THE ACTION OF VARIOUS CONDITIONS ON CARBON MONOXIDE HÆMOGLOBIN. By H. HARTRIDGE.

(*From the Physiological Laboratory, Cambridge.*)

The action of light.

HALDANE in describing the action of light quotes the following experiment[1]. When air containing even as much as 1 % of CO was shaken with blood solution in bright sunlight, no pink colour at all could be observed in the solution; yet when the bottle was taken into the dark the marked pink colour at once appeared on shaking. This experiment showed the powerful dissociation caused by sunlight, which I have entirely confirmed; as an example I may quote the following:

Dark	96 %	Sunlight	40 %	Dark	91 %
Sunlight	42 %	Electric light	88 %	Daylight	80 %
Sunlight	35 %	Dark	92 %	6″ Magnesium	64 %

It would be a convenience if the reaction could be made irreversible, for one could then place the sample under the required conditions and estimate the change that had occurred in a given time. The following experiment was therefore tried: to a solution of COHb was added a few drops of ferri-cyanide; no change in colour occurred and no alteration in the bands if kept in the dark; but if now placed in sunlight, a rapid change took place, the solution turned brown, the methæmoglobin band appeared in the red and the CO bands weakened, till at the end of three minutes the change was complete. The explanation of the above may be found in the fact that ferri-cyanide is without action on COHb. If however the CO is split off, the Hb is at once attacked and changed to

[1] This *Journal*, xx. p. 504. 1896.

methæmoglobin, and the reaction in this way made irreversible. Sodium nitrate was found to act in the same way, and could doubtless be replaced by any of the methæmoglobin forming bodies. Further a few drops N/10, HCl also rendered the reaction irreversible, but was not found to give so sharp a contrast as ferri-cyanide between a solution acted on by sunlight, and a similar one from which light had been excluded.

Three questions naturally arise with regard to this light action:

(1) What rays are responsible?

(2) Are the precautions I have taken adequate?

(3) Is it due to a decreased stability of COHb or an increased affinity of O_2?

With regard to the first, it was considered to be almost self-evident that the active rays must correspond to one or more of the absorption bands; to bring out this point the following experiment may be quoted. Some COHb solution containing ferri-cyanide was placed in a narrow vessel, and round it was poured some solution of O_2Hb of the same strength in order that the light rays to reach the COHb should have to traverse one inch thickness of this, and in so doing would have those rays filtered off which correspond to the absorption bands. The bands of CO and O_2Hb being sufficiently alike for this purpose. If then the reaction was found to be slowed, it would indicate that the active rays correspond to one or more of the bands, but if unaffected, then to the rays between the bands. Now on doing the experiment it was found that the reaction took five times as long as usual, which showed the extent to which the harmful rays had been absorbed. I at once applied this observation by causing the electric lights in my room to be surrounded by jars, in which a dilute solution of blood was placed; this worked well but I found later that ordinary yellow or ruby glass worked equally well.

Having shown that the active rays correspond to an absorption band, the question arose as to which region of the spectrum exhibited the greater activity. Four little glass tubes containing the same mixture of COHb and ferri-cyanide were therefore placed in a spectrum projected by a diffraction grating and Nernst lamp, one was placed in the position of each band; on examination it was found that the solution placed in the ultra violet had changed to methæmoglobin the other solutions remaining practically unchanged. This clearly showed that the ultra violet rays had been responsible for this action on the COHb and explains the value obtained in the first experiment quoted for the

dissociation caused by different light sources; these sources, rich in actinic rays, *i.e.* sunlight, magnesium, etc., cause a big dissociation, while electric light, lamp light, etc., cause very little, because of their poorness in these rays.

I now proceeded to ascertain how far the screening fluid described in the previous paper had been successful in its object; I repeated the double vessel experiment mentioned above, replacing the O_2Hb solution in the outer vessel by some of this solution. As was mentioned this fluid cuts off all rays, except those in the yellow green, and thus exposes only the one band to light action; I now found that some 25 minutes were required to complete the change—in sunlight which was eight times as long as was required for the unprotected solution—and I therefore considered this precaution had been adequate in reducing the dissociation below the limits of experimental error.

As to the nature of the light action, it might be due to instability of the COHb or increased affinity of O_2 for Hb; to test it the following experiments were performed. If the affinity of O_2 is increased in sunlight, it appeared that it should retard the action of reducing agents, longer than if kept in the dark. To a solution of Hb was therefore added a few drops of ammonium sulphide, and half the solution was placed in a tube in the sun, the other half in a tube wrapped in tinfoil, to prevent light action. But it was found that if any difference between the rates of reduction in the two solutions was to be observed at all, it was an increased rate in sunlight; other experiments either confirmed this or showed no change; the increased rate was in the most marked cases only very slight and was probably caused by accidental warming of the solution by the sun.

This leaves one with the alternative that COHb becomes unstable in sunlight. That unaided light action is incapable of breaking the linkage, is shown by the observation, that if the oxygen be removed by a reducing agent, then no dissociation of COHb takes place; showing that the affinity of O_2 for the Hb is necessary in addition to cause the reaction to occur.

Haldane also investigated whether the light action could be due to alterations in the affinity of O_2 for Hb[1]; he prepared mixtures of air and hydrogen and was able to find no influence of light on the resulting saturation of the Hb. Further he tried the effect of X rays on COHb and here again failed to note any change.

[1] This *Journal*, xx. p. 505. 1896.

Influence of dilution.

The question as to whether the laking of blood and the subsequent dilution with water affect the saturation reached at any given tension of CO is one of considerable practical importance. For the spectroscope solutions are so much simpler to work with, in being able to have readings taken direct without corrections for dilution.

Further there is the advantage that solutions get into equilibrium with the gases with so much greater rapidity. The time factor is well shown in one of the following experiments; the method will however be considered in detail as it has been applied to other questions that appear in the course of this paper.

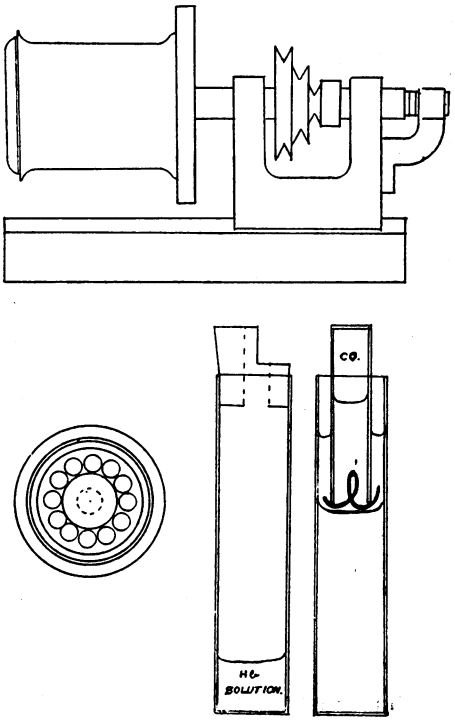

Fig. 1. Apparatus used for testing the effects of dilution, lactic acid etc. on the CO saturation of Hb.

A glass jar, with ground stopper, was fitted to the face plate of a lathe, as shown in Fig. 1. Inside this jar was a carrier to hold 13 narrow test tubes. These tubes were fitted with a cork through which a central hole was bored; half the upper part of the cork was cut away, to ensure free communication between the air in the tube and that contained in the jar. Twelve of the tubes were filled, each with a few c.c. of the material to receive investigation, so that when the jar was rotated the material would spread along the sides and thus offer a continually changing surface to the gas mixture. The thirteenth tube differed in being filled almost to the top with solid wax, the rest of the tube contained water and in this, mouth downwards, stood a small glass tube containing CO gas. It is clear that when the main vessel is placed on its side and fitted to the lathe, the water closing the mouth

of the small tube will run out, and thus allow the CO to escape and mix with the air the jar contains. All this takes place after the jar has been closed, and was found to be a very satisfactory way of filling the jar with a known mixture of CO and air. To facilitate further the rupture of the water seal, a small piece of copper wire, hooked round the open mouth of the small tube that contains the CO, prevented actual contact between this and the wax. Now after the vessel was screwed to the lathe, it was given two or three rapid turns to make certain that the seal was broken, and the CO had all escaped. It was then left for an hour undisturbed, to allow temperatures inside to become equalised, and to let the CO diffuse through the holes in the corks into the air in the tubes, and form one homogeneous mixture. The jar was then rotated continuously for half an hour, to obtain equilibrium between the samples and the contained gases. The jar was then opened and the saturations estimated in the spectroscope.

To return to the question of dilution, the results of the first experiment were as follows:

Undilute blood	...	...	14 %
Laked blood	...	...	26 %
Dilute blood (1 in 50)		...	60 %

It was obvious that the differences in the results were due to high ratio of surface to hæmoglobin offered by the dilute blood, compared with that of the undilute. In the second experiment I therefore used the same amount of hæmoglobin in each tube irrespective of the dilution.

The results I now obtained were:

Undilute blood	...	...	62 %
Laked blood	...	...	66 %
Dilute blood	...	...	65·5 %

The first is still a trifle lower, probably due to the blood becoming a little dry; in the last experiment I therefore added a little normal saline solution to this.

The readings were now:

Blood and saline	...	...	34
Laked blood	...	...	33
Dilute blood	...	...	33·8

This shows therefore that within the limits of experimental error,

the saturations were identical, and therefore one is justified in employing a solution of blood where necessary.

Haldane's experiments[1] on this same question agree with the above.

Influence of lactic acid.

In describing some experiments on the effects of lactic acid on the dissociation curves of oxyhæmoglobin, Barcroft pointed out[2], that unless this acid had the same effect on the compound with carbon monoxide as it had on that with oxygen, the values obtained when there is oxygen want, for the oxygen tension by the carbon monoxide method, would be rendered unreliable. To investigate this point the same apparatus was employed as that used for the dilution experiments above; to the blood solution in the different tubes being added 1 c.c. of a lactic acid solution of given concentration. The Pharmacopœa lactic acid was used in 75 % solution, this was diluted for use 1 in 100.

The results of the experiments are given in the table below, the values are the average found for four tubes at each strength of lactic acid.

	A	B	C	D	E
No lactic acid	23·5	40·5	54	62	34·5
·075 %	23·5	40	55	63	34
·025 %	23·2	41·5	59	63	35

All the experiments are not here given, as it was found in some others done at the same time, that the lactic acid had converted the blood into acid hæmatin thus causing the saturation with CO to rise above the value found for the control tubes; this being due to the fact that the COHb would not be so readily attached by the acid as the O_2Hb. I find therefore that within the limits of experimental error, lactic acid does not affect the saturation of blood with CO.

Influence of species.

This is a question of considerable practical importance because of its bearing on the employment of mice in coal-mines as an indication of poisonous quantities of CO. The evidence of the present workers on this subject, Haldane, Douglas[3] and Krogh[4], shows a marked difference in the saturations of blood of animals of different species,

[1] This *Journal*, xx. p. 512. 1896.
[2] This *Journal*, xli. p. 366. 1910–11.
[3] *Skand. Arch. f. Physiol.* xxv. p. 171. 1912.
[4] *Ibid.* xxiii. p. 217. 1910.

when in equilibrium with the same tension of CO in air; the same difference only not so marked is sometimes to be found even in the blood of different individuals of the same species.

My own experiments which are given below confirm these statements, I employed dilute solution of the blood samples mentioned, the % of hæmoglobin in each being approximately the same; the apparatus was the same as that used in the investigation of the effects of dilution above:

	A	B	C	D	E
Man	7	23	50	62	71
Sheep	7	22	46	60	66
Cat	10	22	48	60	67
Linnet	5	12	36	44	54

	A	B	C	D	E
Man	19	65	50	68	—
Mouse	11	45	36·5	46	—
Stale sheep	22·5	67	45·5	61·5	—

Now these individual experiments are not sufficiently numerous to enable one to make any definite statement with regard to the relative saturations of the different species examined; for this to be done, a number of individuals of each should be tested.

But for my purpose it was sufficient to prove the statement made by Haldane, namely, that in arterial oxygen tension experiments it is essential to employ blood taken from the subject of the experiment. Further I wish to call attention to the saturations found for man and mouse above for these confirm the conclusion of Haldane and Douglas; that mice as a rule become one-third less saturated than man at similar CO tensions. This invalidates the use of mice as CO indicators, the difference between the two species being further accentuated by the fact that at the same time, man usually has to do work.

The question at once arises as to the causes of this phenomonon, as I do not myself accept any theories as to non-identity of hæmoglobin of different animals. I assumed provisionally the presence of some influence from without. Now there are two factors to be examined (1) salt action, (2) acid and alkali; the first as pointed out by Haldane[1] would be certainly a possible cause, from the great influence salts were shown by Barcroft and Camis[2] to have on the hæmoglobin contained in the blood corpuscles; here however the differences are noted not only

[1] *Skand. Arch. f. Physiol.* xxv. p. 175. 1912.

[2] This *Journal*, xxxix. p. 118. 1909–10.

in unlaked blood, but also in a dilute solution, as indicated in my experiments.

Acid has been already investigated, it remained therefore to test salts and alkali. The results of the experiments were as follows:

	A	B	C	D	E
Normal solution	46	31	20	36·5	43
Solution + NaCl	45·5	31	20·5	—	—
,, $+Na_2CO_3$	47·5	32·5	21·5	38	45
,, $+NaH_2PO_4$	—	—	19	35·5	41·5

As far therefore as these salts are concerned, no explanation of this phenomenon has been obtained; slight differences between the normal blood and that containing carbonate are to be observed, but nothing at all comparable with that described above for different species.

The effects of temperature.

The first few experiments at once showed that temperature greatly influences the stability of COHb.

Three tubes containing mixture of CO and air and a little blood solution were shaken in the dark at room temperature till fully saturated. Readings were then taken, and the tubes shaken hard for 15 seconds in water at 70° C. On re-examining the tubes the CO % had fallen very considerably, the results being as follows:

Room temperature 14° C. ...	71	66	57
Water bath 70° C. ...	41	37	33

Further shaking however at the same temperature apparently had no other effect, than to cause the solution to coagulate. I therefore commenced experiments to ascertain the temperature correction necessary in order to convert a given saturation of Hb at one temperature to that at another. And it was clearly of distinct advantage to start at the lowest temperature and work upwards, since the initial experiments had shown how quickly the accommodation to a higher temperature takes place. Further the effects would be precisely the reverse of those that would be found if saturation were incomplete, lastly, if coagulation occurred at the higher temperatures it would not invalidate the previous readings.

The experiments were performed as follows: six milk testing bottles were obtained (Fig. 2), and into each was placed a small quantity of 1 in 40 blood solution; the same solution was also placed in a large vessel in which the bottles could be totally submerged, while being filled with

the required mixture of CO and air. Down the side of this vessel, led a fine glass tube, which having reached the bottom turned abruptly on itself and ended in a fine nozzle, this was connected with a supply of CO and air of 1 to 16 which was controlled by a pinch cock. One of the bottles having been taken, and placed in a special holder, the process of filling took place in the following way: first the bottle was plunged mouth downwards into the water bath till the open end rested on the bottom, the contents of the jar were stirred, and when the solution had reached a constant level inside the neck, the volume of air was read off and noted; the bottle was raised and lowered over the CO supply tube, passing this right up the neck till the nozzle was above the solution; a small quantity of CO was admitted and allowed

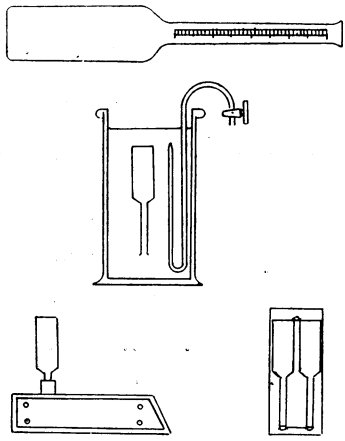

Fig. 2. Apparatus used for investigating the effects of temperature.

to mix with the air inside. The bottle was disconnected from the tube and plunged down to the bottom a second time, and the reading taken. The difference between this and the last being the amount of CO admitted. The bottle was then raised, and a small rubber cork pushed into the neck, a small quantity of the dilute blood solution being enclosed with the gas mixture. The other bottles were filled in the same way as the above, and were then placed in a metal box, and a thick rubber band passed round the lid. The water bath having been prepared at the required temperature, the box was plunged in and the contents thoroughly shaken for about half an hour. The bottles were then removed and readings taken in the spectroscope, the bottles being mouth downwards, so that the blood solution should

drain into the narrow neck and thus offer a very small surface to the contained gases. Further the green solution into which the necks were placed during the observations had also been warmed in the water bath at the same time as the bottles, and thus the blood solution was kept approximately at the same constant temperature.

The temperatures investigated ranged from 0 to 60° C., two readings being made every 10°; these are shown in the table below, and are plotted in Fig. 3.

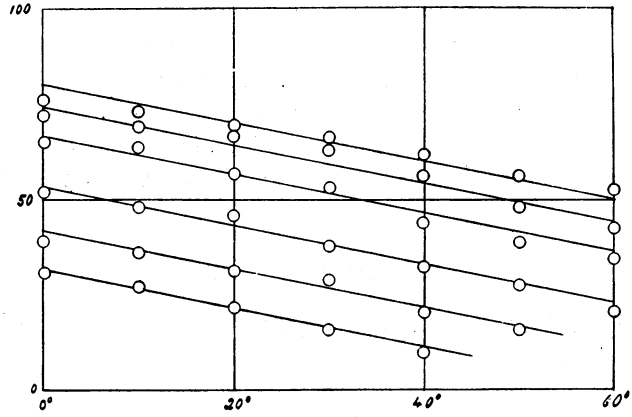

Fig. 3. Temperature curves. CO saturation vertical. Temperature horizontal.

	A	B	C	D	E	F
0°	72	65	76	31	37	52
10°	69	63·5	73	27	36	48
20°	66·5	57	69·5	22	31·5	45·5
30°	63	53	66	16	29	38
40°	56	44	61·5	10	20·5	32·5
50°	48	39	56	—*	16	28
60°	42·5	34·5	52	—*	—*	20·5

* Coagulated.

Therefore for the range observed there is practically a decrease in the saturation of $\frac{1}{2}$% for every degree rise of temperature, this being almost independent of saturation.

This shows the importance of keeping the temperature constant in tension experiments, particularly at points of low saturation; this is a matter therefore of great moment when experiments on arterial oxygen tension are being performed. Now Haldane's experiments on this point do not agree with the above; he found no difference between the

saturation of oxygen and human blood at 37·7° and room temperature[1]. There are however, oddly enough, experiments on mice in the same paper quoted, which do agree[2], the experiments being as follows: the mice were placed in the cold so that their rectal temperatures fell in certain cases below 19°, and it was found that then the CO saturation reached was higher than that obtained at normal temperature; that is to say the same effect as similar experiments performed *in vitro*. The mere effect of temperature may therefore explain them. Other experiments on the other hand by Lorrain-Smith[3] showed similar results for fever in which a rise of temperature would occur; the rise would not however amount to more than a few degrees, and it is possible that some other factor was here coming into play. I find that in their latest paper Haldane and Douglas[4] mention that the precaution should be taken to work at body temperature, in experiments on the arterial oxygen tension; the data of these experiments are however not yet given.

Influence of CO_2.

Haldane, Douglas[5] and Krogh[6] are agreed that CO_2 has no specific action, that is, its presence or absence leaves the final saturations unaffected.

My experiments were performed in the apparatus used in the previous experiments, a slightly different method of filling being however employed. The bottles were filled half full in pairs, with the same mixture of CO and air; one bottle of each pair being filled up with nitrogen, the other with CO_2. The latter gas was not absolutely pure, containing about 20 % of air. The results were:

	A	B	C	D	E	F	G	H	I	J	K	L
CO_2	84	58	50	67	41	73	65	70	78	33	25	22
N_2	89	61	53	70	41	72	63	74	85	36	28	27
					15° C.					60° C.		

This confirms the results of the previous observers, and has the important result of allowing the examination of alveolar air, without any fears of the saturation being influenced by the CO_2 present.

[1] This *Journal*, xx. p. 454. 1896; and xxii. p. 252. 1897–8.
[2] *Ibid.* xxii. p. 238. 1897–8.
[3] *Ibid.* xxii. p. 310.
[4] *Skand. Arch. f. Physiol.* xxv. p. 171. 1912.
[5] This *Journal*, xx. p. 513. 1896.
[6] *Skand. Arch. f. Physiol.* xxiii. p. 221. 1910.

SUMMARY.

(1) The following factors are without influence on the final saturation of hæmoglobin with CO : dilution, lactic acid, CO_2, certain acid and basic salts.

(2) The following have a very marked influence: light, temperature, species.

With regard to the first, I have shown that investigation is simplified if the reaction can be made reversible, by addition of some chemical agent which removes the O_2Hb but leaves the undissociated COHb unaffected. The active rays are found to correspond to the absorption bands, those in the ultra violet being most active. The screening fluid employed in the spectroscope has been sufficient to reduce this light action to a minimum.

The dissociation is due to a change in the stability of COHb, and not to any alteration in the combination with oxygen.

(3) Temperature has a most marked influence; the change in the saturation being about $\frac{1}{2}\%$ for every 1 degree rise of temperature; it is therefore relatively more important at low, than at high temperature.

(4) Equilibrium is reached at different saturations by the blood of animals of different species.