Carbon monoxide, smoking and atherosclerosis

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THE toxic effect of carbon monoxide on the animal organism has probably been known by man since the discovery of fire, and we know it has been recognized as a dangerous poison in ancient times. It was Claude Bernard who first studied its mode of action, but it is J. S. Haldane who is considered as the real pioneering investigator of the physiology and toxicology of carbon monoxide. Together with some of his co-workers he performed the now classical studies on the effects on man of carbon monoxide exposure. His first paper appeared in 1895. Haldane considered, as a result of his investigations, that the only toxic effect of carbon monoxide was its ability to bind to haemoglobin at a much higher degree than oxygen, thus displacing oxygen in oxyhaemoglobin and depriving blood of its oxygen transport ability.

As long as this transport was not seriously impaired, carbon monoxide was regarded as relatively harmless. This was supported by the findings of many physiologists, showing that concentrations of up to 20% of carboxyhaemoglobin had little or no effect at rest on physiological parameters, such as heart rate, cardiac output, respiration, blood pressure, etc. This is just the opposite effect to that observed for moderate hypoxia, where the venous and tissue oxygen tensions are quite normal which is not the case, however, after moderate exposure to carbon monoxide, as was shown by Campbell in England in 1929. This lowering of the tissue oxygen tension is due to the lack of a cardio-respiratory compensating adjustment, and to the displacement of the oxyhaemoglobin dissociation curve to the left, first investigated by J. B. S. Haldane in 1912, and later in more detail by Roughton & Darling (1944) and by others. The displacement has never attained any physiological interest.

An important breakthrough in carbon monoxide physiology was made in 1951 by the Swede T. Sjöstrand, who discovered that carbon monoxide was formed continuously in the human body by the catabolism of haemoglobin. This explains the normal carboxyhaemoglobin concentration of about 0.5% in man, increasing to about 3% by increased haemolysis. This discovery added to the conception of

carbon monoxide as a relatively harmless gas as long as it did not interfere seriously with the oxygen transport of the blood.

During the last 10 years the interest in the physiological and pathological effects of moderate carbon monoxide exposure has increased considerably, mainly due to the concern about the risks of the growing air pollution, especially that due to car exhaust, which is the major source of the 250 million tons of global production of carbon monoxide per year. The gas is released to the air and since the background level does not increase, it is assumed that oxidation to carbon dioxide takes place in the upper atmosphere.

It should be stressed that non-smokers do not run the risk of getting significantly elevated carboxyhaemoglobin levels from car exhaust in the streets, which has been shown in many studies, and clearly demonstrated recently by the findings of carboxyhaemoglobin levels of only 1.4-3.0% in non-smoking taxi drivers in London (Jones *et al.*, 1972). This is in contrast to the much higher carboxyhaemoglobin levels, up to 20%, found in *inhaling* tobacco smokers. Also people exposed to carbon monoxide for hours or days in more or less closed compartments (garages, tunnels, mines, submarines, etc.) with carbon monoxide release may also obtain similar high or even higher carboxyhaemoglobin levels.

Now the question is: do carboxyhaemoglobin concentrations up to 20% exert measurable physiological or pathological effects? A few years ago the answer would have been no, but today it would undoubtedly be yes. The central nervous system seems to be influenced. This was shown in the forties by McFarland and his associates (1970) by demonstrating impaired discrimination of small differences in light intensity at 2 and 4% carboxyhaemoglobin respectively. Also various performance in tests, for instance the estimation of time intervals without having a clock and the duration of auditory signals, are found decreased by some investigators at carboxyhaemoglobin levels about 5% (Beard & Grandstaff, 1970).

Also the myocardium may be affected by small

carboxyhaemoglobin concentrations, since the utilization of available oxygen is very high at rest, and since the binding of carbon monoxide to myoglobin is higher than its binding to haemoglobin. Thus about 5–10% carboxyhaemoglobin concentrations give approximately 3 times higher carboxymyoglobin concentrations (Coburn, 1970) which, of course, to a considerable extent interferes with the oxygen transport function of myoglobin. It has been demonstrated that 5–10% carboxyhaemoglobin in man leads to a decreased coronary arteriovenous oxygen difference (Ayres *et al.*, 1970), and to an increased coronary blood flow. Similar carboxyhaemoglobin levels have been shown to intensify myocardial ischemia and to enhance development of arrhythmia during exercise in subjects older than 40 years (Knelson, 1972), indicating that limited capacity to increase blood flow due to coronary obliterations, increases the susceptibility of carbon monoxide exposure.

Severe damage of the myocardium has also been found by ultrastructural studies in rabbits exposed for 2 weeks to carbon monoxide, leading to a carboxyhaemoglobin concentration of approximately 18% (Kjeldsen *et al.*, 1972). The changes are very similar to changes following severe hypoxaemia, and include the formation of intercellular oedema, destruction of mitochondria, etc.

Our findings of high carboxyhaemoglobin levels in the blood of many heavy smokers, especially in smokers with peripheral arteriosclerosis, have led us to the hypothesis that it might be the carbon monoxide in the tobacco smoke, which is responsible for the much greater risk for smokers of developing arteriosclerosis in comparison to non-smokers.

Today I am going to give the main results of our experimental work which in our opinion definitely prove that carbon monoxide has a damaging effect on the arterial walls, leading to an increased permeability for various plasma components, to the formation of subendothelial oedema and to increased atheromatosis. The results indicate that the much higher risk for smokers of developing arterial disease in comparison to non-smokers is, at least mainly, due to the inhaled carbon monoxide in the tobacco smoke and not to nicotine. There is no evidence from animal experiments that nicotine has an atherogenic effect.

For our animal experiments airtight chambers were constructed in which rabbit cages could be placed, and through which various gas mixtures could be passed. Each of the chambers we use now can hold eighteen rabbit cages. The gas mixtures were made by mixing atmospheric air with carbon monoxide, oxygen, and nitrogen respectively. For the various series we have used different techniques, which I am not going to describe in detail. In the

very first experiment cholesterol fed rabbits were continuously exposed to 170 ppm carbon monoxide for 10 weeks giving carboxyhaemoglobin concentrations about 15%. This resulted in a cholesterol content of the aorta which was 2.5 times higher ($P < 0.001$) than in the control rabbits, which had not been exposed to carbon monoxide, but were also fed cholesterol (Astrup *et al.*, 1967). We have repeated the experiment several times and always found an enhancing effect of continuous carbon monoxide exposure on cholesterol accumulation, and this has now been confirmed in other laboratories, also by using primates (Birnstingl *et al.*, 1970; Webster *et al.*, 1970). By intermittent exposure of groups of eighteen rabbits each to carbon monoxide, 12 or 4 hr a day, we obtained respectively 3 and 5 times higher cholesterol accumulation than by continuous exposure as demonstrated in Fig. 1, suggesting a certain adaptation of continuous exposure.

In another series of experiments (Fig. 2) we exposed groups of eighteen rabbits to various degrees of hypoxia (16% and 10% oxygen respectively for 8 weeks) and found that the cholesterol concentration in the aortic walls from the experimental groups were 3–3.5 times higher than the control groups (Kjeldsen *et al.*, 1968). If, on the other hand, the animals were exposed to hyperoxia (Kjeldsen *et al.*, 1969) (28% and 25% of oxygen respectively) the accumulation of cholesterol in aorta decreased considerably in comparison to the control animals, breathing atmospheric air. The results were highly significant ($P < 0.01$).

We have concluded from these exposure studies that lipid accumulation in the arterial walls of cholesterol-fed rabbits is highly influenced by the composition of the air the animals breathe. The accumulation is increased by hypoxia and carbon monoxide, and decreased by hyperoxia.

Macroscopically as well as microscopically there was no qualitative difference between the lesions in animals exposed to carbon monoxide and the animals exposed to hypoxia. Macroscopically it was easy to distinguish between the aortas from exposed animals and from control animals by the number and size of plaques. Similarly, the microscopic changes were more pronounced in the exposed animals, characterized by a marked lipid accumulation in intima and subintima. Also, in animals not receiving cholesterol, it was possible by very moderate carbon monoxide exposure (9–10% carboxyhaemoglobin) to induce arterial lesions with a pronounced focal subendothelial oedema, indistinguishable from spontaneous arteriosclerosis (Wanstrup *et al.*, 1969). In the electron microscope the changes looked very dramatic (Kjeldsen *et al.*, 1972). Normally, the endothelial membrane in aorta from rabbits is arranged in folds with the cells attached to the base-

FIG. 1. Relative values of aortic cholesterol in cholesterol-fed rabbits exposed to 0.018% carbon monoxide for 24, 12, or 4 hr daily, or to atmospheric air for 10 weeks. Each experiment comprised eighteen rabbits.

FIG. 2. Relative values of aortic cholesterol in cholesterol-fed rabbits breathing air with varying oxygen content for 10 weeks. Each experiment comprises eighteen rabbits.

ment membrane in the internal elastic membranes beneath (Fig. 3). By using the scanning electron microscope the folds are seen to be very regular (Fig. 4), similar to the appearance described in men. After exposure of a rabbit to 180 ppm CO for only 4 hr, giving approximately 16–18% carboxyhaemoglobin, a pronounced change occurred, demonstrated first of all by a very high degree of oedema beneath the endothelial cells (Fig. 5), pushing the cells away from the basement membrane, to which

they are attached by the remains of thin and occasionally thicker sections of cellular material surrounding the liquid, which has intruded between the cell and the membrane to which it was originally attached. The oedema is dominating the picture also beneath the basement membrane, where it fills the space up to the first layers of the elastic membrane. The scanning pictures (Figs. 6 and 7) show that the regular folding disappears with the occurrence of the blisters beneath the cells. In some areas the endothelial cells were separated completely from the basement membrane by the intruding fluid and a plaque was formed (Fig. 8), where the folds had disappeared completely and the endothelial junctions were open. In other areas the cells were disrupted. Where the endothelium was very severely damaged, tiny haemorrhages could be seen and loose aggregations of erythrocytes and thrombocytes were a regular finding at these sites. Similar, but somewhat less pronounced changes, were seen in aortas from rabbits exposed for 2 weeks to atmospheric air with 5% nitrogen, giving an oxygen percentage of 16, corresponding to oxygen tensions occurring at an altitude of about 3,500 m (Kjeldsen, 1972). After this we had no doubt that exposure of rabbits to carbon monoxide as well as to hypoxia could result in the development of severe vascular lesions, which could not be distinguished from spontaneous arteriosclerosis in these animals. Furthermore, cholesterol feeding under these conditions led to a very considerable increase of lipid accumulation.

The problem we now faced was the pathophysiological explanation of the experimental findings and

FIG. 3. Luminal part of thoracic aorta from normal rabbit. (Primary magnification $\times 3300$.)

the relation to the pathogenetic mechanisms in the development of arteriosclerosis in man. The findings that rabbits exposed to carbon monoxide quite often had fluid with a high protein content, 3–4%, in the serous cavities, i.e. pleura, pericardium, and peritoneum, led to the hypothesis that the arterial injuries were caused by increased permeability of the endothelial membranes, leading to subendothelial oedema as demonstrated by the microscopic and electron microscopic findings. To evaluate this hypothesis in 1967 we made a comparison between changes in some physiological and biochemical parameters measured in human individuals during exposure to carbon monoxide for 10 days and later on during exposure to hypoxia over a period of 10 days at the high altitude laboratory at Jungfraujoch in Switzerland, 3,454 m above sea level (Astrup & Pauli, 1968). It was demonstrated, that carbon monoxide exposure (20–25% carboxyhaemoglobin) led to a 50% increase of glomerular filtration rate during the first day of exposure (Pauli *et al.*, 1968); to an increased disappearance rate from the blood of injected radionated serum I^{131} albumin (Siggaard-Andersen *et al.*, 1968), and probably to an increase

in capillary filtration rate measured plethysmographically on the calf (Siggaard-Andersen *et al.*, 1967). This supported the hypothesis of an increased vascular permeability due to carbon monoxide. The transvascular protein flux during carbon monoxide exposure has recently been studied in more detail in my department (Parving, 1972; Parving *et al.*, 1972) by measuring in human individuals the disappearance rate of I^{131} albumin injected intravenously, and by following the protein flux in lymph in dogs. It was confirmed that the disappearance rate of I^{131} albumin, after exposure to carbon monoxide (20–25% COHb) for 3 hr, is increased, on average 50%.

The occurrence of increased permeability during exposure to carbon monoxide could also be demonstrated in dogs, where the lymph flow and the protein flux in the thoracic duct increased considerably. It was of interest that the increase in protein flux was relatively more marked for the high molecular proteins than for the low molecular ones (Parving *et al.*, 1972).

When discussing these results, it should be emphasized that proteins and other macromolecules penetrate the vascular wall under normal conditions

FIG. 4. Surface of the distal part of thoracic aorta from normal rabbit showing regular endothelial folds arranged longitudinally. (Primary magnification $\times 1100$.)

and are transported back to the blood through the lymph. This normal transport through the walls of the total vascular system is quite substantial. For example, in the normal resting man the disappearance rate of I^{131} albumin is about 5–6% per hr, corresponding to the penetration of the total plasma albumin pool through the vascular walls over 16–20 hr. This means that the transport probably cannot be looked upon as being due to accidental leakage through the endothelial membranes. In my opinion the results from our hypoxic/hyperoxic rabbit studies reported here support this point of view, thus indicating that the transport is influenced by the oxygen tension of the blood. Carbon monoxide and oxygen obviously act competitively in the mechanisms controlling the transport, which probably is influenced also in other ways, which I am not going to discuss here.

The results mentioned are consistent with the few, quite old, experimental studies along similar lines reported in the literature. Vascular leakage also occurs following hypoxia, as known from many high altitude expeditions, especially from studies (Singh *et al.*, 1969) during the Chinese-Indian war some

years ago. A considerable number of the Indian soldiers developed acute mountain sickness, often with serious clinical symptoms due to cerebral and pulmonary edema. The pathogenesis seems to involve increased permeability of not only the endothelial but also other cellular membranes. It is well known that men and animals in profound shock have severe acute protein leakage of the vascular system, which at least in some cases is due to the hypoxaemic condition. It is very likely that other pathological conditions related to acute hypoxia, as for instance respiratory distress in new-borns, may have a similar pathogenesis. In some diseases without arterial hypoxaemia, increased transvascular protein permeability has also been found, which I am not going to discuss in detail today, for instance in idiopathic oedema, diabetes, hypertension, in certain allergic conditions, in various inflammatory processes, etc. In my opinion, much more research should be carried out concerning the physiology and especially the pathophysiology of this transvascular protein transport, and it should include the underlying biochemical mechanisms of the systems controlling the permeability. This might lead to a deeper under-

FIG. 5. Luminal part of distal thoracic aorta from carbon monoxide exposed rabbit. Note the protruding endothelial cells and subendothelial blisters separating the endothelial cells from the basement membrane. Note also the considerable widening of the oedematous subendothelial space. (Primary magnification $\times 26,000$.)

standing of some fundamental biological processes and could give a new pathogenetic approach to some diseases.

Let us now look upon the relation of the development of atherosclerosis to a more or less chronically increased vascular permeability. I am not in any way going to discuss in details the pathogenesis of atherosclerosis, but I should like to stress, that one of the most widely-accepted theories concerning the mechanisms involved is the so-called filtration theory. It says, that plasma components filter into the arterial wall from the luminal side, especially when endothelial injuries occur, and under certain circumstances accumulate here forming plaques, with or without the occurrence of lipids. This theory is supported by clinical and experimental findings; and particularly by the observation that most of the lipids accumulated in atheromata are derived from the plasma lipids. The lipoproteins filter into the wall, where under unfavourable conditions the lipids may be released and be deposited. It is quite obvious that the filtration of lipids through the vascular wall will increase when the lipid concentration in plasma increases. Thus the probability of deposition of lipid will increase. The clinical significance of this may be illustrated for instance by the almost linear correla-

tion between serum cholesterol values in middle-aged men and their risk of developing obliterating arterial diseases.

The filtration furthermore depends on the filtration pressure, and here also the clinical association is well known: the risk of obliterating arterial diseases increases with increasing blood pressure within as well as above the normal range.

A third factor of importance for the lipoprotein transport into the arterial wall is permeability. With greater permeability there would be greater filtration. A clinical relation here is the increase of atheromatosis in patients treated with X-rays for cancer. The lesions are localized only in the irradiated areas, where the permeability of the arteries is increased due to the irradiation. Another clinical association seems to be the much higher incidence of obliterating arterial disease in smokers, in comparison to non-smokers, which is in our opinion due to the inhaled carbon monoxide. It has actually been possible to demonstrate (Kjeldsen, 1969) a correlation between carboxyhaemoglobin levels in smokers and the incidence of atherosclerotic disease. This is illustrated in Table 1. The individuals with atherosclerosis (early coronary thrombosis, angina pectoris or peripheral arterial obliterations) have significantly

FIG. 6. Surface of the distal part of thoracic aorta from carbon monoxide exposed rabbit. Note swollen and irregular endothelial folds. Width of endothelial folds is about twice that of controls. (Primary magnification $\times 1130$.)

TABLE 1. Average carboxyhaemoglobin values in atherosclerotic and non-atherosclerotic smokers chosen at random.

Age groups	Atherosclerotic smokers		Non-atherosclerotic smokers	
	Number	COHb%	Number	COHb%
10-19	0	—	24	2.8
20-29	0	—	127	4.2
30-39	6	11.0	192	4.6
40-49	15	6.7	210	4.6
50-59	24	7.4	125	3.7
<60	12	4.5	61	2.9

higher average carboxyhaemoglobin concentrations than smokers without arterial disease. This could, of course, be due to components in the smoke other than carbon monoxide, but since the carboxyhaemoglobin concentrations required to produce severe arterial lesions in rabbits are similar to those found in heavy smokers and since nicotine has only a very moderate effect, if any, on experimentally produced atherosclerosis, I feel justified in concluding that carbon monoxide is the atherogenic agent in the smoke.

An increased inflow of plasma components through the endothelial membrane would probably be of minor importance if the outflow could increase correspondingly. Here, however, difficulties arise. The intima has no lymph vessels, no vasa vasorum, so diffusion and filtration into the medial and external layers of the arterial wall is the only way of eliminating an increased inflow. Mechanical forces might help here, and it has been suggested that the milking effect on the arterial walls by the pulse waves is of importance. A beneficial effect of physical exercise on the development of atheromatosis may, at least partly, be explained in this way.

In my opinion the physico-mechanical concept presented here contains very much of the truth about the pathogenesis of atherosclerosis. The concept does not deny, however, the existence and the importance of metabolic and cellular processes involved when subendothelial oedema and plaque formation occur, and when restoration takes place. Time does not allow me to go into details, but I would like to stress that also in this respect the oxygen supply to the intima seems to be of great importance. There might

FIG. 7. Surface of distal part of aortic arch from carbon monoxide exposed rabbit. Normal arrangement of endothelium in folds tends to disappear. Instead, surface structure exhibits a highly irregular picture with extreme swelling of endothelial cells. (Primary magnification $\times 1130$.)

be an increased formation of lipids during hypoxia, and the removal of already accumulated lipids seems especially to be influenced by the oxygen supply, since it is greatly enhanced by hyperoxia according to some recent results we have obtained. It might be expected that exposure to hypoxia or to carbon monoxide has an opposite effect.

I should like to emphasize that hypoxia of the vessel wall has for many years been considered to promote injuries and atherosclerosis (Hueper, 1944), supposed to occur locally when the blood flow may be turbulent.

I began my lecture by saying that small amounts of carboxyhaemoglobin in the blood have so far not been regarded as having a significantly harmful effect. I do hope that our experimental results have convinced you, that this assumption is wrong and that I am right when concluding as follows:

Even moderately elevated carboxyhaemoglobin levels maintained during months or years may lead to pathological changes in arterial walls and myocar-

dial tissue and may influence the foetal development. The effects are similar to those of hypoxia and lead in the arteries to an increased permeability of the endothelium membrane for macromolecules, to the development of subendothelial oedema and lipid accumulation, and to impaired removal of lipids accumulated here. Carbon monoxide does not only hasten the development of atherosclerosis, but it has also a damaging effect on the myocardium, aggravating the effects of coronary obliteration. The effect of carbon monoxide is caused by a competition with oxygen concerning not only the oxygen transport by haemoglobin and myoglobin, but also concerning the activities of enzymes able to bind carbon monoxide as well as oxygen.

We have furthermore concluded that in man intermittent exposure to carbon monoxide rather than to nicotine, due to tobacco smoking, may be considered the real cause of the much higher risk for smokers of developing arterial diseases in comparison to non-smokers.

FIG. 8. Surface of slightly raised aortic plaque from carbon monoxide exposed rabbit. Plaque is situated between arch and thoracic part. Endothelium covering plaque is quite smooth and shows a characteristic network of fibrin-like material, possibly depicting intercellular junctions. Plaque is surrounded by cobblestone-structured endothelium. (Primary magnification $\times 712$.)

We have been fascinated and challenged by our investigations, not only because of the interesting theoretical problems involved, but also because of their practical aspects concerning a disease, which today dominates the mortality statistics in this part of the world. Research in the coming years will show if our results provide pointers for new developments and new ideas of interest for the theory and practice of medicine.

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