THE PATHOGENESIS

OF

CARBON MONOXIDE ANOXIA.

THESIS

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UNIVERSITY OF EDINBURGH.

by

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CHAPTER I.

INTRODUCTION.

The harmful effects of exposure to carbon monoxide were first observed by Erasistratus about 300 B.C. Since then, evidence of its toxic qualities has gradually accumulated. Aristotle first mentioned headache as a symptom which was emphasized by Cassius in 130 A.D., and about the middle of the third century Aurelianus described disturbances of movement and sensation (285).

The toxicity of the gas has thus been known for over 2,000 years, but in spite of an extensive accumulation of data of research and observation, our knowledge today of its toxicology is by no means complete. Indeed, we have long since recognised its dangerous qualities and have been able in the main to prevent extensive harmful exposure and to combat many of its effects. But remembering that carbon monoxide is formed to a greater or lesser extent whenever carbon is burned in an insufficient supply of oxygen, exposure to the gas as an accidental hazard will increase directly with increased industrial effort and increased use of fuel generally. Carbon monoxide will continue to
be a major accidental hazard and will maintain its established popularity as a suicidal agent through the medium of "illuminating" or coal gas. It is felt that no further explanation need be given for devoting time and energy to the further study of the toxicology of this gas.

The sources of carbon monoxide as well as its clinical manifestations and principles of treatment have been adequately and explicitly dealt with elsewhere. (85). It is now proposed to study the pathogenesis of carbon monoxide in some detail. In the past there has been no complete collective survey of the possibilities of what precisely happened subsequent to the entrance of carbon monoxide into the body. It unites in the lung capillaries with the circulating haemoglobin and is carried to all parts of the body. Thereafter its clinical effects are known and are generally believed to be due to anoxia caused by diminished oxygen carrying power of the blood brought about by the combination of carbon monoxide with haemoglobin at the expense of oxygen. Similarly, it is believed that carbon monoxide is gradually eliminated from the body by the dissociation of carboxy-haemoglobin in the lungs. This process of inhalation, combination with the circulating haemoglobin and subsequent elimination is undoubtedly true but is it the whole truth? Does carbon monoxide dissociate from the circulating
haemoglobin into the tissues to combine with tissue haemoglobin or other substances? Is there any other substance in the blood or in the tissues which is known to combine with the gas in vitro? Is carbon monoxide oxidized in the body to carbon dioxide or is it metabolized at all in the body?

This study is directed towards answering these questions but no claim is made to complete such far reaching issues. It is proposed to discuss the commoner clinical and pathological manifestations and the combination of the gas with blood haemoglobin before attempting to survey its possible influence on tissue haemoglobin, the "pseudohaemoglobin" of Barkan, the cytochrome system, the "Pasteur Effect" and carbonic anhydrase.

In the elucidation of cellular respiration and other cellular metabolic mechanisms, the biochemist frequently makes use of carbon monoxide as an inhibiting agent. To him this inhibition is incidental and has not been recorded in any attempt to show carbon monoxide as a tissue poison. Like cyanide, carbon monoxide inhibits certain haemin-containing enzymatic systems and with the object of ascertaining the chemical constitution and functions of these enzymes the biochemist has indicated combinations and inhibitions important to the present study. In previously published work no attempt has apparently been made to correlate this incidental but all important biochemical
data directly with the carbon monoxide problem.

The absence of complete liaison between the biochemist and the other branches of medical science is all too obvious and expensive to progress, so at present we must be content with the "crumbs fallen from his table". It is felt that the outstanding problems of carbon monoxide anoxia, as indeed of so many poisonous agents will gradually be overcome as our knowledge of the functions of the individual cell increases. A survey has therefore been made of the more recently published biochemical data dealing with the various systems and cellular reactions which may have a bearing on carbon monoxide anoxia.
CHAPTER II.

CLINICAL AND PATHOLOGICAL MANIFESTATIONS.

At the outset it is essential that a definition of the terms to be used should be clearly understood. For instance, the toxic effects of carbon monoxide are so often wrongly referred to under "asphyxia" - a term correctly reserved for states where there is a retention in the blood and tissues of carbon dioxide. (338). "Carbon monoxide anoxia" as a general term, covers the issues most satisfactorily though perhaps not completely.

This at once leads to the four types of anoxia: anoxic, anaemic, stagnant and histotoxic. Undoubtedly carbon monoxide anoxia provides a typical example of anaemic anoxia through its combination with the circulating haemoglobin, thereby diminishing the amount of haemoglobin available for combination with oxygen. Anoxic anoxia is never involved because the percentages of carbon monoxide necessary in the atmosphere to cause death are too low to diminish the available oxygen from that cause alone. Stagnant anoxia, as we shall see, does play an indirect part through vascular stasis associated with circulatory failure. This, however, is secondary; the damage having already been done before circulatory failure.
Table 1.
Table 1.

(Ref. Nos. 85, 128, 189, 285.)

SUBJECT AT REST:-

<table>
<thead>
<tr>
<th>% saturation with CO.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 20</td>
<td>Nil.</td>
</tr>
<tr>
<td>20-30</td>
<td>Negligible; may be slight lassitude and fullness in the head, dilatation of cutaneous blood vessels, and increased pulse rate.</td>
</tr>
<tr>
<td>30-40</td>
<td>Throbbing and sensation of fullness in the head, headache, nausea, giddiness, drowsiness and faintness, muscular weakness, and slight dyspnoea.</td>
</tr>
<tr>
<td>40-50</td>
<td>Giddiness and mental confusion, diminution of sight and hearing, marked muscular weakness and incoordination, increased pulse rate with palpitation and dyspnoea.</td>
</tr>
<tr>
<td>50-70</td>
<td>Complete paralysis and coma; Cheyne-Stokes respiration; depressed heart action and respiratory failure with death if continued.</td>
</tr>
</tbody>
</table>
Table 2.
Table 2.

(Ref. Nos. 98, 138, 236, 239.)

SUBJECT WITH LIGHT ACTIVITY:

<table>
<thead>
<tr>
<th>Atmosphere % CO</th>
<th>Percent. Blood saturation (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.</td>
</tr>
<tr>
<td>.02</td>
<td>8.2</td>
</tr>
<tr>
<td>.03</td>
<td>9.3</td>
</tr>
<tr>
<td>.04</td>
<td>13.6</td>
</tr>
<tr>
<td>.06</td>
<td>17.8</td>
</tr>
<tr>
<td>.08</td>
<td>25.6</td>
</tr>
<tr>
<td>.1</td>
<td>30.1</td>
</tr>
</tbody>
</table>
is manifest. Whether carbon monoxide inhalation, like cyanide, leads to histotoxic anoxia is one of the main considerations of this survey.

**GENERAL CONSIDERATIONS.**

The appearance and severity of the effects of carbon monoxide inhalation are dependent in the first instant upon the degree of saturation of the circulating haemoglobin with the gas, which in turn is dependent upon the concentration in the inspired air. Thus in acute carbon monoxide anoxia with the subject at rest and knowing the blood saturation, the effects may summarized broadly according to Table 1; while the relationship of blood saturation to the concentration of the gas in the atmosphere and the time may be observed from Table 2.

Any degree of muscular activity will affect the character and severity of these effects - in other words, the extent of oxygen deprivation.\(^{(128,189,285)}\). In this connection Henderson et alia \(^{(138)}\) found that carbon monoxide absorption was twice as rapid with moderate exercise (walking) and three times as rapid with fairly hard work than while sitting at rest.

The concentration in the inspired air and the duration of exposure also have a profound effect upon the ultimate effects \(^{(211)}\). Sayers and Davenport \(^{(285)}\) indicate that a given saturation acquired by long exposure and at low concentration produces more
severe symptoms and after effects than by short exposure to a higher concentration. They show also that a person at rest may pass into a state of dizziness and even unconsciousness without experiencing any marked previous effects beyond weakness and dizziness. The following expression has been offered (138) in an attempt to show the relationship between the duration of exposure and the concentration to the probable effects:—

When the duration of exposure in hours $x$ concentration in parts per 10,000

- $= 3$; effect is nil.
- $= 6$; effect is just perceptible.
- $= 9$; headache and nausea.
- $= 15$; dangerous.

**RESPIRATION**

The complexity of the regulation of respiration under normal conditions is not lessened by the study of the effects of inhalation of carbon monoxide. Haldane (128) in his heroic experiments upon himself showed that his respiration was not increased by carbon monoxide until his blood saturation had reached 40% and even then the increase was only slight. This is in accord with the subsequent findings of Sayers and Davenport (285) and Killick (189) while others observed hyperpnoea only when the saturation reached 50% (53, 289). Increased respiration is not, therefore,
a prominent symptom particularly in view of the prominence at 40% saturation of far more alarming effects, such as giddiness, mental confusion, diminution of sight and hearing and above all marked muscular weakness and incoordination. The prominent tendency to faintness and collapse in carbon monoxide anoxia without apparent dyspnoea has been observed repeatedly in the past and Haldane (130) compared it with other forms of anoxia. He claimed that although there is general similarity between the symptoms of carbon monoxide anoxia and anoxic anoxia as experienced at high altitude, the imperfect oxygenation of the arterial blood haemoglobin at altitude leads to marked hyperpnoea without the same tendency to faintness and collapse. Horvath, Dill and Corwin (156) observed increased rate and depth of breathing in two minutes with the inhalation of 4.2% oxygen in nitrogen equivalent to an altitude of about 31,000 feet. McFarland (218) surveying the effects of anoxic anoxia recorded that 16.7% of subjects complained of respiratory changes or distress at 12,000 feet (oxygen pressure about 11.5% atmosphere or 90 m.m. Hg) and 60% complained of hyperpnoea at 16,000 feet equivalent to an oxygen pressure of about 11.5% atmosphere or 80 m.m.Hg. Haldane and Priestly (131) state that there is little increase in respiration while the oxygen in the inspired air falls from 21 to 14% but with percentages lower
Table 3.

<table>
<thead>
<tr>
<th>Column 1</th>
<th>Column 2</th>
<th>Column 3</th>
<th>Column 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data 1</td>
<td>Data 2</td>
<td>Data 3</td>
<td>Data 4</td>
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<td>Data 5</td>
<td>Data 6</td>
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<tr>
<td>Data 9</td>
<td>Data 10</td>
<td>Data 11</td>
<td>Data 12</td>
</tr>
</tbody>
</table>

Note: Table data is placeholders for actual content.
Table 3.
(From Peters and Van Slyke, 259.)

<table>
<thead>
<tr>
<th>Type of Anoxia</th>
<th>Arterial Blood</th>
<th>Venous Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxygen Capacity</td>
<td>Oxygen Content</td>
</tr>
<tr>
<td>None, Normal</td>
<td>20 Vols %</td>
<td>19 Vols %</td>
</tr>
<tr>
<td>Anoxic</td>
<td>20 Vols %</td>
<td>14.8 Vols %</td>
</tr>
<tr>
<td>Anaemic</td>
<td>10 Vols %</td>
<td>9.5 Vols %</td>
</tr>
<tr>
<td>Anaemic CO</td>
<td>15.3 Vols %</td>
<td>14.7 Vols %</td>
</tr>
<tr>
<td>Stagnant</td>
<td>20 Vols %</td>
<td>20 Vols %</td>
</tr>
</tbody>
</table>

The marked figures denote the cause of the anoxia.
than this, pulmonary ventilation increases.

The explanation for this difference lies in the different stimulus to the respiratory centre and carotid body. It will be observed in Table 3 that the oxygen tension of the arterial blood is not altered in carbon monoxide anoxia whereas it is reduced by almost half in anoxic anoxia while the oxygen capacity in the two states is reversed. The stimulus to respiration through the respiratory centre so far as oxygen is concerned is lowered oxygen tension and not lowered oxygen capacity. (55,131,136,147,218). Asmussen and Chiodi (11) in confirming this point, show the effect to be dependent upon the oxygen tension of the carotid body.

In carbon monoxide anoxia the respiratory centre is therefore not stimulated by oxygen lack.

On the contrary, it has been shown that the centre is depressed (53,55,308). The possible causes of this depression will be dealt with later but in the meantime let us review the part played by carbon dioxide and the acid-base equilibrium and trace their influence upon the control of respiration.

In a general way, the respiratory centre may be stimulated in three different ways. Firstly by decreased arterial oxygen tension; secondly by increased arterial carbon dioxide tension, and thirdly by decreased alkali reserve. These stimuli may act directly upon the centre or indirectly through the
nervous connections with the aortic and carotid bodies. Further controls of respiration are effected through the vagus, vasomotor centres and the higher cerebral centres. At altitude the stimulus, as we have seen, is reduced arterial oxygen tension. The hyperpnoea thus produced "blows off" the carbon dioxide in the lungs and affects the acid-base equilibrium so that the blood bicarbonate or alkali reserve decreases proportionately and gives rise to a condition of acarbia. Although the alkali reserve or bicarbonate is reduced we are not necessarily presented with the condition of acidosis. On the contrary, the alkali reserve is decreased as a result of the loss of the acid radicles or acapnia, (124) and it is interesting to note that Sayers and Davenport (285) show as a manifestation of carbon monoxide anoxia, the hand held in the tetany position - a sign of alkalosis. Henderson (136) points out that although the bicarbonate, which constitutes about 20% of the total blood alkali, is reduced, the remaining 80% held by haemoglobin may actually be increased. A further point made by Henderson as a result of the experiments by the Haldanes is that just as increased breathing causes lowering of the alkali reserve, the lowering of the alkali reserve (by the ingestion of ammonium chloride) increases the breathing and lowers the alveolar carbon dioxide pressure.
In the light of this knowledge what is the mechanism of the increased breathing in carbon monoxide anoxia? The arterial oxygen tension is virtually normal. There is no apparent rise in carbon dioxide tension, at least not in the early stages. In experiments with dogs, Haggard and Henderson (124) observed hyperpnoea which was followed by lowering of the carbon dioxide tension. The hyperpnoea was undoubtedly the cause of the reduction of the carbon dioxide tension or alkali reserve. They found further that vagal section was followed by no increased respiration and no decrease in the alkali reserve. Kamei (161) also with dogs, was unable to confirm this; but observed an increased ratio of dissolved to combined carbon dioxide. A more recent analysis with human subjects (284) shows that with carbon monoxide saturation up to 24% there was a slightly increased arterial carbon dioxide content (whole blood) under slightly increased pressure with a proportional decrease in pH. We have observed that after prolonged exposure to carbon monoxide anoxia the respiratory centre becomes depressed rather than stimulated. It seems logical to postulate that this respiratory depression allows the accumulation of carbon dioxide, in turn causing a decrease in pH, which would normally constitute a strong stimulus to the respiratory centre. Clinically, respiration is stimulated in carbon monoxide anoxia when the blood is about 40-50% saturated;
Table 4.

<table>
<thead>
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<th>Column 1</th>
<th>Column 2</th>
<th>Column 3</th>
<th>Column 4</th>
</tr>
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<tbody>
<tr>
<td>Value 1</td>
<td>Value 2</td>
<td>Value 3</td>
<td>Value 4</td>
</tr>
<tr>
<td>Value 5</td>
<td>Value 6</td>
<td>Value 7</td>
<td>Value 8</td>
</tr>
<tr>
<td>Value 9</td>
<td>Value 10</td>
<td>Value 11</td>
<td>Value 12</td>
</tr>
</tbody>
</table>
Table 4.

(Ref. No. 143.)

<table>
<thead>
<tr>
<th>Interruption of circ. up to:-- (minutes)</th>
<th>Cortical</th>
<th>Palpebral pupillary</th>
<th>Cardio-regulat.</th>
<th>Vaso-motor</th>
<th>Respiratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5-10</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10-15</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15-30</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>&gt;30</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
a point which is not necessarily fatal but may be sufficient to embarrass and depress the sensitive tissue comprising the respiratory centre. At this percentage the damage is reversible and with sufficient stimulus increased breathing would follow. But if the saturation progressed there would come a time when the effect upon the centre would be irreversible with failure to react to stimuli irrespective of their quality or magnitude. Death from carbon monoxide anoxia is in fact due to respiratory failure.

It may be argued that the respiratory centre is no more sensitive to carbon monoxide anoxia than the other medullary centres, and to comply with the theory these other centres should also show signs of depressed activity and failure. The sensitivity of the medullary centres to withstand anoxia has been recorded according to Table 4 in which it will be noticed that the cardio-regulatory, vasomotor and respiratory centres are equally sensitive. It will be pointed out at a later stage that the failure of the respiratory centre occurs along with the cardio-regulatory and vasomotor centres.

It must be remembered, however, that excessive loss of carbon dioxide may, in itself, lead to respiratory failure and Haggard (123) using dogs believed this to be the case. If this were true of the human subject we would expect death to be far more common and to occur more readily at altitude where hyperpnoea is so prominent.
CARDIO-VASCULAR SYSTEM

No prediction can be made as to how the heart will react under conditions of oxygen lack without knowing the state of the heart at the material time. There is, however, conformity of opinion that in progressive anoxia, the healthy heart shows decreased conduction time and flattening of the T-wave of the electrocardiogram, \((30,112,116,162,194,197,201,233,283,313)\). Greene and Gilbert \((116)\) pointed out that continued anoxia produces a crisis followed by slowing of the rate with interference of conduction, and they concluded \((117)\) that the effects of carbon monoxide anoxia on the heart were precisely similar to changes induced by anoxic anoxia.

The degree of flattening of the T-wave decreases strikingly as age advances. The change is greatest in young athletic individuals and May \((233)\) offers in explanation the greater adaptability in the young, whereas in the old it may indicate inability to compensate for lowered oxygen tension; or owing to frequent exposure to anoxia the heart may fail to react to the stimulus of lowered oxygen tension.

In acute carbon monoxide anoxia the heart rate begins to increase before there is any increase in respiration. Haldane's \((128)\) pulse rate was 91 per minute with a saturation of about 16%, and 104 when the saturation had reached about 40%; the point at which he recorded symptoms of hyperpnoea. Killick \((189)\) recorded
normal pulse rates (60-65 per minute) under 25% saturation; 65-80 per minute at 30% saturation, and 70-95 per minute at 35% saturation. This is in accordance with other findings (11) in which the pulse rate was increased with 20-30% saturation. On the other hand, anoxic anoxia stimulated respiration, and the pulse rate is increased about the same time or slightly later, the increase being not so marked as with carbon monoxide. Barcroft (18) stated that there is no increase in pulse rate until over an altitude of 16,000 feet equivalent to an oxygen pressure of about 12% atmosphere or 90 m.m. Hg. Barach (16) has since shown a similar relationship.

Grollman (119) states that carbon monoxide anoxia stimulates epinephrine secretion while anoxic anoxia may or may not do so. If this is confirmed it may be shown to have a considerable influence on the early appearance of tachycardia in carbon monoxide anoxia.

With high carbon monoxide saturation of the blood, auriculo-ventricular conduction may be delayed. Elrich, Bellet and Lewey (37) produced heart block in dogs when the saturation exceeded 75% for one hour or more. Resnick (268-270) noted similar delayed conduction with anoxic anoxia which he believed was due to a direct effect of the anoxia on the myocardium but may have been caused by vagal spasm. Other workers believe the direct effect upon the heart muscle (118, 228, 229). In a survey (313) of 22 cases of acute carbon
monoxide anoxia admitted to hospital, only one case of transitory intraventricular block occurred. The prevalence of delayed conduction in the human subject cannot be assessed upon this survey because other transitory effects may have passed off, and any effects occurring in hospital must be regarded as complications of carbon monoxide anoxia. Haggard (123) concluded from his work with dogs that oxygen deficiency brought about by carbon monoxide is not in itself sufficient to cause impairment of auriculo-ventricular conduction. Following respiratory failure, however, the increased anoxaemia from this cause speedily results in the development of heart block through its various stages.

In their explanation of the prominence of fainting in carbon monoxide anoxia, Haldane and Priestley (131) point out that although the oxygen tension maintains respiration more or less normally, the heart is deprived appreciably of its oxygen requirements and cannot carry on with the increased blood flow necessary to satisfy the needs of the tissues generally. If this is so, one would expect the heart muscle to "cry out for oxygen" or in other words give rise to angina. Precordial pain is given as a symptom of carbon monoxide anoxia by Sayers and Davenport (285) and the anginal syndrome has been produced by anoxia in subjects with known disease of the coronaries or a history of angina. (74,162,197,205,275,276). Similarly it has been recommended that subjects with coronary disease
should avoid travelling by air. Myocardial necrosis can undoubtedly occur in carbon monoxide anoxia (see Pathology, Chapter IV) and is quite likely to be more common than is generally supposed through being missed in the early stages of recovery as well as in fatal cases. The foci of necrosis may be small and diffuse and give rise to no marked clinical effects at the time. Even detectable myocardial infarction without pain has been recorded. (14,111,186).

The onset of anoxic anoxia, uncomplicated by carbon dioxide accumulation is followed by a rise in blood pressure. (34,142,144,147,226,230,291,292,338). This rise eventually leads to a fall if the anoxia is progressive (104); presumably due to a reduction of carbon dioxide following hyperpnoea. Van Liere (338) states that the pressure falls if the nerves to the carotid sinus and the vagi are cut, but the rise can be maintained if small amounts of carbon dioxide are given even when the carotid sinus is segregated. This action of carbon dioxide he attributes to be mainly on the vaso-motor centre directly. Brewer (35) contends that dogs respond by a fall in blood pressure with carbon monoxide, and not a rise which he acknowledges as the accompaniment of anoxic anoxia. Surveys (313,373,374) of patients admitted to hospital immediately after severe carbon monoxide anoxia showed reduced blood pressures. This might be expected from cardiac failure and shock, and unfortunately there appears to be no real
evidence of the behaviour of the blood pressure in the earlier stages. Inferences can, however, be drawn from the pulse rate and measurements of the cardiac output. Asmussen and Chiodi (11) have shown that at rest and during work, anoxic anoxia when the oxygen inspired is low enough to reduce the arterial oxyhaemoglobin to 70–80%, produces increased cardiac output; but 20–30% carbon monoxide saturation has little or no effect on the cardiac output but increases the pulse rate considerably. Also it is recorded that the cardiac output showed no more than slight increases up to 30% carbon monoxide saturation, but from 30–50% saturation there was a distinct increase.

Peripheral blood flow is generally increased by carbon monoxide. The increase with about 20% saturation is about 10–15% and almost compensates for the decrease in cerebral arterio-venous oxygen difference. (284) Lennox and Gibbs (202) showed that with increased arterial carbon dioxide tension the speed of flow in the brain was increased and in the leg it was decreased; while decreased arterial carbon dioxide tension produced the reverse effect. The brain thus received preferential treatment as regards the allocation of arterial blood. This could account for the fact that small concentrations of carbon dioxide diminish or completely remove the effects of lowered oxygen tension. (52,103) This beneficial action of carbon dioxide applies also to the blood pressure and to alterations of the oxygen dissociation curve of blood
whether or not it is partially saturated with carbon monoxide. Not only carbon dioxide but also lactic acid, an inevitable concomitant of anoxia, increases the blood flow. (365). Whatever the mechanism it is essential that the speed of flow to the brain be maintained even if need be at the expense of the limbs. Drinker (85) reviews cases of carbon monoxide anoxia where devitalization proceeding to gangrene in the limbs, which ultimately had to be amputated, occurred unaccompanied by thrombosis or other vascular pathology. It seems unlikely, however, that the maintenance of cerebral circulation would be observed so rigidly as to warrant such sequelae. It is more likely that carbon monoxide has an inhibitory effect upon metabolism at tissue level to account for this devitilization.

The maintenance of constant cerebral circulation which in turn maintains a constant respiratory activity lies in the fact that the brain is very restricted in its choice of metabolites for respiratory purposes. Its chief demand for respiratory activity is on glucose (56,204); hence a constant respiratory activity of the brain will depend upon the availability of at least a minimum concentration of glucose and on a constant supply of oxygen. (264)

In prolonged carbon monoxide anoxia or with high saturations of the blood, the heart fails and there is vascular dilatation and stasis. This is most marked in the cerebral vessels and is accompanied by
perivascular haemorrhage and cerebral oedema. (368).

This selection tends to indicate a direct action on the cerebral vessels rather than as a general manifestation of cardiac failure.

**CENTRAL NERVOUS SYSTEM.**

The low threshold of cerebral tissue to oxygen lack is well known. Suffice it to state that cortical tissue does not recover if it is deprived of oxygen for more than 5-8 minutes and the changes in certain other parts of the brain and spinal cord are irreversible after approximately 20-30 minutes oxygen lack. (47, 48).

The clinical effects of anoxia on the cerebral functions are depressant in a manner comparable to the effects of ethyl alcohol and other narcotics. The most recently acquired higher processes are first affected, and as the anoxia progresses the depression spreads to the coarser and earlier developed "centres". This has a profound significance in carbon monoxide anoxia, because the critical senses of sane judgement are impaired at an early stage and the individual, although he may be aware of his danger, may be unable to complete the interpretation of this knowledge sufficiently to move into fresh air, and before long muscular activity is no longer possible anyway. At the time, he is confident that nothing is unusual in his behaviour and it is only after removal from the carbon monoxide and with recovery that he realizes the full significance of the danger.
Loss of the power of concentration and emotional disturbances commonly occur in the early stages and retrograde amnesia is prominent. Impressions of the special senses are often wrongly interpreted. Subsequently the powers of balance and movements requiring muscular coordination are diminished until a stage of coma is reached. Muscular weakness is so prominent and occurs so early that it is not due entirely to effects produced through the central nervous system. With very high concentrations of carbon monoxide the individual may suspect that nothing whatever is amiss until he finally reaches the verge of unconsciousness. It has been stated that in anoxic anoxia there is no significant alteration in behaviour until the diminution of oxygen reaches 50% of normal. (215). The critical altitude in this respect appears to be about 12,000-15,000 feet, (17,216,217,327), but can be considerably raised with the addition of small amounts of carbon dioxide (76,219). Also, if in progressive anoxic anoxia the carbon dioxide tension of the brain is maintained, consciousness is lost without a preceding period of confusion. (108). Normally in anoxia, unconsciousness occurs when the percentage saturation of the haemoglobin of the jugular blood has reached about 25, corresponding on the oxygen dissociation curve to a tension of 19 m.m Hg. (203).

Carbon monoxide gives rise to many types of neurological sequelae but these will be dealt with
Of the special senses, the sight is the most susceptible to oxygen lack. As we have already seen, the ability of the subject of carbon monoxide anoxia to estimate the degree of visual defect is greatly reduced: a fact not appreciated by the individual at the time. Sayers and Davenport (285) give the common ocular changes as disturbances of pupillary reactions, homonymous hemianopias, diplopia, visual field defects, colour blindness, optic neuritis, subconjunctival and retinal haemorrhages. Some of these changes are apparently permanent but most clear up. These authors believe that all the effects through nervous mechanisms find their origin of damage in the nerve nuclei. Others (220) attributed decreased efficiency in eye movements to diminished amounts of oxygen being delivered to the nervous system, subcortical as well as cortical. They consider that the changes in eye movements can be used to detect early effects of oxygen deprivation. Lilienthal and Fugitt (208) provide a delicate test to support their contention that "when the human subject has absorbed enough carbon monoxide to have converted a quarter to a third of his available circulating haemoglobin into carboxyhaemoglobin, the effects of this degree of carbon monoxide anoxia on a variety of physiological functions becomes evident. By contrast, the man who smokes moderately to heavily carries on without appreciable handicap with from a twentieth to a fifteenth of his haemoglobin bound to carbon monoxide. It seems
reasonable to suspect that between these extremes there lies a degree of carbon monoxide anoxia which produces undesirable effects on delicate mechanisms without subjective awareness of any handicap". Their test involves the flicker fusion frequency (FFF) - the critical frequency in cycles per second at which a flickering light appears to be steady. They found that increments in carboxy-haemoglobin of the order of 5-10% resulted in appreciable deterioration of FFF at altitudes which alone did not affect the FFF. McFarland et alia (221) using visual discrimination as a test, found that after three cigarettes the blood saturation with carbon monoxide was 4% and the effect on the subjects' visual sensitivity was equal to that at an altitude of almost 8,000 feet. They computed that at an effective pressure altitude of 6,000 feet the presence of 10% saturation with carbon monoxide depresses visual discrimination equivalent to the depression produced by an exposure to an altitude of 12,000 feet breathing air.

The other special senses appear to be far less sensitive to anoxia and the only other recorded effects are concerned with hearing. Any effects upon auditory mechanisms are of slight degree and are seldom permanent.

Anoxia generally, gives rise to increased cerebro-spinal fluid pressure. (97,249,232,370). The prominence of headache in carbon monoxide anoxia is probably a result of this increase although it may
be due in part to cerebral congestion and oedema (85).

We have observed that nausea and possibly vomiting occur at relatively low saturations. It cannot be assumed that this is due entirely to a direct effect upon the vomiting centre or to reflex mechanisms initiated by the tachycardia or a combination of both.

**OTHER SYSTEMS**

The only effects upon the urinary system seem to be in an attempt to maintain equilibrium elsewhere. This influence is most marked in the control of respiration. The only other recorded effect appears to be a transient albuminuria. (85).

During acute carbon monoxide anoxia it is virtually impossible to assess any minor effects in the endocrine system. Certain sequelae referable to the reproductive system will be discussed later. A similar position exists with the possible effects upon the alimentary system.

The decrease in body temperature occurring in all forms of anoxia may readily be due to the effect of lack of oxygen on metabolism generally.
CHAPTER III.
AFTER EFFECTS: CHRONIC EXPOSURE
AND ACCLIMATIZATION.

AFTER EFFECTS
There can be no doubt that by far the most important after effects of carbon monoxide anoxia are manifest in the central nervous system.

Shilloto, Drinker and Shaughnessy (304) made a survey of 21,143 cases of carbon monoxide anoxia occurring in New York over a ten year period. They found that only 39 cases finally found their way into State Mental Institutions. This survey has been frequently misquoted to include neurological cases as well as mental cases. In fact, there is no mention of neurological cases, nor is there any reference to those cases which died some period after gassing and no account is taken of the mental cases which may have been cared for privately.

The most susceptible part of the brain is that part affecting the psyche of the individual. The types of neurological sequelae may vary, but the commonest appears to be Parkinsonism through damage to the basal ganglia. Muscular hypertonia with or without some degree of peripheral neuritis is less common.

Parkinsonism would undoubtedly be seen more commonly following carbon monoxide anoxia were it not for the
fact that the more vital cerebral centres are so liable to be effected with death occurring soon after the gassing. It can be assumed with safety that in death from carbon monoxide anoxia - in the absence of pre-existing pathology - the basal ganglia always show some degree of degenerative change. (39,146).

Carbon monoxide anoxia causes unconsciousness which may persist for several days, long after the carboxy-haemoglobin has been reconverted into oxyhaemoglobin; or consciousness may be regained with subsequent lapses into coma. These lapses may be consequent upon muscular activity but they are not uncommon with the patient at complete rest throughout. One would expect consciousness to be regained whenever the oxygen lack ceases to exist as in the case with anoxic anoxia, but the fact that recovery can take place after prolonged unconsciousness eliminates any possibility of irreversible damage to cerebral tissue.

Certain visual changes may be permanent and less commonly there may be persistent auditory changes. There appear to be no characteristic sequelae referable to the cardiovascular system although cardiac dilatation and interstitial myocarditis have been recorded as incidental (35). As a result of the Valleyfield disaster many miners complained of precordial pain, palpitation and dyspnoea on exertion (306). Clinical examination showed tachycardia and less frequently cardiac enlargement. It would appear that these manifestations conform to the "effort syndrome" and it was
not possible to estimate whether or not there was any underlying pathology. We have already observed the possibility of small foci of myocardial necrosis quite easily escaping the notice of the patient and his examiner, but no matter how small these lesions may be they may still produce profound qualitative effects. (See Pathology, Chapter IV.)

It has been observed repeatedly that fertility in animals has been greatly reduced following carbon monoxide anoxia. (46, 256, 363). This is apparently due to non-motility or numerical reduction of spermatozoa with subsequent reduction of the weight of the testes. (363). Impotence has rarely been observed. (85).

**CHRONIC EXPOSURE AND ACCLIMATIZATION.**

Carbon monoxide is in no way cumulative in the ordinary sense. To produce chronic effects, chronic exposure to the gas is essential. This very rarely occurs for there is usually intermittent exposure with periods of freedom when complete dissociation occurs. Such are the conditions in industry where the individual escapes from subjection to the gas at the finish of the day's work with resumption the following morning. It is difficult to include data with a view to making a comparison with the well recognized effects of chronic anoxic anoxia. Sustained chronic anoxic anoxia, better known as "mountain sickness," is characterized by cyanosis on the least effort, clubbing of the fingers, emphysema,
muscular weakness, insomnia, vertigo, nausea, syncope etc. (218). Whereas chronic carbon monoxide anoxia is liable to produce more chronic ill-health as shown by headache, dizziness, drowsiness, digestive disturbances, dyspnoea, palpitation and muscular weakness. (29, 234).

As with acute carbon monoxide anoxia, the damage to the tissues is produced during exposure to the gas but the effects may not become apparent until a later date. This applies particularly to neurological manifestations and has a distinct bearing on medico-legal opinion especially in so-called chronic cases. The relationship between acute carbon monoxide anoxia with unconsciousness and hospitalization to the subsequent development of neurological symptoms such as Parkinsonism can readily be shown, but when vague ill-health with headache, dizziness etc., are complained of without loss of consciousness at any time and subsequent Parkinsonism, the relationship may be in doubt.

Parkinsonism through damage to the basal ganglia has been quoted as a result of chronic exposure but other changes have been recorded with almost equal frequency. These include multiple sclerosis, changes referable to single nerves or special senses, and also polycythaemia or anaemia. (85). Chronic exposure in these cases refers to blood saturations causing effects which allow the individual to continue his work or routine without marked discomfort and certainly without causing loss of consciousness. Lewey & Drabkin (206) exposed dogs to a carbon monoxide mixture for \( \frac{5}{2} \) hours per day for 6 days.
per week over 11 weeks. Blood saturations reached only about 20% but the dogs showed a consistent disturbance of postural and position reflexes and gait. These effects were not marked clinically and the animals retained a friendly disposition throughout. Subsequent histological examination showed changes in cerebral grey and white matter and in heart muscle.

Beyond descriptions of damage to the central nervous system and the heart, very little data is available regarding systematic effects of chronic carbon monoxide anoxia. Susceptibility of the alimentary system has been shown in rats in which chronic exposure was maintained. (257). It was observed that food intake dropped 23%; effective peristalsis was reduced by 33%, and the egestion time was increased 22%. On the strength of these results Van Liere quotes carbon monoxide as a histotoxic agent so far as the gut is concerned. (338).

Acclimatization to carbon monoxide anoxia is a phenomenon as yet only partially understood. Its existence was first indicated by Faure in 1856. (338). By acclimatization is meant that symptoms are lessened during successive exposure to the same concentrations of carbon monoxide, or alternatively that to produce the same clinical manifestations a greater concentration of carbon monoxide must be inhaled. Further, the relationship between the concentration of carbon monoxide breathed and the degree of saturation of the blood in the same individual alters with successive exposures.
How may this process of acclimatization be accomplished? Various theories have been offered in explanation. Barcroft (18) suggested an alteration in the haemoglobin dissociation curves but such a change has not been observed. (132,188,190). Haldane and Priestly (131) advanced the hypothesis of "secretion" of oxygen inwards by the lung alveoli. They postulated, from their experiment with mice and subsequently confirmed on human subjects, that this process is not active in low concentrations of carbon monoxide; and their figures with mice indicate no "secretion" of oxygen even when the carbon monoxide saturation is well above 30%, which is far greater than is necessary to produce acclimatization. The stimulus for oxygen "secretion" by the lung is said to be oxygen lack but it has been shown that acclimatization to carbon monoxide anoxia occurs in the absence of any oxygen lack as judged by the degree of blood saturation. (32,189). Further evidence against this theory has been given by other workers. (31,75). Killick (189) suggested a selective action of the alveolar membrane in preventing carbon monoxide from entering the blood and an alternative possibility of "excretion" of carbon monoxide outwards. Killick then proved this theory, too, to be untenable.

We appear to be left with the possibility of disappearance of carbon monoxide from the blood into
the tissues. Thus, once again, we are confronted with the question, does carbon monoxide unite with substances in the body other than the circulating haemoglobin? If such a substance existed and if it increased in quantity during the development of acclimatization, the whole problem of acclimatization would become clearer. Muscle haemoglobin may fill this rôle for it has been shown to increase in quantity during prolonged exposure to carbon monoxide. Combination of carbon monoxide with muscle haemoglobin would tend to show a lower level of saturation of the circulating haemoglobin; the alteration of this level depending upon the degree of increase of the muscle haemoglobin. (See Chapter VI).

The manifestations of acclimatization may be different in experimental animals from those observed in man. It has been repeatedly shown that the smaller animals exhibit a profound increase in circulating RBCs and haemoglobin (41–45, 247), whereas with the human subject the evidence is conflicting in this respect: some observers have recorded an increase as with animals (239) while others have recognised no change. (189, 191).

Chronic exposure of mice to carbon monoxide leads to cardiac hypertrophy (44, 45, 338), a result, no doubt, of increased blood viscosity associated with polycythaemia. Campbell (41) states that acclimatization takes place too readily in small animals to be depend-
ent entirely on increased circulating RBCs and haemoglobin. He claims that the tissues, particularly the heart, can withstand anoxia and function satisfactorily.
CHAPTER IV.

PATHOLOGY.

The appearances of the body after death from carbon monoxide anoxia depend on many factors. For the moment let us consider the effects likely to be produced by acute anoxia with death occurring within a few hours of the beginning of exposure.

The body frequently has a lifelike appearance: an illusion brought about by the pinkness of the skin. This was first observed by Donato in 1556 (285) and is due to the cherry red colour of the blood. The pink colour becomes even more evident in dependent areas as hypostasis develops. Occasionally similar pink patches appear in non-dependent parts and herpitiform areas have been recorded. (85).

On dissection the bright cherry red colour of the tissues is at once observed and is most striking in the muscles, and is due to the carbon monoxide combined with the blood contained therein. The possibility that this colour may be due in part to the combination of carbon monoxide with the muscle haemoglobin has been apparently overlooked for no reference has been found indicating such a possibility. It has been observed that carbon monoxide does unite with the muscle haemoglobin but Killick (191) states that there is no direct evidence
of this combination. It is found at autopsy, even when hypostasis is fully developed, that the muscles are equally affected; those which are not dependent and contain but little blood being equally as brightly red as those occupying dependent positions and engorged with blood.

There is no evidence that carbon monoxide increases or diminishes the coagulating power of the blood. The time of appearance and progress of post-mortem hypostasis is therefore unaffected. The onset and progress of rigor mortis is also unaffected.

We have surveyed the clinical effects of carbon monoxide anoxia on the heart and we have noted that some observers at least regard the gas as having a direct action on the myocardium. Careful examination of the heart at autopsy tends to confirm that belief not only in animals but also in man. Working with dogs it has been shown that 75% saturation or over for one hour produces haemorrhages and necrosis of the myocardium. (87). In man the lesion in acute cases is more diffuse and takes the form of an interstitial and parenchymatous myocarditis (141, 207) without any evidence of vascular blockage (329). It is impossible for anoxia alone to bring about these changes in the living animal because the far more sensitive central nervous system would suffer first and precipitate a fatal issue before any other
tissues would show such devitalization. In addition, in animals chronically exposed to give saturations never in excess of about 20%, degenerative changes and necrosis of individual muscle fibres have been observed repeatedly. (44, 87, 206). These examples seem to exclude the possibility of pure oxygen lack through anaemic anoxia but it would be unwise to assume that similar effects necessarily occur in man. However, some substantiation of this assumption is to be gained from the survey of Mayers (234) on 50 garage workers chronically exposed to carbon monoxide. These workers complained of chronic ill-health and 18% included symptoms of cardiac disturbances. The average pulse rate for the whole series was just under 102 per minute and some complained of palpitation, shortness of breath and precordial pain; manifestations similar to those observed following acute carbon monoxide anoxia.

Petechial haemorrhages are a common finding at autopsy and are observed frequently over the serous surfaces of pleura, pericardium and peritoneum. Other common sites include the meninges and throughout the brain substance; in the latter always associated with oedema. Thus the smaller vessels, particularly the arterioles, are most susceptible and the histological changes most universally recorded are of a degenerative nature, usually fatty or hyaline. Once again these effects are brought about by anoxia or by a direct action of the carbon monoxide on the tissue constit-
-uting the vessel wall. Pre-existing arteriolar sclerosis is naturally a further factor in producing serious or fatal issues in the elderly, but it cannot be inferred that carbon monoxide anoxia produces or even predisposes to the development of vascular sclerosis. Thrombosis occurring in these small vessels must be due to the damage to the vessel wall for we have seen that the clotting of the blood is unaffected by carbon monoxide saturation of the haemoglobin.

The pathology of the brain may be divided conveniently into vascular lesions and lesions to the nerve tissue. The vascular changes include congestion, stasis, petechial haemorrhages, thrombosis and oedema.

Removal of the calvarium presents a bloody effusion with gyral flattening. Petechiae may be present over the meninges and throughout the grey and white matter and there may be haemorrhage into the ventricular systems in which the cerebro-spinal fluid is increased in quantity. Vascular congestion is always prominent throughout the brain substance and is a manifestation of the preferentially increased blood supply to the brain and vascular dilatation through local action, and to a certain extent through cardiac failure. Campbell (41,43,44) has emphasized the prominence of cardiac failure in animals but the cerebral vascular lesions in animals and in man are far more prominent than vascular congestion and oedema elsewhere.
Thrombosis is not an invariable finding and its occurrence appears to be due to changes produced in the vessel wall.

The central nervous system has been minutely examined in dogs rapidly killed by carbon monoxide. (368). The circulatory changes included dilatation, stasis, perivascular haemorrhages and severe diffuse oedema. The nerve cells showed various degrees of chromatolysis and some were shrunken and stained diffusely. The most susceptible areas were cells of the cortex, corpus striatum, dorsal motor nucleus of the vagus and the dorsal sensory areas of the medulla; while the least susceptible were the nucleus ruber, nuclei of the oculomotor, trochlear, abducens and facial nerves and the large polygonal cells in the reticular formation of the medulla. Less severe carbon monoxide anoxia produced similar lesions but of less severity. Some dogs, moribund at the time of exposure, were killed 16 to 165 days later. In these there was marked vascular dilatation with endothelial proliferation particularly at the site of the haemorrhages. Large cystic areas were found in the medullary substance of the brain and the marked cellular reaction in relation to these areas consisted of fat laden neuroglia. Many nerve cells had recovered but in the deeper layers of the cortex, chromatolysis, shrinkage and dark staining were still
present. In addition, there were focal areas of myelin degeneration throughout the entire nervous system including the peripheral nerves.

The same observers made a comparison of these lesions with those produced by pure oxygen lack-anoxic anoxia. In the series of rapid death from carbon monoxide anoxia the animals were killed through exposure for 20-30 minutes and in the anoxic anoxia series the animals died within 11-14 minutes. The damage to the central nervous system was similar in character in each case but differed in degree: in the carbon monoxide series the lesions were far more marked.

In the human subject the pathology of the central nervous system is essentially the same in character but with a different distribution (39, 146). The most susceptible area is the globus pallidus. Hill and Semerak (146) surveyed 32 cases in which death occurred within a few days of gassing. All showed bilateral lenticular degeneration. A similar review of 10 cases reveals similar lesions (39). When recovery occurs where consciousness has been lost, some degree of degeneration of the globus pallidus is the rule, and it has been found in dogs that 75% saturation with carbon monoxide is the critical level and they always show pathological lesions of the brain at this
level.\(^{(27)}\).

In the globus pallidus a cystic area appears with a wall of neuroglial activity. The presence of this area gives rise to the characteristic clinical syndrome so commonly seen following acute and even chronic carbon monoxide anoxia and ascribed to damage to the basal ganglia. It seems that damage with subsequent cyst formation in the globus pallidus may give rise to no definite clinical manifestations. Such a case is recorded \(^{(39)}\) where the individual attempted suicide by coal gas. He was a young adult and recovered after a period of unconsciousness. Subsequently he had "fits" of unknown character but with no definite clinical evidence of any lesion of the basal ganglia. Three months after this suicidal attempt he made another and died. At autopsy there was bilateral cyst formation in the globus pallidus and its walls consisted of thick glial tissue, with its age being consistent with the first attempt three months before. Alexander \(^{(2)}\) states that although pallidus necrosis arises within a short period of carbon monoxide anoxia, the clinical effects don't necessarily appear for some time; usually one to several years. If this is so we are presented with an almost insuperable medico-legal problem. If the lesion to the basal ganglia were progressive such a contention would be supported but it appears that the damage to the nerve cells is done
at once and the reparative changes effected by the neuroglial elements are localised.

It is interesting, if not entirely relevant, to note that some of the degenerative changes observed in the brain of animals and man through anoxic anoxia are irreversible and are reckoned to become summated in animals repeatedly subjected to anoxia (8,335). Carbon monoxide may produce a similar effect especially in individuals chronically exposed, and in whom definite symptoms and lesions in the central nervous system ultimately become manifest.

It appears that the cerebral lesions in carbon monoxide anoxia are due to a number of factors: pre-stasis and stasis, haemorrhage, thrombosis, nutritional changes in the vessel wall and dilatation. It has been claimed that this dilatation is due partly to atony of the vessels and paralysis of the vaso-constrictors (285). The selectivity of damage to the globus pallidus cannot be explained on these factors alone and it has been pointed out that the blood supply to this part of the brain is minimal at the best of times. The globus pallidus has less capillary density than other parts (2,285), and is sparingly supplied with blood by endarteries. Alexander (2) maintains that there is great variance in the origin of vascular supply to the various segments of the globus pallidus. The common origin is the anterior choroidal or pallido-hippocampo-capsular artery. He claims that because of its recurving course, great length and small calibre, it is very liable to thromb-
which thrombosis from any other cause usually occurs in other regions of the brain. The lesion in the globus pallidus is one of ischaemic necrosis, so it seems reasonable to regard this poor vascular distribution as a factor in its causation. Support to such a contention is at once available in recorded cases where similar lesions have been produced by other forms of anoxia, i.e., nitrous oxide anaesthesia (59,60,61,214). Another factor which appears to have been overlooked is to be found in the utilization of oxygen by the cerebral tissues constituting the globus pallidus. The oxygen uptake of this tissue or alternatively its cytochrome oxidase content is only about one fifth that of the cerebral cortex, (82). This estimation was determined irrespective of blood supply, but at the best of times the blood supply is minimal with the result that the intracellular oxygen utilization has become minimal; the supply and demand being far less than for other parts of the brain. This may be taken to mean that even under normal conditions the threshold of the globus pallidus for oxygen is extremely low as compared with other parts and that any decrease in the oxygen supply would be liable to produce ischaemic necrosis. This additional factor in all forms of anoxia is at once evident. Further influence of this factor is made clear in experimental poisoning of animals by sub-
stances which inhibit the activity of cytochrome oxidase. Cyanide and sulphide in sublethal doses have been shown to produce similar necrotic lesions in the globus pallidus. (3, 93, 158, 271). These substances produce their histotoxic anoxia without affecting any other form of anoxia. The prevalence of pallidal necrosis could be explained fully if it were shown that carbon monoxide inhibited cytochrome oxidase in the living animal – a combination of anaemic and histotoxic anoxia. Further, it has been shown that substances inhibiting tissue respiration at other levels are able to produce pallidal necrosis. Barbiturates, for instance, can produce ischaemic necrotic lesions in this area (2, 63, 110.) and their action/to inhibit the negative or dehydrogenase process of tissue respiration without involving any anoxic factors.

A fuller discussion of these processes is made under Chapter VII.
CHAPTER V.

CIRCULATING HAEMOGLOBIN

In 1778 Troja described the cherry red colour of the blood in carbon monoxide anoxia but it was in 1857 that Claude Bernard showed that the anoxic effects were due to combination of carbon monoxide with the circulating haemoglobin. (285).

It is not proposed to enter into the physiology of the respiratory functions of the circulating haemoglobin. Suffice it to state that there is only one method of entry of oxygen and carbon monoxide into the body and that is through the lungs. The process of entry of these gases from the alveoli into the blood is one of simple diffusion and is dependent primarily upon the affinity of the haemoglobin for the gas present. Blood haemoglobin has far greater affinity for carbon monoxide than for oxygen. This has been estimated at between 200 and 300 times (83, 189, 211, 302). The difference in these affinities means that the compound of haemoglobin with carbon monoxide is far more stable and less readily dissociated than the compound with oxygen.

Neglecting the time factor for a moment, the final saturation of the haemoglobin with carbon monoxide is proportional to the partial pressure of the gas in the atmosphere in relation to oxygen. Keeping in mind the great affinity of haemoglobin for carbon monoxide it is at once obvious that small concentrations in the atmosphere can produce high degrees of
Figure 1.
Figure 1.

(From Killick 1940)

Rate of increase of blood saturation (as percentage of equilibrium value) during exposure of the human subject to carbon monoxide anoxia.
saturation incompatible with life. With smaller concentrations there comes a time when equilibrium is reached between the carbon monoxide in the alveoli and the blood saturation. (Figure 1 and Table 2). A theoretical fallacy to the establishment of a true equilibrium has been pointed out by Roughton and Root (279). They observe that a true equilibrium cannot be reached if carbon monoxide is being steadily lost from the blood to combine with other substances in the tissues.

When the subject is removed from the contaminated atmosphere, so long as respiration is taking place, the carboxy-haemoglobin dissociates in the lungs and the carbon monoxide is eliminated from the body. The main treatment is directed towards the dissociation of carboxy-haemoglobin — a process regulated by respiration and circulation. A full understanding of the many influences involved in the gas combining and dissociation properties of the circulating haemoglobin is essential before the carbon monoxide problem can be fully realized, for upon these influences lies the answer to the subsequent effects of carbon monoxide upon the body.

In the first place we are dealing with a chemical combination of haemoglobin with the gas (oxygen or carbon monoxide) and not a process of adsorption. Haemoglobin is constituted of an iron (haem) containing molecule and a protein (globin).
Figure 2.
Figure 2.

(From Douglas, Haldane, Haldane. 1912.)

Dissociation curves of oxyhaemoglobin in the presence of 40 m.m. pressure of carbon dioxide at 38°C.
Figure 3.
Dissociation curves of carboxy-haemoglobin in the presence of air (20.9% oxygen) at 38°C.
molecule. Hill (145) suggested a method of combination of oxygen and carbon monoxide with haemoglobin. He contended that these gases combine with the haemoglobin in the dissociated form, i.e. the gas combines with the iron-containing molecule which then recombines with the protein molecule. In explanation he points out that oxyhaemoglobin is a stronger acid than reduced haemoglobin which suggests that in reduced haemoglobin the protein molecule and the iron-containing molecule are combined by two separate linkages, while in oxyhaemoglobin the two molecules are combined by only one linkage; the spare linkage of the protein requiring sodium or some other positive ion and the spare linkage of the iron-containing requiring a gas for its saturation.

Species differences in the affinity of haemoglobin for gases and their subsequent dissociation are often marked. (125, 145, 302). (Figures 2 and 3). Similarly, individual differences must be borne in mind for such an influence is in fact responsible for the different susceptibility of individuals exposed to the same concentration of carbon monoxide. Although different susceptibility was noted by Borrichius in the 17th. century (285) it was not investigated until comparatively recently. Haemoglobin however, cannot be considered to be the only factor responsible. Douglas and the Haldanes (83) believed the globin molecule to be responsible. This has since been disproved and an explanation has been offered by Hill (145).
Figure 4.
Calculated oxygen dissociation curves of human blood at different temperatures and with 40 m.m. Carbon dioxide pressure. (Note similar shape.)
He suggests from the hypothesis that the available alkali inside the red corpuscle is completed for by the oxyhaemoglobin (stronger acid), the reduced haemoglobin, and the acid or carbon dioxide, that different ratios of the hydrogen to the basic ion concentration inside the corpuscle may explain these individual differences.

There is no indication in man that age and development bear any relationship to the dissociation curves of haemoglobin but it has been shown that the oxygen dissociation curve of the blood of the young duck is shifted to the right compared with the blood from the adult duck: the curve of the latter fitting the theoretical curve. (274) Human foetal and adult bloods do differ, however, in their resistance to alkali owing to the presence in foetal blood of two kinds of haemoglobin – one resistant (80%) and the other non-resistant (20%). The resistent haemoglobin has all disappeared by about six months. (135).

Certain physical factors affect the dissociation curves. Paul Bert in 1878 showed the influence of temperature upon the oxygen dissociation curve (131) and a similar influence has been shown with carbon monoxide (83,133,286). (Figure 4). High humidity has the effect of increasing the affinity of haemoglobin for carbon monoxide. (286). The influence of light is marked and will be more fully discussed when dealing with other haemin proteins, Chapter VII. Darling and Roughton (65) indicated that
the oxygen dissociation curve is shifted to the left as the percentage of methaemoglobin increases; the effect being qualitatively the same but quantitatively less than that produced by carboxy-haemoglobin. They found also that the shifts produced with methaemoglobin and carboxy-haemoglobin are additive so methaemoglobin cannot be held responsible for the "Haldane Effect" (vide infra.). They believed the shift to be due to the formation of compounds intermediate between reduced haemoglobin (wholly ferrous) and methaemoglobin (wholly ferric); the conversion of one of more of the four ferrous atoms in the haemoglobin molecule to ferric leading to an increased affinity of the remaining ferrous atoms for oxygen. A similar effect upon the affinity for oxygen has been observed with dilution. (150). At a dilution of from 5 to 200 times there was a threefold increase in the affinity for oxygen, the dissociation curve being nearly the same as blood with 40 m.m. carbon dioxide pressure at the same temperature. It has been observed that the affinity for oxygen is lowered by altitudes of 4,000 metres and over (12) but there is no evidence of the affinity for carbon monoxide being affected by similar conditions.

In the living subject the elimination of carbon monoxide from the blood is hastened by increased pulmonary ventilation. This constitutes the main objective in treatment and one of the methods of producing a stimulation of respiration is by increasing the alveolar carbon dioxide. Carbon dioxide
Figure 5.
Figure 5.

(From Stadie, Martin, 1925.)

Rate of elimination of carbon monoxide from partially asphyxiated dogs under different conditions.
Figure 6.
Figure 6.

(From Stadie, Martin, 1925.)

Oxygen dissociation curves of blood 50% saturated with carbon monoxide showing the effect of acidity on the partial pressure of carbon monoxide.
administration is really the sheet anchor in treatment and one of its actions is to bring about a lowering of the pH. Lowering the pH has a two-fold beneficial effect: besides producing increased pulmonary ventilation it also causes a shift in the carbon monoxide dissociation curve to the right. The inhalation of carbon dioxide is the physiological method of bringing about this alteration in the pH but the rationale of carbon dioxide inhalation in treatment is not confined to its effect upon the pH. It has frequently been misunderstood that the increased rate of elimination of carbon monoxide has been brought about entirely by hyperventilation but Stadic and Martin (308) have shown that with a constant ventilation rate, lowering the pH of itself increased the rate of elimination. (Figure 5). The influence of acidity on the partial pressure of carbon monoxide is perhaps more fully appreciated in Figure 6.

Haldane and Priestly (131) record an interesting paradoxical effect in their discussion of the properties of haemoglobin. When blood is exposed to a mixture of oxygen and carbon monoxide, the combined curve for varying pressures being insufficient to saturate the haemoglobin, the dissociation curve for varying pressures of either gas ceases to be a rectangular hyperbola and acquires a remarkable hump.
Figure 7.
Blue: Carbon monoxide at a pressure insufficient to saturate the blood and the oxygen pressure gradually raised from zero. (Calculated on known affinities of CO and O2 on blood used.)

Black: actual observations.

Red: Curves for carboxy-haemoglobin calculated.
Figure 8.
Figure 8.

(J.B.S. Haldane, 1912.)

The "Haldane Effect."
(Figure 7). This is shown by the fact that at very low pressures of carbon monoxide, the pressure of the carbon monoxide helps the oxygen to combine with haemoglobin. The reverse with low pressure of oxygen also holds good.

J.B.S. Haldane in 1912 showed that when haemoglobin was partially saturated with carbon monoxide and the remainder saturated with oxygen, the oxygen dissociation curve was shifted to the left; the degree of shift being proportional to the degree of saturation with carbon monoxide. (Figure 8). In other words, in carbon monoxide anoxia the available oxygen combined with haemoglobin is held more firmly as the degree of saturation with carbon monoxide increases. This effect, confirmed by numerous workers since (18, 145, 259, 278, 308) has become to be known as the "Haldane effect" and has been held responsible for the many otherwise unaccountable clinical effects of carbon monoxide anoxia. For instance, an individual lying in coma may regain consciousness from carbon monoxide anoxia and talk intelligently but upon movement, even in fresh air, he commonly relapses into coma. In elucidating the "Haldane effect", Stadie and Martin (308) compared the position of 40% saturation with carbon monoxide to anaemic anoxia in pernicious anaemia with 40% haemoglobin. (Figure 9). In each case there is 8 volumes % oxygen available and the tissue requirements are about 4 volumes % in each case.
Figure 9.
Figure 9.

(From Stadie, Martin. 1925.)
But it will be observed that with carbon monoxide this will be available at 12 m.m. and in the pernicious anaemia case at 28 m.m. It is presumed that the partial pressure in blood (capillaries) required by the tissues is about 12-20 m.m.Hg. The plight of the individual with 40% saturation with carbon monoxide is obviously far more precarious than that of the individual with pernicious anaemia with 40% haemoglobin.

We see that the stability and dissociation of the compound carboxy-haemoglobin are influenced by many factors. The partition is affected by humidity, light, temperature and dilution. The elimination or dissociation in vivo is affected by species, respiration and pulmonary circulation, blood pH, the partial pressure of carbon dioxide, altitude and the presence of methaemoglobin. We have recognised the bearing of the "Haldane effect" upon the whole problem.

The principles of treatment are directed towards the elimination of carbon monoxide and replacement with oxygen. These two factors are combined by the inhalation of 5-7% carbon dioxide in oxygen. The value of such a procedure can be assessed from Figure 10, in which the original work of Henderson and Haggard is summarized. (137). The inhalation of pure oxygen, although hastening elimination, is not
Figure 10
Figure 10.

(From Henderson, Haggard. 1922.)

Elimination of carbon monoxide from dogs subjected to carbon monoxide anoxia.
so effective. Henderson and Haggard found that mixtures of air and carbon dioxide were more effective than pure oxygen but not nearly so effective as the oxygen-carbon dioxide mixture. Numerous others have subsequently agreed with the beneficial effect of the latter mixture but found pure oxygen more beneficial than the mixture of air and carbon dioxide. (250, 251, 337, 343). The disappearance of the gas from the blood takes place rapidly at first and then gradually slows down as the saturation decreases (279). In untreated cases 5% saturation has been found persistently present 7-8 hours after the end of exposure (289) and a similar persistence has been recorded for several days. (191).

We know that carbon monoxide enters the body through the lungs and combines with the circulating haemoglobin. There can be no doubt that our knowledge of this initial process of carbon monoxide anoxia is thus complete. Whatever happened to the gas in the body will be dealt with in time but in the meantime let us consider what quantity is excreted by the reverse process. In other words, is the quantity of carbon monoxide dissociated from the haemoglobin in the lungs equal to the amount originally inhaled? If equality does exist a further question is automatically answered; namely, that carbon monoxide is not metabolized in any way by the body. The problem would remain, however, as to
whether or not carbon monoxide combines with other pig-
ments or substances in the tissues.

J.S.Haldane in 1900 (129) estimates that not
more than 3% if any carbon monoxide was retained or
oxidized in the mouse per hour over a period of 26-
29 hours. The methods of estimation available at
that time make such a conclusion unwarranted. In
1922 (130) he stated that carbon monoxide is not
oxidized or otherwise decomposed in the body and that
it passes into the body and out again without there
being the slightest loss. The possible error in his
methods at this time makes his statement equally
unwarranted however true the substance of which may
subsequently have been proved.

It has been shown that when normal human
subjects inhaled 150 c.c. carbon monoxide, 60-70%
was eliminated from the blood within the first hour
and was recovered in the expired air. (279,336)
Roughton and Root (279) next investigated the fate
of the remaining 30-40%. To hasten the dissociation,
the subjects breathed oxygen after the first hour
after the inhalation of the carbon monoxide and
continued for four hours. At the end of this
period it was estimated that about 96% of the total
carbon monoxide was recovered in the exhaled air. In
view of the experimental errors of the carbon monoxide
determinations (280,281) and the possibility of small
leaks in the masks, rubber bags, processes of transfer
etc., so nearly complete recovery seems as good as
could be expected and indicates that all the carbon monoxide absorbed by the body must have been reversibly combined with haemoglobin of the blood and similar substances in the cells. During the later phases the carbon monoxide lost from the blood must be less than that currently found in the expired air, the balance being made up by the carbon monoxide which dissociates from combination with substances outside the blood stream and diffuses into the latter.

In discussing the possible extra-circulatory reversible combinations of carbon monoxide, Roughton and Root mention muscle haemoglobin but concluded, seemingly without qualification, that this combination could account for only an infinitesimal proportion of the 30-40% of carbon monoxide lost during the first hour. (See Chapter VI). They mention too, as an insignificant factor the stagnant RBCs "stored" in the spleen, skin and bone marrow. Of more relevance they suggest the "pseudo-haemoglobin" of Barkan. This "pseudo-haemoglobin" was described by Barkan in 1938. He regards it as an intermediate in the breakdown of haemoglobin to bile pigments. It is to be found in greatest quantity, therefore, in the liver and has been postulated to have a far greater affinity - 10 times - for carbon monoxide than has blood haemoglobin. In support of the possible part played by this "pseudohaemoglobin" it has been shown by determining the distribution
of radio-active carbon monoxide that the liver curve showed a high initial phase as compared with other regions such as the thigh and spleen-heart areas with a prolonged diminution of excretion. (336).

Further helpful evidence may be expected from the developments to be made in the use of radio-active carbon in the near future but this will be limited, at least at first, to distribution of carbon monoxide and not to its possible detrimental effects to tissue oxidation during its extra-circulatory sojourn.

It seems probable from this recent work by Roughton and Root that there is no loss of carbon monoxide from the time it enters the body to the time it is eliminated. There is evidence, however, that extra-circulatory reversible combinations do take place with the circulating haemoglobin acting as the transporting host in both phases of this process. These authors used small quantities of carbon monoxide which would give low blood saturations. With higher saturations there may be far greater loss to extra-circulatory combinations especially to those substances involving competition between oxygen and carbon monoxide for their saturation. This competition, as with blood haemoglobin, would be decided by the relative affinities for the two gases as well as by the pressures at which they were available.
Respiration of isolated frog muscle has been found to be stimulated by carbon monoxide (49, 50, 91, 92, 295, 311) and this has been held to be due to oxidation of carbon monoxide to carbon dioxide. (91). The action of isolated tissue in an unphysiological environment cannot be regarded as a fact when applied to the whole organism, and such a conclusion is not borne out by the more fundamental investigations of Roughton and Root, who went further and showed that there is no enzyme in the RBCs capable of oxidizing carbon monoxide to carbon dioxide and that carbonic anhydrase of the RBCs has no "formic anhydrase" properties.
CHAPTER VI.

TISSUE HAEMOGLOBIN

Boerhaave observed red pigment in muscle in 1775 (267) but investigation into tissue pigments with a respiratory function was not published until 1887 when McMunn made known the results of his classical experiments (224). His theories of the presence and properties of these pigments, "myohaematin" and histiohaematin" brought forth voluble scorn from his fellow physiologists at the time with the result that further investigation was delayed and McMunn himself became discouraged.

Regrettably little work has since been done and it is alarming that haematologists today confine their attentions purely to the blood haemoglobin when assessing the anoxia of the anaemic patient. Blood haemoglobin is the initial host in transporting oxygen in the higher organism but the cells of many tissues cannot utilize the oxygen without intermediate substance which include the cytochromes, and for the maintenance of their respective functions certain tissues require tissue haemoglobin. It is remarkable, then, that the haematologist has not devoted more effort to the furtherance of his knowledge of the requirements of the cell for oxygen: the only true criterion of anoxia.

Tissue haemoglobin is distributed throughout the red muscles and has become known as muscle haem-
Table 5.

<table>
<thead>
<tr>
<th>Material</th>
<th>Tensile Strength</th>
<th>Elongation</th>
<th>Impact Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steel</td>
<td>60,000 psi</td>
<td>25%</td>
<td>25,000 ft-lb</td>
</tr>
<tr>
<td>Aluminum</td>
<td>40,000 psi</td>
<td>15%</td>
<td>10,000 ft-lb</td>
</tr>
<tr>
<td>Brass</td>
<td>30,000 psi</td>
<td>20%</td>
<td>15,000 ft-lb</td>
</tr>
<tr>
<td>Copper</td>
<td>50,000 psi</td>
<td>10%</td>
<td>12,000 ft-lb</td>
</tr>
<tr>
<td>Magnesium Alloy</td>
<td>45,000 psi</td>
<td>15%</td>
<td>18,000 ft-lb</td>
</tr>
<tr>
<td>Titanium</td>
<td>75,000 psi</td>
<td>5%</td>
<td>5,000 ft-lb</td>
</tr>
</tbody>
</table>

Note: Values are approximate and subject to variation based on material grade and manufacturing process.
**Table 5.**

**MUSCLE HAEMOGLOBIN-BLOOD HAEMOGLOBIN.**

<table>
<thead>
<tr>
<th>Property</th>
<th>Muscle Haemoglobin</th>
<th>Blood Haemoglobin</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversible combination with</td>
<td>Oxygen and Carbon monoxide</td>
<td>Oxygen and Carbon monoxide</td>
<td>148, 333</td>
</tr>
<tr>
<td>Visible spectrum:</td>
<td>5815 A</td>
<td>5770 A</td>
<td>149, 163</td>
</tr>
<tr>
<td></td>
<td>5446 A</td>
<td>5420 A</td>
<td></td>
</tr>
<tr>
<td>Affin. for oxygen</td>
<td>Very high</td>
<td>moderate</td>
<td>333</td>
</tr>
<tr>
<td>Rel. CO2/O2 affin.</td>
<td>Low</td>
<td>High</td>
<td>332</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>17,500</td>
<td>68,000</td>
<td>331</td>
</tr>
<tr>
<td>Oxidation to &quot;met-&quot;</td>
<td>Very easy</td>
<td>Less easy</td>
<td>330</td>
</tr>
<tr>
<td>O2 dissoc. curve (Fig. 11)</td>
<td>Hyperbolic</td>
<td>Sigmoid</td>
<td>246</td>
</tr>
<tr>
<td>Velocity constants:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O2 combination. &quot;dissociation.&quot;</td>
<td>19,000, 37</td>
<td>4,000, 40</td>
<td>244</td>
</tr>
<tr>
<td>CO combination. &quot;dissociation.&quot;</td>
<td>300, .04</td>
<td>130, .004</td>
<td></td>
</tr>
</tbody>
</table>
Figure 11.
Comparison of the oxygen dissociation curves of blood and muscle haemoglobin.
-oglobin or myohaemoglobin. It belongs to the same prosthetic group as blood haemoglobin with 0.345% iron content. (246). The general properties of the two pigments are summarized in Table 5, and the oxygen dissociation curves are compared in Figure 11.

In 1921, Günther (121) pointed out the distinction between red and white muscle. The red muscle derives its colour from the muscle haemoglobin contained within its fibres; the white muscle being devoid of this pigment. The distribution of muscle haemoglobin is directly proportional to the activity of the muscle. In the human embryo the muscles (including the heart) have no colouration before the third month; between the third and seventh month there is a certain increase; and thereafter there is no change until after birth when there is an increase with activity. (121). In the growing dog the level increases in the leg muscles until the high value of the adult is reached; whereas the diaphragm, with a uniform degree of activity throughout growth, shows a uniform haemoglobin content. (360). House dogs have a lower content than working dogs. (362). Muscular paralysis through section of the sciatic nerve in the dog showed a marked reduction of muscle haemoglobin in the affected leg and after 7 weeks this reduction reached 50% in some muscles. (361). In resting cat soleus muscle the haemoglobin was reduced within 2 minutes. (245, 246). Observation on autopsy material
has led to the conclusion that a similar relationship between activity and muscle haemoglobin distribution exists in the human subject. (366).

It is contended that muscle haemoglobin is concerned with the rapid exchange of oxygen and carbon dioxide between the blood and the contracting elements. (187). The reserve power of any given muscle seems to be determined partly by the haemoglobin it contains. In this way muscle haemoglobin functions as a short term oxygen store to tide the muscle over from one contraction to another. Millikan (245) observed that when contraction takes place, the oxygen demand starts to rise in less than 0.2 second, reaches maximum value within 1 second from the onset of contraction and falls nearly to its resting value within 10 seconds of the end of contraction, when contraction has been of a few seconds duration only. There are many examples of this property of oxygen storage. For instance, seal muscles, rich in haemoglobin, are isolated from the general circulation during the dive and they store enough oxygen to allow the muscles to carry on "anaerobically" during the first 5-10 minutes of the dive. Similarly, the oxygen stored in the muscle haemoglobin of the pectoral muscles of the penguin is consumed in a 5 minute dive. (297). Hill (149) calculated that enough oxygen is stored by muscle haemoglobin to allow the heart to continue from one heat to another - an operation requiring 2.7 c.m. of oxygen per gram per second, and the amount stored by muscle haemoglobin
in 2.9 cm per gram per second. Hill's explanation depends upon Keilin's observations (163) that in small animals with rapid heart beat, as in the wing muscles of insects, catalytic activity is more important than storage so the muscle haemoglobin is replaced by cytochrome.

This aspect of distribution has been summarized by Millikan (246). He states that muscle haemoglobin is "generally found in large quantities in those muscles requiring slow, repetitive activity of considerable force. The highest concentrations are found in muscles whose complete cycle of activity takes the order of a second, and whose action must be maintained over long periods. Examples are the heart muscles of larger mammals, breast muscles of the larger flying birds such as the pigeon, and leg muscles of running animals such as the horse and the dog. Muscles which contract regularly more than two or three times per second usually have much cytochrome but little or none of the haemoglobin. Examples are the wing muscles of flying insects, and the hearts of small birds and mammals such as canaries and mice. On the other hand, muscles which are called upon for repetitive activity of no great power or intensity with large intervals of rest between contain large quantities of cytochrome but no muscle haemoglobin.

Unlike the cytochrome system, muscle haemoglobin
is not a catalyst in the strict sense; but from its oxygen affinity and its speed of activity it is between blood haemoglobin and the catalyst, cytochrome oxidase. It has been suggested to act as a connecting link between oxygen carrier and oxygen catalyst. (246).

Millikan (246) estimates that about 25% of the body's haemoglobin is locked up in the muscle fibres as muscle haemoglobin and is not able to circulate. In the dog this has been estimated at 15-50% of the total. 10-80 grams of muscle haemoglobin under widely different conditions were found with total circulating haemoglobin represented as 100 grams. (360,362).

Gaßcheidlen in 1869 showed that muscle haemoglobin combined with carbon monoxide (120) and the spectrum of this compound has been demonstrated to be different from that of the compound with blood haemoglobin. (267). Although the affinity of oxygen for muscle haemoglobin is 2.5 - 5 times greater than for blood haemoglobin, the affinity of muscle haemoglobin for carbon monoxide is still 20 times greater than for oxygen. (244). So far as these affinity relationships are concerned, the position is therefore qualitatively analogous to that of blood haemoglobin. Killick (191) gives an erroneous impression by stating that although carbon monoxide combines with muscle haemoglobin, its affinity for muscle haemoglobin is far less than for blood haemoglobin and only a small quantity
of the gas inhaled unites with the haemoglobin in the muscle. She then quotes Gscheidlen's work of 1869 when he estimated this quantity to be about $\frac{1}{20}$ of the carbon monoxide absorbed. The knowledge of carbon monoxide anoxia was infinitesimal in 1869 and nothing was known of the distribution of extra-circulatory haemoglobin at that time. It is remarkable that Killick should draw such conclusions from such obviously unfounded records and even more remarkable that Roughton and Root (279) should accept Killick's conclusions without reference to more relevant and accurate recorded data. The affinity of muscle haemoglobin for oxygen and carbon monoxide is not influenced in its effect by the different affinity of blood haemoglobin for these gases in the way Killick has inferred.

The fundamental point is that carbon monoxide has far greater affinity than oxygen for both blood haemoglobin and muscle haemoglobin: 200-300 times greater for the former and 20 times for the latter. The main difference lies in the fact that blood haemoglobin obtains its gas for combination from the alveolar air while muscle haemoglobin obtains its gas apparently by diffusion from the circulating haemoglobin. The ease of transfer of oxygen from blood or circulating haemoglobin to muscle haemoglobin is dependent upon the ease of dissociation of oxygen from the blood. The influence of partial saturation of the circulating haemoglobin with carbon monoxide upon the
dissociation of oxygen has been discussed and it will at once be realized that in carbon monoxide anoxia the effect upon the muscle haemoglobin is due not only to differences in the affinities of the two gases for the haemoglobin but also to the fact that oxygen is held more firmly by the transporting host — "Haldane affect". There is no indication to show whether or not the compound of carbon monoxide with muscle haemoglobin behaves in a similar way to and comes under the same influences as the compound with blood haemoglobin. If this were so the oxygen dissociation curve for muscle haemoglobin would be shifted to the left and the tissues would suffer from a double "Haldane effect".

Killick (191) recognises that carbon monoxide combines with muscle haemoglobin in vivo but states that there is no direct evidence as to the effects produced by such a combination. The prominence of muscular weakness and collapse as a common clinical manifestation may be attributed partly to this combination. We saw that the cherry red colour of the muscles at autopsy is generalized and unrelated to the quantity of blood contained therein; that individual heart muscle fibres show necrosis even with small saturations; and that devitalization of tissue may occur requiring subsequent amputation. All these effects indicate some additional factor to the anaemic anoxia produced by carbon monoxide inhalation and may be due to its combination with muscle haemoglobin or similar substance.
If it is to be regarded that "pseudohamoglobin" of Barkan is an intermediate between haemoglobin and bile pigment, it will have to be observed that such a substance is possibly constituted in part from the breakdown of muscle haemoglobin as well as blood haemoglobin, for they are apparently "excreted" in a similar way. This has been shown by injecting muscle haemoglobin by various routes with its subsequent excretion as bile pigment in the urine. (362).

The properties of muscle haemoglobin indicate that it may have a bearing upon acclimatization to carbon monoxide anoxia. In dogs, prolonged severe anaemia caused a slight reduction in the level of muscle haemoglobin but inactivity had a far greater and quicker effect upon this level. (361). Anoxic anoxia as experienced at altitude produced increases in both blood and muscle haemoglobin in dogs. This increase averaged 44% in blood haemoglobin and 66-70% in muscle haemoglobin. (159). In the human subject the occurrence of mountain sickness has no relationship to the degree of anoxaemia in the arterial blood, the oxygen and carbon dioxide tensions in the alveolar air, or to the amount of haemoglobin circulating; and it has been concluded that it is probable that the tissue systems of transporting oxygen, namely muscle haemoglobin and the cytochromes, play an important role in the mechanism of this syndrome. (25). All these observations emphasize the importance of considering acclimatization in terms of mechanisms which operate at
tissue level. Among them, an increase in muscle haemoglobin is important and is perhaps one of the most fundamental adaptive mechanisms in organisms which are born and raised at high altitudes and represent adaptation in its true significance. (159).

No work has been recorded upon possible alteration in the level of muscle haemoglobin in chronic exposure to carbon monoxide. Increases in the circulating haemoglobin have been repeatedly observed and it seems a reasonable prediction that similar or even greater increases are to be found in muscle haemoglobin.
CHAPTER VII.

CYTOCHROME SYSTEM.

Before embarking upon a discussion of the relevant aspects of the cytochrome system to the present study it is as well to have some detailed knowledge of the system. This requires a certain amount of historical investigation, for the initial work done in different schools was unnecessarily complicated by differences in terminology.

McMunn in 1887 (224) first described a respiratory pigment which he called "histiohaematin". This was subsequently renamed cytochrome in 1925-1926 by Keilin (163,164) and he indicated the three types a, b, and c. In the meantime Warburg in 1924 observed an iron catalyst to which he gave the name "atmungsferment". (344). Then in 1929 Keilin investigated an enzyme in yeast and heart muscle which he believed was identical with Warburg's "atmungsferment". He called this "indophenol oxidase". (166). It was then argued that the two were not identical (355) but as knowledge has increased the opposing views have merged and the name given to the ferment is "cytochrome oxidase". In 1914 Battelli and Stern had recognised the oxidase in animal tissues but it was not associated with any other tissue pigments and its nature was not determined (27).

The cytochrome system is constituted of the three cytochromes, a, b, and c and the ferment cytochrome oxidase. The system functions in a way that tissue cells
may utilize molecular oxygen: a faculty denied them were it not for some "activating" mechanism.

Cytochrome c is the only one of the system to have been isolated so far. (252). It is composed of a haem derivative with a protein bearer giving a molecular weight of about 16,500 (173) and containing 0.43% iron. (173,334).

The properties of the components and of the completed system have largely been arrived at by analogy but they are none the less distinct. The complete cytochrome system can readily be prepared from heart muscle (173,178) and this preparation has been largely responsible for the present extensive data relating to the system. The preparation is of colloidal structure and it appears that the cytochrome oxidase will exist only in such a structure in cell-free conditions: any attempt to separate it from this structure have failed. Physiological conditions must exist before any criteria can be taken for functions of the complete system (173,178,184,192,193).

Pure cytochrome c has been isolated from the heart preparation and further properties have been ascribed to it. It is thermostable. Reduced and oxidized cytochrome c are divalent and trivalent iron compounds. It is not autoxidizable and does not combine with carbon monoxide. It becomes autoxidizable at pH below 4 or above 12, and at 13 it combines reversibly with carbon monoxide. Biologically, cytochrome c is
Table 6.

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>9</td>
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</tbody>
</table>

Data for Table 6.
Table 6.

(Ref. Nos. 115, 322.)

<table>
<thead>
<tr>
<th>Visible absorption bands (mμ) in heart muscle:</th>
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<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Cytochrome a</td>
</tr>
<tr>
<td>Cytochrome b</td>
</tr>
<tr>
<td>Cytochrome c</td>
</tr>
<tr>
<td>Cytochrome a3</td>
</tr>
<tr>
<td>CO compound:</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
</tr>
<tr>
<td>Cytochrome a3</td>
</tr>
</tbody>
</table>
oxidized specifically by cytochrome oxidase and can be reduced by the succinic dehydrogenase system \((173,178)\). Its iso-electric point has been estimated at \(\text{pH} 10\) \((334)\).

Cytochromes \(a\) and \(b\) have not been isolated in the pure state, but they are undoubtedly similar substances to cytochrome \(c\) with similar properties and similar spectra. (Table 6).

The nature and function of the cytochrome system is based on Szent-Gyorgyi's study of the oxidation of catechol \((252)\). Ferrous iron was rapidly oxidized to ferric iron when added to catechol. Since the system was devoid of free ferric ions at the commencement, the oxidation was due to an intra-molecular electron shift from the ferric iron to the catechol within the complex. It was found that the reaction between the ferrous-catechol complex and oxygen was strongly inhibited by carbon monoxide. There apparently existed a competition between oxygen and carbon monoxide for the complex which depended on the relative concentration of the two gases. It was concluded that the first step of the reaction consisted in a reversible oxygenation of the iron-catechol complex. There must have followed an intra-molecular electron shift between the oxygen and the central ferrous atom. The ferric thus formed accepted an electron from the catechol and was thereby regenerated to the ferrous form. The overall result of this cycle was the oxidation of catechol to quinone and the combination of the charged oxygen with the
hydrogen ions of the water to form hydrogen peroxide.

The reaction of the cytochromes involves the transference of an electron in a similar way; the three cytochromes a, b, and c being arranged in series. The electron is transferred from one iron compound to another without involving oxygen or hydrogen ions in the oxidation. The process is initiated in the presence of active cytochrome oxidase which is specific for this series. The whole system, constituted of the four substances, is contained within the cell and is responsible for the utilization of molecular oxygen transported to the cell by the circulating haemoglobin.

The process is commenced by the oxidation of cytochrome oxidase and then apparently in a step up manner the three cytochromes become oxidized and subsequently reduced. This constitutes a reversible redox system, Fe'''' \rightleftharpoons Fe'''. The cytochromes a, b, and c in the living cell are oxidized not by oxygen but by oxidized cytochrome oxidase. Inhibition of the oxidase will, of course, prevent the oxidation of the cytochromes: the cell is deprived of its oxygen no matter how ample is the supply and under what tension, and we are confronted with histotoxic anoxia. The effect of cyanide is to prevent the reduction of the cytochromes with a precisely similar end result.

The order of the cytochromes in this series is not definitely known but is believed to be according to the following scheme: oxygen \rightarrow cyto-
Figure 12.
Figure 12.

(a) Ref. no. 15.

(b) Ref. no. 252.

(c) Ref. no. 15.
-chrome oxidase $\rightarrow$ cytochrome a $\rightarrow$ cytochrome c $\rightarrow$ cytochrome b. (15,252). Evidence favouring this order lies in their redox potentials as follows: - (physiological pH and E° in volts). (22,24,114,252,324).

$$a = + 0.29$$
$$c = + 0.123 \text{ to } -0.262.$$  
$$b = - 0.04.$$  

We now have the completed system on the positive side of cellular respiration. The negative side dealing with cellular metabolites of respiration is comprised of the dehydrogenase system. It is not known if there is a connecting link between the two systems (134) but it has been suggested that cytochrome b may function as that link, for it has the required potential and it is a protein. Potter (261) believes that no such link is required.

The complete scheme of the system and its method of function are recorded diagramatically in Figure 12 (a) & (b).

In 1939 Keilin and Hartree (177) recognised cytochrome a 3 spectroscopically and they believed this to be identical to cytochrome oxidase. For instance, both are thermolabile being affected by temperatures above 52°C; their oxidations and reductions can be followed spectroscopically in the living cell; they are rapidly and efficiently reduced by biological reducing systems; the spectra of their carbon monoxide compounds are similar; they both combine reversibly with
the same substances, namely KCN, H2S, NH2OH, CO and azide; and they occur in the same situations. It remains to be proved definitely whether these two are identical and the main points against are threefold. Firstly, strong light has no effect on the spectrum of the a3 compound with carbon monoxide; secondly, there exist cells, the respiration of which is poisoned by cyanide and by carbon monoxide, yet they are devoid of cytochrome or of its component a; and thirdly, there has been a failure to demonstrate a direct or indirect reduction of the oxidized component a3 by addition of reduced cytochrome c under strictly anaerobic conditions and in complete absence of other reducing substances. This may be due to the fact that the reaction may take place only in the presence of molecular oxygen and a3 would be reoxidized as rapidly as it is reduced by c. Hence the reduced a3 could hardly be expected to be visible spectroscopically.

Whether cytochrome a3 and cytochrome oxidase are identical or not has but little bearing upon the present issue but the argument has at least furnished us with the relevant properties of the oxidase. We have observed that carbon monoxide combines reversibly with cytochrome oxidase but let us look back for a moment to the earlier work of Warburg (344, 345, 347, 354) and Keilin (166). Warburg showed inhibition of the respiration of yeast by carbon monoxide and its reversibility by light, and Keilin then observed that cytochrome oxidase, present in yeast and heart muscle,
was inhibited in a similar manner with reversibility by light. It is upon these original observations that we have been able with better spectroscopical methods to build up a comprehensive picture of the distribution of the cytochrome system throughout animal tissues. It must always be borne in mind that analogy plays the major part in forming this pattern of cellular metabolism and that to diverge from physiological conditions is liable to cloud the issue with false conclusions.

In general, cell respiration may be divided into three types: main, accessory and residual.\(^{(252)}\). Main respiration is manifest by intact organs and tissues and proceeds probably completely through the cytochrome – cytochrome oxidase system. It comprises essentially the combustion of sugar breakdown products by the haemin system. Accessory respiration encompasses all those oxygen consuming processes which do not involve cytochrome oxidase and is not dependent upon the intact cell. The amount of oxygen required in this type in vivo is negligible compared with the total and with the main respiration. It is largely sensitive to cyanide. Residual respiration is cyanide resistant and occurs in certain organisms with which we are not concerned. An exception is retinal tissue, the respiration of which will be discussed in Chapter VIII. Our interest lies chiefly with main respiration involving the complete cytochrome–
cytochrome oxidase system.

No reference has been made to the other haemins, namely peroxidase and catalase; the reason being that these enzymes contain iron in a stabilized ferric state and are not inhibited by carbon monoxide. (252).

It seems that the haemins have been derived from chlorophyll; the earliest being the green or pheophorbid haemins; then the mixed colour or pheohaemins of which cytochrome oxidase is cited as an example (243), and the red or protohaemins including blood pigment. (252). Free haematin has been observed spectroscopically in living cells and has been speculated as being a precursor of cytochrome (165). Incidentally, these haemins are believed to be responsible for the thermostable peroxidase activity of haemoglobin and peroxidase-free cells such as bacteria, yeast etc. (168). Keilin (166) found cytochrome present in ascaris eggs which are aerobic and found none in the adults which are practically anaerobic. He also observed it to increase during development of the egg of Gastrophilus equi while the haemoglobin diminished. He concluded that cytochrome was more than a vestigial pigment devoid of function. We saw in the preceding chapter that the presence of cytochrome in muscle depended to a large extent on the nature of the activity required by that muscle: where catalysis is more important than
Tables 7, 8, 9.
Table 7.  (Ref. No. 322.)

<table>
<thead>
<tr>
<th>Rat tissue</th>
<th>Cytochrome Oxidase units/mg. dry tiss</th>
<th>Cytochrome c mg/gm. dry t.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>9.7</td>
<td>2.34</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.7</td>
<td>1.36</td>
</tr>
<tr>
<td>Brain</td>
<td>3.5</td>
<td>.88</td>
</tr>
<tr>
<td>Skel. musc.</td>
<td>2.3</td>
<td>.35</td>
</tr>
<tr>
<td>Liver</td>
<td>1.7</td>
<td>.24</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.6</td>
<td>.21</td>
</tr>
<tr>
<td>Lung</td>
<td>1.3</td>
<td>.14</td>
</tr>
<tr>
<td>Testis</td>
<td>1.1</td>
<td>.03</td>
</tr>
<tr>
<td>Diaph. musc.</td>
<td>.72</td>
<td>.18</td>
</tr>
<tr>
<td>Large int.</td>
<td>.36</td>
<td>.02</td>
</tr>
</tbody>
</table>

Table 8. (Ref. No. 262.)

<table>
<thead>
<tr>
<th>Cytochrome c, fresh rat tissue. Microgms/gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
</tr>
<tr>
<td>Kidney</td>
</tr>
<tr>
<td>Skel. musc.</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Brain</td>
</tr>
<tr>
<td>Spleen</td>
</tr>
<tr>
<td>Lung</td>
</tr>
</tbody>
</table>

Table 9.

<table>
<thead>
<tr>
<th>Cytochrome oxidase content as estimated by Qo2:-- (Rat tissues:--).</th>
</tr>
</thead>
<tbody>
<tr>
<td>References:--</td>
</tr>
<tr>
<td>Heart</td>
</tr>
<tr>
<td>Kidney</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Brain</td>
</tr>
<tr>
<td>Skel. muscle</td>
</tr>
<tr>
<td>Spleen</td>
</tr>
<tr>
<td>Lung</td>
</tr>
</tbody>
</table>
storage the cytochrome was abundant at the expense of muscle haemoglobin.

The original theory of McMunn is worthy of quotation on the development of the cytochromes in relation to haemoglobin. (224). He stated that "they are as important as haemoglobin, if not more so in some animals, and they have the right of priority in time, as they were developed at an earlier period than haemoglobin, speaking from a phylogenetic point of view ...... It is not improbable, but indeed likely, that by a process of physiological selection these respiratory proteids may have become more complex, and their molecular instability therefore greater as the animal body became more elaborated, and a necessity arose for the setting apart of respiratory proteids for abstraction of oxygen from the air. In this way haemoglobin may have arisen ......"

The distribution of the cytochrome system has been recorded for many animal tissues but the data obtained cannot be interpreted directly into human valves. In the main it can act as a definite guide remembering that qualitative species differences in all probability exist in the same way as with haemoglobin. It would appear from Tables 7-11 that all animal tissues contain cytochrome to some extent. It is universally accepted that the
Table 10.

(Ref. No. 272.)

Relative Cytochrome c contents:-

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Man</th>
<th>Rabbit</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney cortex</td>
<td>45</td>
<td>354</td>
<td>1430</td>
</tr>
<tr>
<td>Liver</td>
<td>125</td>
<td>607</td>
<td></td>
</tr>
<tr>
<td>Brain cortex</td>
<td>232</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>Submax. gland</td>
<td>190</td>
<td>378</td>
<td></td>
</tr>
<tr>
<td>Colon mucosa</td>
<td>39</td>
<td>97</td>
<td>136</td>
</tr>
<tr>
<td>Lung</td>
<td>45</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

Table 11.

(Ref. No. 160.)

Cytochrome c - microgs/gm.

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>microgs/gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigeon breast</td>
<td>494</td>
</tr>
<tr>
<td>Beef heart</td>
<td>191</td>
</tr>
<tr>
<td>Pig shoulder</td>
<td>96</td>
</tr>
<tr>
<td>Beef tongue</td>
<td>88</td>
</tr>
<tr>
<td>Beef shoulder</td>
<td>61</td>
</tr>
<tr>
<td>Beef kidney</td>
<td>29</td>
</tr>
<tr>
<td>Beef liver</td>
<td>14</td>
</tr>
<tr>
<td>Beef brain</td>
<td>9</td>
</tr>
<tr>
<td>Beef stomach</td>
<td>6</td>
</tr>
<tr>
<td>Beef ovary</td>
<td>5</td>
</tr>
<tr>
<td>Beef spleen</td>
<td>4</td>
</tr>
<tr>
<td>Beef lung</td>
<td>3</td>
</tr>
<tr>
<td>Human uterus</td>
<td>trace</td>
</tr>
</tbody>
</table>
heart is about the richest with the notable exception in these tables of Pidgeon breast. Unfortunately no complete estimate has been recorded for human tissues owing no doubt to the difficulty in obtaining really fresh autopsy material. It seems reasonable to assume, however, that in the distribution of the cytochrome system, man will approximate to the rat and the rabbit. The only inference intended in this comparison is to indicate that the heart is richest.

All red muscles contain muscle haemoglobin and the cytochrome system; the relative proportions of each depending upon the nature of the work required. Human heart muscle provides an example where an abundance of each is necessary while voluntary muscles as a general rule require but little of the cytochrome system with the diaphragm and tongue as exceptions. (341). The non-muscular tissues contain cytochrome but no muscle haemoglobin. In small embryos the cytochrome oxidase content is minimal as a rule (1, 248, 262, 341, 369), and increases with age. For instance the chick embryo contains none for several days and it then increases with the adult quantity being reached about the twelfth day. (262).

Let us now review the action of carbon monoxide on these isolated tissues bearing in mind once again the hazards attending isolated tissue reactions. Various methods have been
employed in showing the inhibitory effect of carbon monoxide upon cellular respiration and some of these methods make greater use of analogy than others. Qualitative reactions can be accurately gauged spectroscopically, not only by the appearance of the compound of carbon monoxide with cytochrome oxidase but also by the absence of oxidation of cytochrome c. Quantitative surveys of the inhibitory effect have been determined largely by the decrease in oxygen consumption \((Q_02)\) of the tissue under consideration using the Warburg technique or some of its later developments. One of the original methods was by the estimation of lactic acid which increases as anaerobic conditions are approached. (102,345). This method, using the Pasteur reaction, is completely inaccurate as an indication of the decrease in the catalytic activity of cytochrome oxidase and will be fully dealt with in Chapter VIII. The use of the "Nadi" reagent is unreliable in as much as it can be oxidized by other substances such as copper. Spectroscopic observation and the oxygen uptake, therefore, will guide us in assessing the inhibitory effects of carbon monoxide.

Inhibition of yeast respiration by carbon monoxide was first shown by Warburg in 1926-1927 and at the same time he found that the inhibition was reversed by light. Keilin in 1929 carried this a step further and showed that the inhibitory
effect was brought about by the combination of carbon monoxide with cytochrome oxidase. At the present time it is generally accepted that carbon monoxide inhibits cellular respiration in isolated tissues by combining with cytochrome oxidase, thereby preventing the oxidation of reduced cytochrome c, and that this reaction is reversed by light. Many different tissues have been employed in showing this reaction since Keilin first extracted the complete cytochrome system from heart muscle. In fact the reaction has come to be used as a method of detecting the cytochrome system.

The affinity of carbon monoxide has been estimated to be less than that of oxygen for cytochrome oxidase but the ratio differs considerably with the various workers. Green (115) gives the affinity for oxygen as 5-9 times greater than for carbon monoxide. Approaching this from another angle let us observe the relative concentrations of carbon monoxide necessary to produce any degree of inhibition. Warburg (252) estimated the ratio of oxygen to carbon monoxide at 50% inactivation to be 7:30. Stotz, Altschul and Hogness (323) showed that one part of oxygen to four of carbon monoxide caused 55-65% inhibition, and that 87% carbon monoxide caused complete inhibition. Winzler (437), using bakers' yeast, estimated that the dissociation constant between cytochrome oxidase and carbon monoxide is 4.15 m.m.Hg of carbon monoxide and the "apparent oxygen dissociation constant" is 1.85 m.m.Hg of oxygen. It can safely
be assumed that the affinity of cytochrome oxidase for oxygen is greater than for carbon monoxide and that the ratio of these affinities is probably lower than that suggested by Green. These estimations refer to these gases in a gaseous state and when combined with the circulating haemoglobin the position is considerably altered. The affinities will remain the same but the partial pressures of the two gases combined with haemoglobin and available to the oxidase cannot be assessed on partial pressure of the gases in the air to which the animal is exposed. A hazard to be expected in assessing the affinities in the living animal has been indicated by Warburg (352, 353) and justified by Schmitt (294). Although the function of the oxidase is the same in all cells, its chemical properties may vary considerably in different types of cells; the difference being due probably to slightly different configuration of the haemin-like active centres.

We are well enough equipped now to approach the subject in the living animal. Oxygen and carbon monoxide are transported to the cytochrome system by their combination with the circulating haemoglobin so let us review the position at tissue level. The cytochrome system is unaffected by oxygen tension (252) but depends upon an adequate oxygen supply. In yeast suspensions the oxygen consumption rate is independent of the oxygen
pressure but this does not apply if there is bound oxygen in the form of oxyhaemoglobin. When bound oxygen is present there is an increase in the rate of oxygen consumption as the oxygen pressure falls (28). The oxygen supply to the tissues is decreased by the presence of carboxy-haemoglobin and the remaining oxygen is held more firmly by the haemoglobin (Haldane effect). Haldane (126) claimed that since the amount of carbon monoxide needed to produce a given degree of inhibition depends upon the oxygen pressure it follows that both combine with the respiratory ferment in the same way. Cytochrome oxidase cannot be assumed, however, to act in a precisely similar manner to haemoglobin and it is purely catalytic so far as the oxygen supply to the tissues is concerned. Any degree of combination with carbon monoxide will further deplete the already scanty oxygen supply to the tissues in carbon monoxide anoxia.

Haldane's experiments (126) were conducted on different types of organisms. Using moths devoid of haemoglobin he found that their movements were inhibited by carbon monoxide even when they had an ample sufficiency of oxygen. Similarly with cress seed he found that germination was inhibited by carbon monoxide. He indicated the probability that the cells of the moth contain several catalysts with different affinities for carbon monoxide and oxygen, only one, however, being the limiting
factor to oxidation above a partial pressure of carbon monoxide. This was borne out by the different effects of carbon monoxide on moths and cress. In 80% carbon monoxide the moth needed 7-8 times as much oxygen as when the oxygen is diluted with nitrogen, and the cress seed only 2½ times. The actual oxygen pressures in the tissues during the experiments were of course unknown while those of carbon monoxide must have been the same as in the gas mixture. With rats the issue was complicated by haemoglobin but even when almost all the haemoglobin was combined with carbon monoxide (98.3%) there was sufficient oxygen dissolved in their blood to maintain normal life with occasional clumsiness of the hind legs. In all cases the addition of more carbon monoxide caused hyperpnoea, convulsions and death. Haldane concluded that cells contain a catalyst of oxidation which is poisoned by carbon monoxide; the affinities of the catalyst differing in different species and perhaps in different tissues.

Experiments conducted along similar lines showed revival of whole animals by illumination. Fleischmann (95) observed that translucent young Tenebrio larvae became immobilized in the dark in a mixture of 80% carbon monoxide and air. Mobility was restored by illumination with strong light and the experiment could be repeated up to six times with the same animals.
Table 12 (a) and (b).
### Table 12.

(a) Oxygen uptake of sliced ox brain:—
(Glucose substrate)
(Ref. No. 82.)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Oxygen Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>1700</td>
</tr>
<tr>
<td>Cerebellar cortex</td>
<td>2550</td>
</tr>
<tr>
<td>Corpus striatum</td>
<td>1980</td>
</tr>
<tr>
<td>Cornu ammonis</td>
<td>1260</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1170</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>360</td>
</tr>
</tbody>
</table>

(b) Oxygen uptake of minced dog brain:—
(Ref. No. 151.)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Oxygen Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>116</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>136</td>
</tr>
<tr>
<td>Midbrain</td>
<td>92</td>
</tr>
<tr>
<td>Medulla</td>
<td>69</td>
</tr>
<tr>
<td>Thalamus</td>
<td>101</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>107</td>
</tr>
<tr>
<td>Spinal cord.</td>
<td>50</td>
</tr>
</tbody>
</table>
Dealing first with the central nervous system we find it well supplied with the cytochrome system (253), and for the cat the cytochrome oxidase activity has been recorded for the different parts in the ratios, cortex: medulla: spinal cord = 100: 34:12 (63); and grey matter: white matter: nerve = 26.2:43:1 (154). Grey matter generally has the highest oxygen uptake and the greatest amount of cytochrome oxidase but this differs considerably as shown in Table 12(a) & (b).

Attention has already been drawn in Chapter IV to the minimal cytochrome oxidase content of the globus pallidus compared with other situations of grey matter and the characteristic necrosis of this tissue found in death from carbon monoxide anoxia. It is not concluded from this that combination of the oxidase with carbon monoxide is the sole factor involved, but it is noteworthy that other substances which are definitely known to produce inhibition of tissue respiration produce similar lesions. These include cyanide and sulphide which inhibit the cytochrome system and barbiturates with their effect upon the negative dehydrogenase element. We have seen that the blood supply to the globus pallidus is very scanty normally and that in carbon monoxide anoxia this is made more precarious by stagnation. Decrease in oxygen pressure, according to Baumberger (28) is followed by an increase in the consumption rate or Qo2. Others, however, have observed no such
change with diminished oxygen tension. (90, 342). Nevertheless, what little oxygen remains available in the haemoglobin will be utilized in a comparatively short time, and since the circulation rate at this level is slowed down the carbon monoxide will diffuse from the blood into the tissues. Under similar conditions the same process would occur in all tissues were it not for the fact that the blood supply and circulation rate elsewhere are maintained at a higher level. Campbell (43) has shown that carbon monoxide does in fact diffuse out of the blood stream into the tissues until equilibrium is reached, and then it is attracted back into the circulation and carried back to the lungs. Damage to the globus pallidus in subjects chronically exposed to carbon monoxide of such small concentrations to exclude deficiency of oxygen alone can perhaps be ascribed to the inhibition of cytochrome oxidase in the way described. Anoxic anoxia if severe and prolonged enough without causing death should show a similar selection of effect upon the globus pallidus due purely to insufficient oxygen being present to satisfy the needs of the cytochrome system. Sayers et alia (289) recorded such a result with dogs; the degree of necrosis being less than that produced by carbon monoxide while life was still retained. This has been shown also by estimating the oxygen uptake of various tissues in vitro after subjecting the living animal to acute anoxic anoxia. Cardiac
muscle was decreased by 35% while a less marked decrease was observed with liver, cerebral cortex and kidney. (62,100,101,282). The further effect of carbon monoxide has been shown with sliced rat tissues.(69). The tissues were exposed to anoxic anoxia (5% oxygen in nitrogen) and carbon monoxide anoxia (5% oxygen in carbon monoxide). The oxygen uptake was recorded and the carbon monoxide mixture produced greater inhibition as follows:- testicle 42.8%, kidney 33.8%, spleen 33%, and cerebral grey matter 21.7%.

Haggard (1922) using chick embryo nerve cells showed no inhibitory influence by carbon monoxide. This work has been used repeatedly in the argument against any histotoxic properties possessed by carbon monoxide. The result of this work proves nothing, however, because the chick embryo as we have recorded contains no cytochrome oxidase for several days.

It is not suggested that the only site of the inhibiting effect on respiration in the brain is located in the globus pallidus. All grey matter particularly would be affected in a similar way but perhaps not to the same degree. There is evidence that the respiratory and vaso-motor centres are depressed by anoxia from some cause or other but not by any reduction in oxygen tension to which these centres are not susceptible.
In Kinnear Wilson's disease (hepato-lenticular degeneration) the globus pallidus is frequently the site of a necrotic lesion. The disease is apparently due primarily to an upset in copper metabolism. Glazebrook (109) in recording two cases suggests that pallidal necrosis may be due to inhibition of enzymatic activity by the upset in copper metabolism. It is interesting that copper has been found to be essential for the regeneration and activity of elements of the cytochrome system. (54, 298, 299, 322).

The respiration of isolated medullated nerve is inhibited by carbon monoxide (294, 295). This was partially, and in some cases completely, reversed by illumination indicating the combination of the gas with a substance similar to cytochrome oxidase. The effect upon the oxidase in isolated frog nerve was observed by using different gas mixtures. (51). 97% carbon monoxide in oxygen inhibited respiration while 97-98% nitrogen in oxygen had no effect.

Angina pectoris as a symptom and myocardial necrosis as an autopsy finding following severe and prolonged exposure to carbon monoxide have been discussed. We have observed also that angina and cardiac arrhythmia have persisted long after recovery, and that chronic exposure to small concentrations, insufficient in themselves to produce any anoxia through combination with blood haemoglobin, is sometimes followed by microscopic
necrotic foci in individual muscle fibres. We also know that heart muscle is rich in muscle haemoglobin and the cytochrome system. It is suggested that these clinical and pathological findings are possibly manifestations of the inhibiting effect of carbon monoxide upon the cytochrome system through combination with cytochrome oxidase.

Fisher and Irving (94) have proved this to be so in the case of the embryo of the speckled trout. Their subject material consisted of a complete physiological system in which the heart was easily visible and the frequency of its beat practically regular. 90% carbon monoxide in oxygen produced gradual reduction in frequency until a period of depression was reached - 50% of the original. Thereafter there was either a gradual decline further or the rate was maintained at the low level. The inhibition was removed by light promptly and in some cases completely, while dim light or darkness restored the inhibition promptly. The light effect could be repeated many times. The speed of onset of the inhibition by carbon monoxide resembled the slow development of inhibition by cyanide and reduced oxygen tension but the effect of light and dark was contrast by their immediate action.

The decrease in fertility in animals subjected to carbon monoxide anoxia has been referred to in Chapter III and it was indicated that quantitative reduction and non-motility of
spermatozoa had been observed and offered as an explanation. Spermatozoa contain the cytochrome system (196,225,372,373), and the content of the various portions bears a direct relationship to their activity as follows:

head : mid-piece : tail = 1.2 : 14.4 : 29.1 while the complete sperm shows 25. It may be speculated that inhibition of respiration of the spermatozoa may be responsible for infertility.

Almost all tissues of the body other than those already mentioned and including liver, spleen, kidney, testicle and bone marrow have been subjected to carbon monoxide by the tissue slice technique; and in each case there has been inhibition of respiration. (69,328,357,358).

Drinker (85) quotes the advocates of methylene blue by injection as a therapeutic measure in carbon monoxide anoxia. He states that the rationale of giving methylene blue is to substitute a catalyst for the cytochrome oxidase, but since he doesn't believe that cytochrome oxidase is affected in carbon monoxide anoxia it is not surprising that he disapproves of its use. The actions of methylene blue are obviously worth investigating for all that, and if any beneficial effect is to be gained by its administration it is a further piece of evidence in favour of the inhibitory effect of carbon monoxide on the cytochrome system.

Methylene blue is a dye which can be reversibly
oxidized and reduced and acts as a catalyst for some oxidative processes taking place in the living cell. (262). Warburg et alia (350) believed its catalytic effect responsible for the oxidizing of bivalent Fe to trivalent Fe, but it has since been held responsible for the oxidation products of carbohydrate metabolism, (21, 26, 69). Using tissue slices and measuring oxygen consumption it has been observed that with testicle, kidney, spleen and cerebral grey matter, methylene blue had no effect unless respiration was first inhibited by cyanide or carbon monoxide, and then there was an increase (69). Brooks (38) conducted direct experiments on 80 rats and found that when made unconscious by inhalation of gaseous cyanide or carbon monoxide, their rate of recovery was considerably accelerated by intraperitoneal injections of methylene blue. Recovery in the case of cyanide required 36% and in that of carbon monoxide only 57% of the time required for recovery of the corresponding controls. It cannot be claimed that this effect was due to substitution for the inhibited cytochrome oxidase but the beneficial effect of the therapy seems unquestionable.

The transfusion of fresh blood seems an obvious form of therapy in carbon monoxide anoxia. Drinker (85) indicates that it is of no benefit and is contra-indicated as a further tax upon a failing circulation. This could perhaps
be overcome to some extent by the initial withdrawal of carboxy-blood before or at the same time as transfusion of fresh blood; or could it be that carbon monoxide had already combined with extracirculatory substances so that replacement by fresh blood could not overcome cellular anoxia at once?
CHAPTER VIII

THE "PASTEUR EFFECT".

Pasteur discovered that many cells form lactic acid in the absence of oxygen (184), and the "Pasteur effect" came to be defined as the action of oxygen in diminishing carbohydrate destruction and in suppressing or decreasing the accumulation of products of anaerobic metabolism (79).

Organisms and tissues may be classified broadly into three different types: strict aerobes equipped only with respiratory metabolic systems; strict anaerobes equipped only with anaerobic fermentative metabolic systems, and facultative organisms equipped with both respiratory and fermentative systems. Most doubly equipped organisms possess in the "Pasteur effect" a regulatory device that enables them to use as occasion demands, either their aerobic or their anaerobic systems. By the operation of this effect their fermentative apparatus is blocked in the presence of sufficient oxygen, and energy is furnished almost exclusively by the far more efficient and powerful respiratory apparatus. When oxygen is lacking, however, the fermentation system is brought into operation. (212).

Lipmann (212) offers the following analogy to illustrate the energetic structure of a facultative anaerobe. A power plant uses as a source of energy
cheap water power; this may be compared to the "cheap" respiratory energy. But because of seasonal variations of the flow of water, power may not be entirely reliable and hence, as a safeguard against a deficiency in the supply of power, a more expensively operating steam engine is built into the plant; this may be compared to "expensive" fermentation. For obvious reasons the plant will be equipped with a switch mechanism - the "Pasteur effect" - which keeps the steam engine from functioning so long as the water flow supplies sufficient energy, but throws it into operation when water power is lacking.

Lipmann concludes theoretically that \( \frac{1}{12} \) to \( \frac{1}{9} \) of the total possible energy is made available to the cell by the anaerobic fermentation of the glucose molecule. This indicates that to draw the same energy from fermentation as from respiration the cell must use 9-12 times as much substrate. In reality the anaerobic energy is rarely equal to the aerobic. As a rule a fully developed facultative anaerobe uses aerobically only 4-8 times as much substrate as aerobically, this reaching on the average half the energy level of the aerobic state. From these considerations the economical and regulatory aspect of the "Pasteur effect" becomes evident.

Since Pasteur's initial discovery, two
experimental facts have become known. Firstly, a great number of cells (most normal animal cells) show respiration and no lactic acid formation when examined in air or in oxygen; and secondly, that in total absence of oxygen, lactic acid is formed in large amounts. These two facts have led to the conclusion that the occurrence of lactic acid formation is caused by a decrease in respiration, the assumption being that respiration and lactic acid formation are reactions coupled in such a way that whenever respiration decreases, the cell tries to compensate by an increase in lactic acid formation for the amount of energy lost through the inhibition of respiration. The quantitative relation between the oxygen consumed and the lactic acid formed appears to be one molecule of oxygen causing one or two molecules of lactic acid to disappear. (184). The effect of cyanide and carbon monoxide on numerous cells seemed to support this interpretation (346, 348); for both in cyanide and carbon monoxide an increase in the splitting process was always found together with the inhibition of respiration.

It was claimed in the preceding chapter that estimation of lactic acid formation was totally inaccurate as an assessment of the degree of inhibition of cytochrome oxidase. The reason for this inaccuracy can be appreciated from the
following experiment which serves as a typical example of the "Pasteur effect". Geese erythrocytes were examined at 5 vols.% carbon dioxide with 20 vols.% oxygen in cyanide and an inhibition of respiration of 62% was observed; the lactic acid formation, which at the same oxygen tension in the absence of cyanide was zero, rose to 71% of the maximal anaerobic fermentation. If, however, the same cells were examined in the absence of cyanide and respiration was decreased by exposing the cells to 6 vols.% oxygen instead of air, then in spite of a 70% inhibition of respiration, no lactic acid formation appeared. Similar results have been recorded for brain tissue. Kempner (184) concludes from this that, if in cyanide as well as at an oxygen tension of 6 vols.%, one finds an inhibition of respiration of 60-70% but increased production of lactic acid only in the case of cyanide, one must discard inhibition of respiration as a determining factor in interpreting the mechanism of the "Pasteur effect" and also that of the effect of cyanide. It then follows that the cause of the lactic acid fermentation which occurs in complete absence of oxygen is not that the cells no longer respire and therefore obtain their energy in another way, but that the lactic acid fermentation catalyst itself is directly or indirectly affected by the absence of oxygen; or vice versa, that the disappearance or non-appearance of lactic acid
fermentation in the presence of oxygen is not due to the hypothetical resynthesis of one or two molecules of lactic acid through one molecule of oxygen consumed in respiration, but to the reaction of oxygen with the lactic acid fermentation enzyme, regardless of whether the oxygen is transferred by one of the catalysts of respiration or by some other oxygen carrier in the cell. A reciprocal relation between the oxygen affinity of the lactic acid fermentation enzyme and the oxygen affinity of the respiratory enzyme does not exist.

On the other hand, if the inhibition of respiration through cyanide must be discarded as the cause of lactic acid formation in cyanide/air, then lactic acid formation in cyanide can only be explained by an effect of cyanide on the catalytic system of the lactic acid fermentation itself, occurring simultaneously with the inhibiting effect on the catalytic system of respiration. The inhibitory effect of cyanide on respiration is due to a reaction of cyanide with the iron of one of the respiratory catalysts so that the combination of the catalyst with oxygen is prevented. The effect of cyanide on lactic acid fermentation can be explained in the same way. Assuming that the lactic acid fermentation is inhibited under aerobic conditions by the reaction of one of the lactic acid fermentation catalysts with oxygen, the effect of
Figures 13 and 14.
Figure 13.

<table>
<thead>
<tr>
<th>Cytochrome oxidase-CO spectrum:</th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>Ref. Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat heart muscle:</td>
<td>450</td>
<td>510</td>
<td>589</td>
<td>241,314.</td>
</tr>
<tr>
<td>Pasteur enzyme-CO spectrum:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat retina:</td>
<td>450</td>
<td>515</td>
<td>578</td>
<td>316.</td>
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Figure 14.

(Ref. No. 315.)

\[
\text{OXYGEN} \rightarrow \text{CYTOCHROME OXIDASE} \rightarrow \text{CYTOCHROMES} \rightarrow \text{SUBSTRATES}.
\]

\[
\text{PASTEUR ENZYME} \rightarrow ? \rightarrow \text{FERMENTATION SUBSTRATE}.
\]
cyanide on cellular metabolism would be due to the fact that not only the activity of the respiratory catalyst, but at the same time the activity of the fermentation catalyst is prevented.

Correspondingly the effect of other substances which change the reactions of cellular metabolism can be explained. For instance, carbon monoxide which inhibits respiration and increases lactic acid formation would, like cyanide, act by displacing the oxygen from the respiratory system as well as from the enzyme system of lactic acid fermentation.(184).

Let us now consider the lactic acid fermentation catalyst, or more simply termed the Pasteur enzyme. Its reversible combination with carbon monoxide has been proved (40,242,315,316) and the spectrum of the combined complex is similar but not identical to the spectrum of the cytochrome oxidase compound with carbon monoxide.(241,316). Figure 13. The carbon monoxide compound is light sensitive in both cases (199).

We are dealing, then, with a heavy metal catalyst; in fact a haemin protein like cytochrome oxidase.(40). These two intracellular haemin proteins are endowed with the power to react directly with molecular oxygen. That is, oxygen is "mobilized" for its two chief tasks in aerobic cell life by two autoxidizable haemin proteins acting in an
analogous manner but independently of each other.

Figure 14. The effect of a variation of the oxygen tension on the overall phenomena of cellular respiration and aerobic fermentation will depend upon the affinity which the iron contained in the two types of enzymes has for oxygen and carbon monoxide (315).

Carbon monoxide and oxygen compete for the ferrous form of the Pasteur enzyme and the ferric form inhibits fermentation. The distribution of the iron of the enzyme between the two gases is determined by the ratio of their partial pressures and by their affinity to the metal in its particular linkage. (316). If these gases do not vary in their effect on the two processes it must be because the affinity of the enzymes for the gases is equal. On the other hand the magnitude of the "Pasteur effect" in a given cell will depend on the concentration of the Pasteur enzyme, provided that it operates under conditions in which it is saturated with respect to substrate.

As with the cytochrome system, so with the Pasteur enzyme, we find that physiological surroundings are essential before experimental results can be interpreted to apply in any way to conditions existing in the living animal. (10, 71, 78, 79, 359).

In general it would appear that in higher animal tissues the cytochrome oxidase has a greater affinity for oxygen and a lesser
affinity for carbon monoxide than the Pasteur enzyme in the same tissue (199, 200). In fact the Pasteur enzyme has a greater affinity for carbon monoxide than it has for oxygen so far as partial inhibition is concerned. A carbon monoxide:oxygen ratio of 8:5 is strongly in favour of complete inhibition (316).

The distribution of the Pasteur enzyme throughout animal tissues has not been fully assessed but at least it has been found in the following tissues:- brain (40,63,67,81,89,222,318,319), nerve (155), cardiac, smooth and skeletal muscle (305), liver (40,62), kidney, spleen and testicle (40), ileum and colon (73), erythrocytes (290) and retina (64,199,200,317). It has been found to be absent in jejunal mucosa (73) and bone-marrow (357,358), while the retina contains Pasteur enzyme but no cytochrome (198,199,200,316).

It is not proposed to delve deeply into the possibility of carbon monoxide inhibiting the Pasteur enzyme in the living animal, but suffice it to postulate that there is a further substance responsible for extra-circulatory combination and that from its greater affinity for carbon monoxide there is every reason to believe that it does play a part in the effects of carbon monoxide anoxia. These effects are difficult to postulate and segregate from other possible histotoxic effects, but at least one line of
approach which presents itself is the utilization of glucose. Glucose metabolism increases as the Pasteur enzyme becomes inhibited or as anaerobic conditions are approached. We have observed that the brain received a preferential blood supply apparently in an attempt to maintain an adequate supply of glucose as well as oxygen; but Dixon (80) found that with cyanide, rabbit cortex showed a 250% increase in utilization of glucose. Preferential blood supply could not possibly meet an increased demand of this dimension. McGinty (222) with dogs confirmed this and measured the blood flow. A typical result indicated a rise from 167 c.c. per minute to 324 c.c. within the first five minutes and then a fall to 112 c.c. per minute within the next fifteen minutes. Recovery took place. Carbon monoxide would probably give similar results but the increased blood flow would be comparatively short lived through failure of circulation. This failure of circulation will have the double effect of diminishing the supply of glucose to the brain and allowing lactic acid to accumulate. The oxygen uptake of certain parts of the brain (grey matter) is many times greater than others and the demand for glucose is correspondingly greater under normal conditions. If the distribution of Pasteur enzyme in the brain bears any relation-ship to the oxygen uptake the globus pallidus would be one of the first areas of grey matter to suffer
from lack of glucose to maintain its "expensive" energy level from fermentation. Damage could result from either diminished glucose or lactic acid accumulation; the further effect of the latter would be to cause further dilatation of cerebral vessels (365) with stagnation and a further diminution in the supply of glucose.

Anoxic anoxia as experienced at high altitude produces but little effect upon the blood lactic acid level; and after a period of time, sufficient presumably for acclimatization, sea level values are found. (36,338).
CHAPTER IX.

CARBONIC ANHYDRASE.

Henrigues in 1928 calculated that carbon dioxide is given off from blood more rapidly than the straightforward equation would suggest and suspected the presence of a catalyst. Van Slyke and Hawkins recognised the presence of such a catalyst in 1930 and in 1931 Brinkman and Margaria proved its presence in blood, and found its activity was completely abolished by cyanide. This led to further confirmation and the isolation of the catalyst, to which the name carbonic anhydrase has been given. (37,235–238,309).

Meldrum and Roughton first produced carbonic anhydrase in pure form (236) and its catalysis has been defined as hastening both reactions of the reversible process $\text{H}_2\text{CO}_3 \rightleftharpoons \text{H}_2\text{O} + \text{CO}_2$. In fact it hastens this reaction 5–7 times. It is a zinc-protein substance containing at least 0.3% zinc and probably 0.33% (157,182); and 14.95% – 15.8% nitrogen (182,300) with a molecular weight of 30,000. It is stable at pH ranges from 6–10 (236). Its action is inhibited by cyanide, sulphide, azide, heavy metals and by carbon monoxide (236,239).

The relative distribution in the blood of man, rabbit and rat has been estimated at 0.55:1.21:1.7
and all showed a content abundantly in excess of minimum physiological requirements (239). Carbonic anhydrase is also present in nervous tissue (7,8,9) and in gastric mucosa (66,182). The cerebrum apparently contains a greater quantity than the spinal cord and the possibility of a functional relationship between the content of carbonic anhydrase and "functional levels" of the central nervous system is pointed out. There is a distinct relationship in parts of the brain to the rate of metabolism and it is thought that this relationship may be as a result of metabolism (7). In other words, if carbonic anhydrase in the tissues is absorbed from the red blood cells then the tissue with the best blood supply will have the highest carbonic anhydrase content. In this connection Hodgson found that the absolute amount of carbonic anhydrase is decreased in anaemia and increased in cyanosis (152). Meldrum and Roughton (239) investigating other tissues estimated that the content appeared to be proportional to the tissue haemoglobin. The most tenable theory as to the function of the enzyme in the tissues is that it facilitates the removal of carbon dioxide from the tissues (9).

We have seen that carbon monoxide causes inhibition which is light sensitive to some extent. The affinity for carbon monoxide is apparently not great but there is no competition with any other
gas as with the haemin ferments. Instead, the presence of carbon monoxide in air or oxygen will apparently inhibit the effect of carbonic anhydrase irrespective of its partial pressure in relation to oxygen. The evidence is far too scanty to make any drastic conclusions as to the effects of carbon monoxide or carbonic anhydrase in the living animal but at least we can estimate the possibilities.

Let us suppose for a moment that the carbonic anhydrase activity is inhibited by carbon monoxide in vivo and observe what effects we might expect. This would apply to the tissues as well as the blood and the whole process of elimination of carbon dioxide would be "stopped down" while the level in the blood would be largely maintained. This "stopping down" effect gives rise to other relevant possibilities. For instance we have observed that any increase in the arterial carbon dioxide tension hastens the elimination of carbon monoxide in a two-fold manner. Firstly by increasing pulmonary ventilation and secondly by hastening the dissociation of carboxyhaemoglobin (Figure 15). Any influence which diminishes the rate of the reversible process $\text{H}_2\text{CO}_3 \leftrightarrow \text{H}_2\text{O} + \text{CO}_2$ will possibly have an effect upon the ultimate elimination of carbon monoxide from the body.
Carboxy-haemoglobin dissociation curve in the absence of oxygen and at different pressures of carbon dioxide.
Figure 16.

Figure 17.
Figure 16. (From Haldane, Priestley. 1935.)

The influence of carbon dioxide on the oxygen dissociation curve.

Figure 17. (From Haldane 1912.)

The influence of carbon monoxide on the oxygen dissociation curve.
In a similar way, carbon dioxide causes a shift to the right in the oxygen dissociation curve (Figure 16) and it is interesting to compare these curves with those of oxygen dissociation when the blood is partially saturated with carbon monoxide, or in other words, compared with the "Haldane effect". (Figure 17). It is observed that the curves are the same shape and there is a shift to the left as the percentage saturation with carbon monoxide increases on the one hand, and a similar shift to the left as the partial pressure of carbon dioxide decreases on the other hand. It is postulated that inhibition of carbonic anhydrase by carbon monoxide may have some influence in producing the "Haldane effect."
CHAPTER X.

GENERAL SUMMARY.

This survey was prompted by the belief that the toxic effects of carbon monoxide were not due entirely to its combination with the circulating haemoglobin. An attempt has been made to convey that belief in a critical description of the clinical and pathological manifestations combined with a study of the after-effects, chronic exposure and acclimatization.

It was next necessary to review the gaseous combinations of the circulating haemoglobin and the influences affecting these combinations and dissociations. It can safely be assumed that all the effects of carbon monoxide anoxia are due primarily to its initial combination with the circulating haemoglobin; for any extracirculatory combinations which may take place are dependent upon the circulation of haemoglobin for the transportation of carbon monoxide.
It seems that the total quantity of carbon monoxide inhaled and combined with the circulating haemoglobin is excreted by the lungs and can be recovered in the expired air. From this it is concluded that carbon monoxide is not metabolized in any way by the body. It was further observed that 60-70% of the total was recovered within the first hour, and the remainder took several hours to be released, even when the process of elimination was speeded up by the inhalation of oxygen. Such a decrease in the rate of elimination would be expected if carbon monoxide diffused from the circulating haemoglobin to combine with substances in the tissues. Subsequently, as the carbon monoxide saturation of the circulating haemoglobin decreased the extra-circulatory combination would dissociate and the carbon monoxide would be attracted back into the circulation and carried to the lungs for "excretion."

A number of extra-circulatory substances which could combine with carbon monoxide in this way has been described. The "pseudo-haemoglobin" of Barkan has a far greater affinity for carbon monoxide than blood haemoglobin has, and evidence is presented showing that it probably does combine with carbon monoxide in the living animal.
Other possibilities include muscle haemoglobin, cytochrome oxidase and the Pasteur enzyme. The fullest possible knowledge of these substances has been given before attempting to show any relevant actions they may possess. They all combine with carbon monoxide in vitro and the combination is by competition with oxygen for saturation of the haem molecule with which they are constituted. Their affinities for carbon monoxide and oxygen differ but muscle haemoglobin and the "Pasteur enzyme" have a greater affinity for carbon monoxide than for oxygen, while the affinity of cytochrome oxidase for these gases is reversed. These affinities refer to in vitro experiments where the gases are available in gaseous form but in the living animal the gases are made available by their combination with the circulating haemoglobin. For instance, if cytochrome oxidase were exposed to an atmosphere containing about 0.1% of carbon monoxide, no combination would take place and the activity of the oxidase would be uninhibited; but if a human subject were exposed to such a concentration his blood in time would reach about 70% saturation and the cytochrome oxidase of his tissues would be subjected to an even greater relative concentration of carbon monoxide than the blood saturation would suggest.
No originality is claimed in postulating that muscle haemoglobin and cytochrome oxidase are affected in carbon monoxide anoxia. Combination with muscle haemoglobin has been accepted although its true significance has never been fully appreciated. This has been discussed not only in its quantitative relation to extra-circulatory combination but also its qualitative relation to function in acute anoxia and acclimatization. Some authors have recognised the possibility of inhibition of cytochrome oxidase but because of its greater affinity for oxygen in vitro the possibility was discarded. There is no evidence of a previously recorded attempt to associate inhibition of the Pasteur enzyme with the carbon monoxide problem and it is felt that the present attempt has been warranted.

A short survey of the properties of carbonic anhydrase has been given. In virtue of it being a zinc protein and not a haemin protein, its inhibition by carbon monoxide is subject to somewhat different conditions. There is no competition between oxygen and carbon monoxide, but the partial pressure necessary to cause inhibition is probably never great enough in the living animal subjected to carbon monoxide anoxia. Nevertheless, further experimental work is indicated before this possibility can be finally rejected.
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