THE RELATION OF THE ACTION OF CARBONIC OXIDE TO OXYGEN TENSION.

By JOHN HALDANE, M.A., M.D., *Lecturer in Physiology, University of Oxford. Grocers' Company Research Scholar.* (Two Figures in Text.)

[*From the Physiological Laboratory, Oxford.*]

THE following investigation took its origin from an enquiry in which I am at present engaged into the nature and action of the suffocative or poisonous gases met with in the air of coal-mines. Among these gases is carbonic oxide. So far as my experience goes, however, carbonic oxide never occurs alone as an impurity, but always in connection with an excess of diluent gases, chiefly nitrogen; so that when carbonic oxide is present in the air of a coal-mine, there is also a more or less considerable reduction in the percentage of oxygen. It is known, however, firstly, that part at least of the action of carbonic oxide is due to its property of entering into combination with the hæmoglobin of the blood corpuscles, and so putting them out of action as oxygen carriers; and, secondly, that the proportion of carbonic-oxide-hæmoglobin formed in blood brought into contact with a gas-mixture containing carbonic oxide and oxygen, depends not merely on the tension of carbonic oxide in the mixture, but also on the tension of oxygen. The larger the proportion of oxygen in the gas mixture the smaller will be the amount of carbonic-oxide-hæmoglobin formed in the presence of a given proportion of carbonic oxide. Hence it might be expected that in air containing a diminished proportion of oxygen, carbonic oxide will be more poisonous; and that in air containing an increased proportion the poisonous action will be less.

A very simple experiment served to show that this is the case.

Two bottles, each of about three litres capacity, were filled, one with oxygen, and the other with air. Into each bottle 15 c.c. of carbonic oxide were introduced through a tubulated stopper. The bottles were then closed, and the gases mixed by thorough shaking with a little water which had been left for the purpose inside. The water having been removed a young mouse was introduced into each of the bottles, which were again closed. Within five minutes the mouse in the bottle containing air showed marked signs of loss of power over the limbs, and two minutes later it was lying on its back comatose. Fresh air was then at once blown through the bottle with a bellows. Two minutes later the mouse had regained power over its limbs, and about twelve minutes later it seemed quite in its normal condition again. Meanwhile the mouse in the bottle containing oxygen remained quite unaffected. After an hour and a half it was taken out. For some time previously it had been shivering, and was evidently suffering from cold, as it had got rather wet in the bottle, but it showed no symptom of poisoning. Both mice remained perfectly well when replaced in their cage.

It seemed of importance to determine as accurately as possible within what limits raising or lowering of the oxygen percentage affects the poisonous action of carbonic oxide. For this purpose the following arrangement was employed (Fig. 1).

A mouse was placed in the bottle *A*, which had a capacity of about 200 c.c.: through this bottle a perfectly steady air-current in the

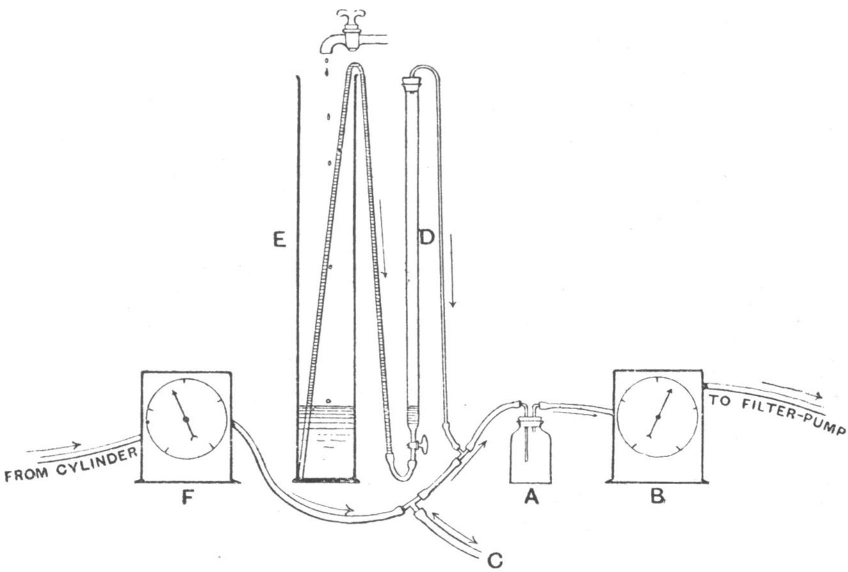

FIG. 1.

direction shown by the arrows was maintained by means of a filter pump driven by water supplied at a constant pressure from a cistern. The air current was measured by the small meter *B* (the accuracy of which was ascertained) and was drawn into the tubing at the opening *C*. Between *C* and *A* a T tube was placed, communicating by one of its limbs with narrow glass tubing, which was connected in the manner shown by means of a paraffined cork with the upper end of a burette *D*, containing carbonic oxide. The nozzle of the burette was connected with a siphon, dipping into a tall glass jar *E*. Over this jar was a water tap. When water was allowed to drop from the tap into the jar the level of the water in the latter, and in the burette gradually rose, and a measured quantity of carbonic oxide could thus be steadily and slowly driven from the burette into the air current. By regulating the water-tap the rate of outflow of carbonic oxide could be regulated at will. The water-tap was not connected directly with the burette because a steady outflow of water could not be obtained unless the rate of flow was considerable.

When it was desired to substitute oxygen or any other gas for the whole or part of the air the oxygen was allowed to pass in from a cylinder of compressed gas through the meter *F*. When pure oxygen was required the flow was so regulated that a slight excess of oxygen passed outwards by the opening *C*. By means of this arrangement it was possible rapidly to substitute oxygen for air without the slightest interruption or alteration in the rate of flow through the bottle and meter *B*.

The carbonic oxide employed was made in fairly large quantities at a time by heating oxalic acid with sulphuric acid, and kept in a large bottle fitted up as a gasholder. The gas was purified by passing it through a large tube of moist potash-lime. Each new stock of carbonic oxide was accurately analysed to ascertain the degree of purity of the gas. Sometimes, as in the example given below, it was found that, in spite of care, appreciable quantities of air had become mixed with the carbonic oxide. In calculating the percentages allowance was in each case made for the impurity. The oxygen was determined with pyrogallic acid and potash, and the carbonic oxide by ammoniacal solution of cuprous chloride. The apparatus was the same as that which I have used in the analysis of gases from coal-mines.[1]

Example of an analysis.

Carbonic oxide	92·27	
Carbonic acid	0·07	
Oxygen	1·56	} = 7·66 of air.
Nitrogen	6·10	}
	100·00	

[1] *Proc. Roy. Soc.* 57, p. 244, 1895, and *Transactions of the Federated Institution of Mining Engineers*, 1895.

A month later, after nearly the whole of this bottleful of gas had been used, the residue was found to contain 0·15 % of carbonic acid and 1·96 % of oxygen (corresponding to 9·4 % of air), so that there was only a very slight additional contamination of the gas by air diffusing from the water.

As good reasons exist for suspecting that the gas employed in some of the recorded experiments on carbonic oxide was impure and contained carbonic acid or air, it seemed necessary to take every precaution as regards the gas employed.

In air about 0·06 % of carbonic oxide is sufficient to produce distinct symptoms in mice. The following are the notes of an experiment.

Young black and white mouse about half grown. Rate of ventilation 1·70 litres per minute. Rate of CO delivery 1·00 c.c. per minute. Corrected percentage of CO = ·058 %[1]. Temperature 13° C.

5 p.m. Mouse placed in bottle and left in the air current, no CO being added.

5.38 No change. Quite active. Movements as sharp as usual. Climbs up glass tube, cleans its fur, &c. as usual. CO now added.

5.42 Slight hyperpnœa, the respiratory movements being more visible.

5.47 Mouse less active. Sits up on hind legs, panting slightly. Does not clean its fur, &c. as usual.

5.51 CO off.

5.57 Cleaning its fur vigorously, climbing up the tube, and is altogether much more lively. No longer panting, and appears to be quite as usual again.

5.59 CO again started.

6.4 Panting distinctly. On trying to climb up it gets exhausted, and sits panting.

6.13 Remains very quiet. Sitting in one position, and apparently panting.

6.24 Seems drowsy and torpid. When roused so that it tries to climb up the glass a tendency to slip down is apparent, so that there is some loss of power in the limbs.

6.30 Bottle containing mouse was put in warm water at about 35° C.

6.35 Panting much less, but torpor and feebleness remain.

6.36 Bottle put in cold water at 12°. Panting began again in about a minute.

[1] The stock of carbonic oxide used in this experiment had the following composition:

Carbonic oxide	97·42	
Carbonic acid	0·05	
Oxygen	0·48	air 2·53.
Nitrogen	2·05	

6.55 After the last observation had been repeated several times the bottle was placed in warm water at 26° and left.

7.6 Quite as torpid and feeble as ever. CO off.

7.12 Has regained its full power over limbs, climbed up tube without exhaustion, and then began to clean its fur.

7.15 Quite as lively as usual again.

The following experiment will afford an idea of the effects of a somewhat higher percentage of carbonic oxide.

Young mouse, not quite fully grown. Rate of ventilation 1·03 litres per minute. Rate of CO delivery 2·34 c.c. per minute. Corrected percentage of CO 0·221 °/₀. Temperature 14·5°.

10.55 CO started.

10.59 Hyperpnœa and great loss of power over legs. Cannot stand. Lies on belly with legs stretched out. Seems hardly conscious.

11.12 Continues very helpless and drowsy looking. Remains lying on side when put there, but does not remain on its back.

11.20 Same condition, but respirations seem less frequent (108). [Normal frequency = about 140.]

11.35 Respirations 90. Seems feebler.

11.46 Respirations 74 and are feebler. Remains on back when put there.

11.57 Respirations 66. Has lain on its back since last observation.

12.8 Respirations 44.

12.27 Respirations 35.

12.49 Respirations 16 and gasping in character.

1.20 Died almost imperceptibly. Since last observation respirations had gradually become shallower, until at last they died away.

On post mortem examination the liver and other organs had the characteristic bright red appearance seen in carbonic oxide poisoning. On diluting a little of the blood with water the colour of the solution indicated that the blood was about two-thirds or three-fourths saturated with CO.

With larger proportions of carbonic oxide than in the last experiment death is correspondingly more rapid, and is accompanied by convulsions, as in the case of asphyxia from breathing an atmosphere deprived of oxygen. If death occurs within a few seconds the blood in some parts of the body may, as Heger has already observed[1], contain very little carbonic oxide hæmoglobin, death being too rapid to allow the whole of the blood to become saturated.

The observation that so small a proportion 0·06 °/₀ of carbonic oxide

[1] *Journal de Médecine de Chirurgie et de Pharmacologie*, 1894, p. 106.

produces distinct symptoms is not new, but has previously been made by Hempel.[1]

It is probable that, particularly with such small animals as mice, death by poisoning with very small proportions of carbonic oxide is due partly to fall of the body temperature consequent on diminished metabolism and heat production. The following experiment, in which the production of carbonic acid by a mouse was estimated, supports this view.

The apparatus was the same as that already described (fig. 1) except that the air entering the bottle was deprived of carbonic acid by soda lime, while the carbonic acid in the outgoing air current was estimated by means of the absorption apparatus described by Mr Pembrey and myself[2].

Time		CO_2 given off in grammes	Air breathed by mouse
First	15 minutes	·0552	Pure air
Second	,, ,,	·0570	Pure air
Third	,, ,,	·0531	0·16 % of CO
Fourth	,, ,,	·0264	0·16 % of CO
Fifth	,, ,,	·0272	0·16 % of CO
Sixth	,, ,,	·0227	Pure air
Seventh	,, ,,	·0405	Pure air
Eighth	,, ,,	·0508	Pure air

The carbonic oxide produced the characteristic loss of power over the limbs, &c. Recovery was rather slow. During the seventh and eighth periods shivering was observed, as if the animal were suffering from cold. Probably its temperature had gone down under the influence of the carbonic oxide. The bottle containing the animal was kept in a bath at 15·5° C., and the rate of ventilation was 0·48 litres per minute.

It will be seen that the carbonic oxide produced a very marked diminution in the respiratory exchange, and that the diminution lasted for about half-an-hour after the animal had ceased to breathe the carbonic oxide.

Experiments with Carbonic Oxide in Oxygen.

The oxygen used in these experiments was that supplied in cylinders by Mr Orchard. It contains about 97 % of oxygen. The

[1] *Zeitschrift für analytische Chemie*, XVIII. p. 399.
[2] *Philosophical Magazine*, 1890, p. 306.

following is the result of an analysis of a sample from the cylinder employed in most of the experiments.

Oxygen	96·91
Carbonic acid	0·25
Nitrogen	2·84
	100·00

I have breathed this oxygen pure for considerable periods without its producing any appreciable effects. It has no smell suggestive of chlorine or other impurity.

In oxygen it requires about ·8 % of carbonic oxide to distinctly affect a mouse, and very much more to produce death. The effect of dangerous percentages (over 5 %) seems to vary considerably according to circumstances. When the percentage is gradually increased the mouse appears to be capable of adapting itself to a considerable extent to the atmosphere. The following two experiments may be quoted.

Young mouse, not quite fully grown. Initial ventilation ·28 litres per minute. Bottle kept in bath at 28°.

12.20 Mouse put in bottle and ventilated with oxygen.

12.37 CO started. Corrected percentage = ·85.

12.41 Slight hyperpnœa, and seems a little drowsy.

12.46 Distinct sluggishness, and apparent slight loss of power, noticed when it jumps up on the side of bottle.

12.55 Condition same. Ventilation reduced, so that CO percentage = 1·76.

12.58 Hyperpnœa and marked loss of power. On trying to climb up it stops to rest, or slips down.

1.5 Seems no worse. Lies or sits in awkward positions, as if very drowsy. When roused gets up on legs.

1.14 No worse. Began to clean its fur, but stopped again at once.

1.20 Ventilation increased from ·13 to ·66 litres per minute (i.e. to five times its previous amount). Oxygen turned off and air substituted. Percentage of CO now = ·34 (i.e. a fifth of its former amount.)

1.22 Seems decidedly more feeble, and rapidly losing power.

1.25 Remains lying on its side or back in a comatose state.

1.26 CO exhausted. The mouse had recovered completely a few minutes later.

Same mouse. Ventilation throughout = ·102 litres per minute. In bath at 28°.

2.5 Mouse put in and ventilated with pure oxygen. After a short time went to sleep.

2.12 CO started. Percentage = 2·31.

2.15 No change in the mouse. Sitting asleep.

2.17 Laying head down; seems unnaturally drowsy and losing power over limbs.

2.22 Lies with legs spread out, but will not remain on its back.

2.28 Condition same. CO increased to 2·97 %.

2.39 Not apparently more feeble. Still will not lie on its back. Sometimes wakes up and tries to climb.

2.41 CO increased to 7·2 %.

2.47 Still does not remain on its back, and seems no worse. CO exhausted.

3.0 Completely recovered. Now cleaning its fur.

These experiments sufficiently show that in oxygen carbonic oxide is very much less poisonous than in air. This fact can also be very conveniently demonstrated with the apparatus described by alternately supplying the animal with air and oxygen, while the delivery of carbonic oxide and the rate of ventilation remain absolutely unaltered. If about ·2 to ·5 % of carbonic oxide are added to the ventilating current the animal will be seen to alternately become worse or recover again according as air or oxygen is supplied. With a somewhat higher percentage death occurs very rapidly when air is supplied.

Experiments with diminished oxygen percentages.

To obtain an atmosphere containing a diminished percentage of oxygen, hydrogen or nitrogen was partially substituted for air by means of the arrangement already described. The hydrogen employed gave no arsenic reaction with Marsh's test, and portions from the same cylinder had previously been used for experiments on man without any ill effects being produced.

Mice, as compared with men, appear to be specially sensitive to reduced percentages of oxygen. A mixture of one-third of hydrogen, or nitrogen and two-thirds air causes panting and uneasiness. Cyanosis and loss of power over the limbs is caused by a mixture containing two-thirds of hydrogen. With considerably reduced percentages of oxygen it is thus difficult to distinguish the symptoms produced simply by lack of oxygen from those due to carbonic oxide. Nevertheless it is quite evident that the poisonous action of carbonic oxide is very markedly increased by a diminution of the oxygen percentage. The following experiment may be quoted.

Half-grown brown mouse. Ventilation 1·00 litres per minute. Temperature 12·5°.

1.7 Mouse put into bottle and ventilated with pure air.

1.9 Continues quite lively. Hydrogen now added at the rate of ·33 litres per minute, so that oxygen percentage was reduced from 20·9 to 13·9.

1.12 Mouse panting a little, and is not so lively. Still cleans its fur, &c. but often stops to pant.

1.21 Mouse remains the same. No loss of power. Hydrogen off.

1.24 Panting ceased. Much more lively.

1.25 CO turned on. Percentage = 0·247.

1.27 Panting.

1.29 Limbs getting feeble.

1.32 Limbs sprawling. Mouse sits resting on its belly. Will not lie on side or back.

1.35 No further appreciable change in mouse. Hydrogen turned on at same rate as before.

1.36 Mouse much worse. Lies on back.

1.36½ Respirations gasping in character. Mouse lying on back with limbs twitching.

1.38 Seems to be dying. Hydrogen off.

1.39 Improving. Will not now lie on back. Twitching and gasping respirations have disappeared. Limbs still sprawling.

1.40½ Hydrogen on again.

1.41½ Gasping respirations.

1.42½ Remains on back or side. Quite comatose.

1.46 Hydrogen off.

1.51 Remains in same condition. Respirations getting less frequent, CO stopped.

1.58 Still quite comatose. Taken out. Felt very cold, therefore warmed in the hand, when it gradually began to revive, and was replaced in its cage in cotton-wool.

At 2.50 it was still feeble, but when seen again eight hours later it was perfectly well.

A similar very marked increase in the poisonous action of carbonic oxide was observed in several other experiments with hydrogen and air: also in an experiment in which a mixture of 31·4 % of pure nitrogen and 68·6 % of air was employed.

The conclusions drawn from these experiments with carbonic oxide in air containing a reduced percentage of oxygen have of course a special practical interest in connection with carbonic oxide poisoning as it occurs in coal-mines and elsewhere, but I hope to deal more fully with this subject in another paper.

Discussion of the Results.

In the case of pure oxygen the tension of oxygen is nearly five times as great as in air, so that to produce equal saturation of the hæmoglobin of the corpuscles with carbonic oxide in oxygen and in air, the tension of carbonic oxide would presumably require to be five times as great in the oxygen as in the air. Hence on the hypothesis on which the investigation started one might expect that carbonic oxide would turn out to be about five times as poisonous in air as in oxygen—that is to say, that five times as high a percentage of carbonic oxide would be required in oxygen to produce the effect of a given percentage in air.

Now the experiments made with the apparatus described above clearly showed that carbonic oxide is much more than five times as poisonous in air as in oxygen. In the latter gas the poisonous action is reduced to about a tenth or less. Evidently then some other factor has to be taken into account besides the relative tensions of oxygen and carbonic oxide. That this factor exists was further shown by the following experiment:

A thick-walled bottle of 3·1 litres capacity was filled over water with oxygen, to which was added 30 c.c. of carbonic oxide, so that the oxygen contained about 1 % of carbonic oxide. After the latter had been thoroughly mixed with the oxygen a full-grown mouse was placed in the bottle, which was again tightly closed by means of a tubulated cork, the tube of which was connected with a filter-pump and gauge, so that the pressure in the bottle could be reduced as required. By means of a clamp the bottle could be shut off from the pump when any desired pressure was obtained.

4.40 Mouse put into bottle.
4.42 Some hyperpnœa.
4.56 No further change. Walks and climbs about normally. No distinct loss of power over limbs, though perhaps a little sluggish.
4.56–57 Pressure reduced to about 50 % of an atmosphere.
5.1 Hyperpnœa more marked. More tendency to stop and pant.
5.5 No further change. Pressure reduced to 27 % of an atmosphere.
5.8 Panting greater. Looks drowsy and totters when walking.
5.11 Marked weakness of limbs. Tends to sprawl on its belly or lie on its side but can creep about when roused.

5.15 No further change. Pressure reduced to about 17 % of an atmosphere.

5.20 No marked change. Pressure reduced to about 10 % of an atmosphere.

5.25 Perhaps a little more helpless, but no marked change. Air was now let in to atmospheric pressure, when the mouse rapidly improved, and recovered completely on being taken out.

In this experiment the oxygen tension and carbonic oxide tension were diminished simultaneously, and in equal proportions. The effect of the carbonic oxide nevertheless increased.

To account for this relation between oxygen tension and the effect of carbonic oxide the hypothesis suggested itself that the higher the oxygen tension the less dependent an animal is on its red corpuscles as oxygen carriers, since the oxygen simply dissolved in the blood becomes considerable when the oxygen tension is high.

Experiments with increased Oxygen Pressure.

If the above hypothesis were correct one would expect to find that by raising the oxygen tension sufficiently high it would be possible to abolish entirely the poisonous action of the carbonic oxide. There is, however, a limit to the possibility of raising the oxygen tension, since, as shown by Paul Bert, at about five atmospheres, or somewhat less, oxygen acts as a poison. The question therefore was whether at a less tension than this the action of carbonic oxide could be abolished. To investigate this the following arrangement was employed (Fig 2).

Three thick-walled measuring cylinders, *A*, *B*, *C*, each of about 650 c.c. capacity, and provided with tightly fitting paraffined corks, tubulated in the manner shown, were connected together by means of thick-walled rubber tubing. *A* was further connected, in the manner shown, with a cylinder of compressed oxygen, and a mercury pressure gauge *E*, and with the vessel *C*. Screw clamps were placed on the tubing at *E* and *F*, and there was a three-way glass tap at *D*. The joints, corks, etc. were carefully secured, so as to withstand the required pressure. The vessel *B*, having been first filled with water, was connected with the gas-holder of carbonic oxide, and filled with carbonic oxide, the water being sucked over into *C*. The tap *D* was then closed. A mouse was now placed in *A*, which had been previously filled with oxygen. The cork having been pressed home and secured, the pressure in the whole system was gradually raised to the required amount by cautiously turning the regulating screw of the oxygen cylinder. The clamp *E* was now closed, and *D* turned so as to connect *A* with *B*. Carbonic oxide could then be driven from *B* into *A* as required, by means of pressure from the oxygen cylinder.

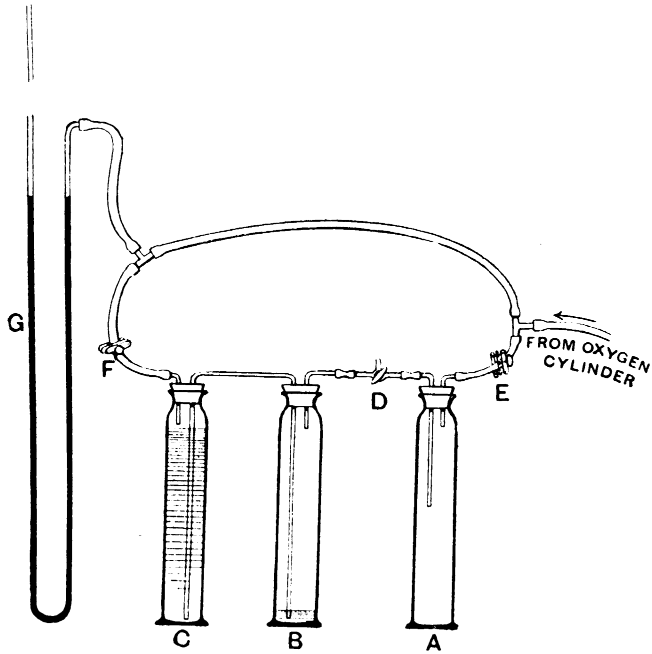

FIG. 2.

The following are the notes of an experiment.

7.15 Large mouse put in.

7.21 Pressure raised to 70·5 cm. of mercury.

7.26 Mouse unaffected. Apparatus tight. 30 c.c. of CO (giving a tension of 6 % of an atmosphere of CO in *A*) driven into *A*.

7.28 Slight hyperpnœa.

7.29 35 c.c. of CO sent in, so that tension of CO in *A* about 15 % of an atmosphere.

7.31 Hyperpnœa more marked. Often jumps up in vessel, as if uneasy.

7.32 50 c.c. of CO sent in so that CO tension in *A* = about 27 % of an atmosphere.

7.35 Mouse gets easily exhausted after any effort, such as jumping up in the bottle. 75 c.c. of CO sent in, so that tension of CO in *A* = nearly 50 % of an atmosphere.

7.38 Mouse as before; can walk about quite well when *A* is held horizontally, but is exhausted easily by any effort. The whole of the CO now sent in, *D* and *F* closed and *E* opened. Pressure in $A = 139{\cdot}5$ cm., so that tension of CO in $A = 139{\cdot}5 - 70{\cdot}5 = 69$ cm., or nearly an atmosphere.

7.47 No change in mouse. Walks about, and climbs on the tube when *A* is held horizontally, but is easily exhausted and somewhat sluggish.

7.47–49 Pressure in *A* diminished to 63 cm. by opening *D* outwards. Mouse rapidly became helpless and unconscious, and began to gasp. Died at 4.51.

Post-mortem. Liver and other organs bright red. A drop of blood largely diluted with water gave the characteristic cherry-red colour, which was hardly appreciably altered on shaking some of the solution with coal-gas. The blood was thus practically saturated with carbonic oxide.

This experiment shows that at a tension of two atmospheres of oxygen the poisonous action of carbonic oxide on mice is abolished. Apart from its action in putting the red corpuscles out of action as oxygen carriers carbonic oxide would thus appear to be a physiologically indifferent gas, like nitrogen[1].

I have several times repeated the experiment just described, but have never found it fail. In some experiments the mouse was kept for half-an-hour or more in the vessel, until the carbonic acid tension must have been nearly great enough to affect the animal.

It might be thought that the death of the mouse is perhaps due to the liberation of gas within its body, from too sudden diminution of pressure. Control experiments with pure oxygen or air showed, however, that this is not the case. Even a very sudden diminution of pressure, from giving way of a cork, did not injure the animal. Diminution of the pressure of a mixture of oxygen with a third of carbonic oxide does not, however, always kill a mouse, as shown by the following experiment.

A young half-grown mouse had been kept since 1.25 at a pressure of 128 cm. of mercury in an atmosphere consisting of about two-thirds of oxygen and one-third of carbonic oxide. At the end of this time it remained quite lively.

[1] Experiments interpreted as leading to an opposite conclusion have been recently described by Marcacci (*Archives Italiennes de Biologie* XIX. p. 205, 1893) and Piotrowski (*Archiv f. Anat. and Physiol.* 1893, p. 205). Neither of these authors, however, furnishes analyses of the gas employed by them, or any other guarantee of its freedom from such probable impurities as carbonic acid and air.

1.49 Pressure reduced to 106 cm.
1.50 Decidedly worse. Legs tend to sprawl.
1.52 Pressure reduced to 78 cm.
1.55 Lying with its head on the bottom of the jar, but does not remain on its back when put there.
1.57–2.8 Pressure gradually reduced to 0.
2.15 Mouse more torpid, but still does not remain on its back. Removed from the glass vessel and brought into the air. Immediately got worse. Lay on its back and had slight convulsions. Breathing ceased at 2.16 except for occasional gasps. Appeared once or twice to be dead.
2.18 Respirations becoming more frequent.
2.23 Still unconscious and does not move limbs.
2.27 Shows signs of returning consciousness, and power over its limbs.
4 p.m. Completely recovered.

Young mice are probably more capable of resisting asphyxia from carbonic oxide poisoning than older ones. This is what might be expected from the fact that young animals are difficult to drown, and may live for a short time in an atmosphere deprived of oxygen.

To remove a mouse with safety from the atmosphere of carbonic oxide in the pressure apparatus it is necessary to replace the carbonic oxide by oxygen without in the operation letting the pressure down to less than about two atmospheres. When the carbonic oxide has been washed out the animal can be safely removed to air.

In support of the hypothesis that carbonic oxide is, apart from its influence on hæmoglobin, a physiologically indifferent gas I may quote here the following experiment, made on an animal devoid of hæmoglobin.

Two mixtures are made, the one of 20 volumes of oxygen and 80 of carbonic oxide, the other of 20 volumes of oxygen and 80 of carbonic acid. Into each of these mixtures a cockroach (Blatta orientalis) is brought. In the carbonic oxide mixture the animal is not sensibly affected, even after a week[1]. In the carbonic acid mixture, on the other hand, a cockroach almost instantly exhibits convulsive movements, and

[1] In one experiment a cockroach was kept without injury in a carbonic oxide mixture for 18 days. At the end of this time the residual atmosphere had the following composition:

Carbonic oxide	71·41
Oxygen	14·21
Nitrogen	9·34
Carbonic acid	5·04
	100·00

becomes quite motionless at the end of from 20 to 30 seconds[1]. It appears to be dead, but nevertheless recovers after a time if taken out before too long. This experiment shows in a striking way the contrast between the essentially indifferent gas, carbonic oxide, and the essentially poisonous gas, carbonic acid.

It is now necessary to discuss more in detail the probability of the hypothesis advanced above that the abolition of the poisonous action of carbonic oxide when the oxygen tension is raised to two atmospheres is due to the fact that the animal can live on the oxygen simply dissolved in the blood.

In the absence of directly obtained data as to the coefficient of absorption of oxygen in blood we may provisionally take as a basis for calculation the number ·0262, given by Zuntz[2] as probable from the results of experiments by Paul Bert on the solubility of nitrogen in blood. Arterial blood contains usually about 20 % of its volume of oxygen, combined in the red corpuscles. Of this oxygen about six or eight volumes are, under ordinary circumstances, used up in the circulation, so that six or eight volumes of oxygen would appear to be necessary to supply the normal requirements of the tissues. Now on the above assumption blood will retain in simple solution about 2·6 % of its volume of oxygen in presence of an atmosphere of pure oxygen, or about 0·5 % in presence of air. At two atmospheres of oxygen blood will dissolve 5·2 % of oxygen. This dissolved oxygen will, moreover, probably be particularly easily available for the wants of the tissues, since its tension is very high, and it will therefore probably pass very easily and completely through the capillary walls.

Now 5·2 volumes per cent. of oxygen will be scarcely as much as is required by the animal. Nevertheless the latter possesses an easy means of making this reduced quantity suffice—namely by increasing the rate of the circulation. During any serious exertion the respiratory exchange of such animals as have been investigated is enormously increased—often to about ten times the normal value, or probably more. To cover this increase the rate of circulation must apparently be also very largely increased, probably by several times. Even were the whole of the oxygen of the arterial blood used up the quantity would not be anything like sufficient to supply the needs of the tissues during exertion, unless the rate of circulation were largely increased.

[1] An almost equally rapid effect is produced by putting the animal in pure hydrogen, and thus depriving it of oxygen.

[2] *Hermann's Handbuch*, IV. 2, p. 16.

If then the rate of circulation were similarly increased in an animal deprived of the use of its red corpuscles, but breathing in an atmosphere of oxygen at double the normal pressure, the requirements of the tissues for oxygen could easily be covered. It is also possible to see how, as in the last experiment on page 213, an animal might still live for a short time when deprived of the use of its corpuscles in an atmosphere at ordinary pressure consisting of two parts of oxygen and one of carbonic oxide.

There can be little doubt that with high carbonic oxide tensions, such as those employed in the pressure experiments just described, the corpuscles are practically saturated with carbonic oxide, and so put completely out of action[1]. Both theoretical considerations, and the examination of the blood of various animals which have died on lowering the pressure, confirm this assumption. In a future paper I hope, however, to communicate more direct evidence on this and a number of further points connected with, and suggested by, the action of carbonic oxide on mice and other animals, including man.

The theory advanced seems to explain completely both the abolition, at high oxygen tensions, of the poisonous action, and the effect of simultaneously diminishing the oxygen and carbonic oxide tension. As might be expected the effect of simultaneous diminution is most striking with high initial oxygen tensions. With initial tensions of less than an atmosphere the oxygen simply dissolved in the blood is of less relative importance to the animal, so that the symptoms come to depend chiefly on the relative affinities for hæmoglobin of the two gases, and the effect on the animal of simultaneous proportional diminution of their tensions is less marked, as in the experiment described on page 209. Finally, the theory explains the fact that the slight effects produced by carbonic oxide even in presence of oxygen at two atmospheres of pressure do not increase on raising the carbonic oxide tension above about 10 % of an atmosphere.

[1] It might be thought that possibly the relative affinities of oxygen and carbonic oxide for hæmoglobin are different at high pressures from what they are at low pressures, and that consequently the corpuscles are not saturated with carbonic oxide at the pressure employed in these experiments. To test this hypothesis I put a dilute solution of blood in the pressure apparatus, with a pressure of two atmospheres of oxygen, and observed its changes of colour on letting in carbonic oxide. But even with a tension of as little as 10 % of an atmosphere of carbonic oxide the solution gave on shaking the same tint as part of the same solution saturated with carbonic oxide. The saturation of the blood was thus practically complete at this tension, in spite of the high oxygen pressure.

Chief Conclusions.

1. The poisonous action of carbonic oxide diminishes as the oxygen tension increases, and *vice versâ*. At a tension of two atmospheres of oxygen this poisonous action is abolished in the case of mice.

2. The disappearance of the poisonous action is due to the fact that at high oxygen tensions the animals can dispense entirely with the oxygen-carrying function of hæmoglobin.

3. The poisonous action of carbonic oxide is entirely due to its power of combining with the hæmoglobin of the red corpuscles, and so putting them out of action as oxygen-carriers.