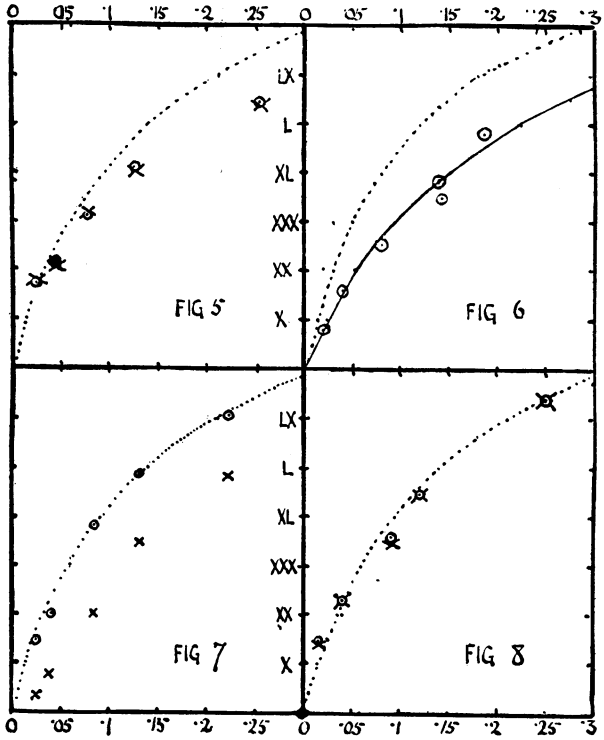
Rat	Observer	Blood %	Spleen %
1	Hanak	50	34
	Harkavy	50	36
2	Hanak	53	32
	Harkavy	51	38
3	Hanak	16	16
	Harkavy	17	18

Of greater interest than the experiments done on rats are those done on rabbits. For their initiation we have to thank Dale and Burn who made a suggestion for testing our view, that the spleen pulp was largely out of the circulation unless the spleen were made to contract by some special stimulus. It was the injection from time to time of a dose of adrenalin into the ear vein, in which case contractions would follow and so in each relaxation mix the spleen pulp up with corpuscles from without. The series was never pushed to completion because the first few experiments gave us the key to what seemed a more satisfactory control, revealing the fact that in half-hour exposures, although if the animal is kept quiescent the deficit in CO between the hæmoglobin of the spleen the general circulation is considerable, yet the spleen and the systemic circulation rapidly get into equilibrium if the rabbit is handled in such a way as to make it "kick about" as rabbits do.

In Figs. 7 and 8, which illustrate the point, the exposures were half-hour ones; in Fig. 7 the rabbits were allowed to stay very quiet, in Fig. 8 they were handled, the bloods contained the same amount of CO in each case, relatively to the dose, but whereas in Fig. 7 the spleens show a deficit of CO hæmoglobin of 15 p.c. (more or less) in Fig. 8 the spleens and the bloods are equally saturated. A single comparison of 15 minutes' exposure showed the difference between the animal which

was handled and that which was not, the figures are as follows. The concentration of CO was ·083 p.c.

Rabbit 1 handled, arterial blood 27 % sat. spleen 21 %
„ 2 quiet, „ „ 16 % „ „ 0 %



Figs. 5–8. Ordinate and Abscissa as in Figs. 1–4. Exposures: Fig. 5, rabbits quiescent, 1 hour; Figs. 7 and 8, ½ hour rabbits; 7, quiescent; 8, active; Fig. 6, guinea pigs, equilibrium between blow and atmosphere, dotted line in animals, thick line in vitro.

This experiment shows some points of contrast with the half-hour experiments. The arterial blood has not in 15 mins. had time to attain equilibrium with the air which would mean about 35 p.c. saturation, but the blood of the rabbit which exercised itself violently as the result of being handled contained considerably more CO than that of the quiet rabbit. The second point is that here again we have a spleen which like those of some guinea pigs contained no carbon monoxide. Thirdly, whilst there is a great difference between the CO saturations of the hæmoglobin in the two spleens, even that of rabbit 1 had not quite as much CO as the blood.

Two final series were undertaken for the purpose of ascertaining the speed at which equilibrium is obtained in rabbits which were encouraged to express their feelings in the form of violent muscular exercise. In ten minutes' exposure the equilibrium between the hæmoglobin in the spleen and that in the blood can be established as shown in Fig. 5, though this is not always the case; in the last series in which the dose was kept constant and the time of exposure varied, equilibrium was not reached in five minutes (one rabbit), and therefore, as might be expected, it was not reached in two minutes (one rabbit). Nevertheless even in this short exposure the deficit was not great.

The following comparison is instructive as showing that the hæmoglobin in the spleen pulp of a rabbit can in two minutes, if the rabbit kicks, attain to about the same degree of saturation as may be delayed for two hours if it does not.

Exposure minutes	Conc. of CO in air	COHb in blood (A)	COHb in spleens (B)	Diff. (A–B)
2	·12 %	26 %	23 %	3 %
120	·025 %	20 %	16 %	4 %

Without further experiment it is impossible to say whether the muscular exercise or the mental condition which prompted it was the cause of the establishment of contact between the spleen pulp and the circulation, but a hypothesis which has much to recommend it is that in these experiments we did what Dale and Burn suggested, but not in the way which they conceived, that in fact we had used the animals' own adrenals to secrete adrenalin; this may be so, but equally it may be that the spleen was stimulated directly through the sympathetic system, and of course the two methods of stimulation very commonly run concurrently. The invasion of the CO may be augmented by the considerable fluctuations in blood-pressure which no doubt occur under such circumstances.

One point may be mentioned which has no reference to the spleen, namely the relation between the saturation of the arterial blood and the pressure of CO in the air to which the animal was exposed. In each of the Figs. 1–8 the curve dotted in is a rectangular hyperbola, and in most of the experiments the blood-points fall upon it with as great an accuracy as could be expected, a comparison of the work of Nicloux(2) on dogs and pigs.

A comparison may be drawn between the curve which is obtained in the animal and that which would be obtained by equilibration of the animal's blood with the atmospheric air which the animal was breathing.

This comparison is set out approximately for guinea pigs in Fig. 6—approximately, because the guinea pigs used for the equilibration experiments were not the same animals as those used for the absorption of CO in vitro, and there are slight differences for different animals as has been shown for rats, men, etc., by Douglas, Haldane and Haldane(3). Each point on the equilibration curve represents a determination on the blood of a different animal. The pressure of oxygen in the alveolar air is of course calculable by the method suggested by Haldane and Lorrain Smith(4) and indicate an alveolar oxygen pressure of 11–12 p.c. of an atmosphere in guinea pigs. A comparison between Fig. 6 and Figs. 1–4 will show that in certain cases the blood-points fall below the dotted line, indicating that the alveolar pressure approximates more nearly to the atmospheric. These points are usually with high pressures of CO in the atmosphere and might, no doubt, be interpreted as the result of oxygen secretion, but they could in most instances be explained on the assumption that the animals were rendered dyspnœic, which in fact they often were. We made no actual tests of the degree of dyspnœa.

Summary.

1. The observation is confirmed that when rats are placed in atmospheres containing carbon monoxide, the hæmoglobin in the blood comes into equilibrium with that gas much more rapidly than it does with the hæmoglobin of the spleen pulp. This observation is extended to guinea pigs and rabbits.

2. In the case of guinea pigs, between four and six hours may elapse before the hæmoglobin in the spleen pulp attains equilibrium with that in the blood, in the case of rabbits over two hours may elapse—a fact which suggests the almost complete occlusion of the blood stream from the spleen and the reduction of the rhythmic contractions to something quite nominal.

3. If the rabbits are lifted and "kick about" the CO goes into the spleen relatively rapidly so that the spleen pulp may become charged in two minutes with as great a percentage of CO hæmoglobin as it might otherwise acquire in two hours.

4. It is suggested that under such circumstances stimuli passing along the splanchnics, either directly to the spleen, or to the adrenals or both, cause contractions of the spleen, whether these are rhythmic or spasmodic is uncertain.

5. When the guinea pigs, rats or rabbits are exposed to air containing

CO, for long enough to establish equilibrium, the general law is observed,

$$\frac{[COHb]}{[O_2Hb]} = K\frac{[CO]}{[O_2]};$$

as between the arterial blood and the alveolar air, there are cases with the higher concentrations of CO in which the saturation of the arterial blood is less than the law indicates. The calculated alveolar pressure of oxygen is as a rule about 12–13 p.c. of an atmosphere.

We are much indebted to Mr Barcroft for suggesting this research and for help in carrying it out. We should like to take this opportunity of thanking Professor Langley for giving us the facilities of his laboratory, and the Medical Research Council to whom much of the apparatus used in this research belonged.

REFERENCES.

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(2) Nicloux. Bull. Soc. Chem. Biol. 1, p. 114. 1919.
(3) Douglas, Haldane, J. B. S. and J. P. This Journ. 44, p. 275. 1912.
(4) Haldane, J. P. and Lorrain Smith. Ibid. 22, p. 231. 1897.

NOTE BY J. BARCROFT.

Professor Langley in criticising the above kindly pointed out to me that the spleens of different animals differ greatly in the amount of muscular tissue contained in the capsule and that the particular animals used were all such as had rather little muscle. As Drs Harkavy and Hanak had left Cambridge I performed with the help of Miss Sands an experiment on cats, which have very muscular spleens. The scheme of the experiment was as follows. A concentration of 0·14 p.c. CO was "put up" in the chamber in which were five kittens, and also myself. I was breathing air piped to "respiratory" valves from outside so as not to imbibe CO. A kitten was killed by decapitation so sudden as to be instantaneous, at each of the times stated below and the CO in the bloods and spleens pulp analysed:

Time from zero (minutes)		5	10	20	40	60
% COHb	Blood	20	39	56	61	63
	Spleen pulp	—	0	29	52	58

affording a general confirmation on kittens of the experiments on rats, guinea pigs and rabbits.