PAPERS AND ORIGINALS

Some Physiological and Pathological Effects of Moderate Carbon Monoxide Exposure*

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Carbon monoxide is produced from technological and natural sources in an estimated global amount of at least 250 million tons a year. Fuel combustion by motor vehicles is the major source. 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.

Early Studies

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 was recognized as a dangerous poison in ancient times. It was Claude Bernard who first studied its mode of action, and he showed that blood treated with carbon monoxide was unable to bind oxygen. J. S. Haldane (1895) is considered as the 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, and his first paper appeared in 1895. Haldane thought, 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. This conclusion was derived from the fact that animals such as mice could live for days with their haemoglobin completely bound to carbon monoxide if the dissolved oxygen in plasma was increased sufficiently by placing the mice in a high pressure chamber.

As students we learnt that these and other classical carbon monoxide studies gave valuable information about normal physiological mechanisms concerning oxygen uptake by the blood and its release. Medical interest in carbon monoxide, however, was dominated by its toxic and dangerous effects when inhaled in such amounts that it blocked the transport of oxygen by haemoglobin. So long as this was not seriously impaired carbon monoxide was regarded as relatively harmless. Many physiologists had shown that concentrations of up to 20-30% of carboxyhaemoglobin had little or no effect on physiological parameters, such as heart rate, cardiac output, respiration, blood pressure, etc., at rest. This is just the opposite effect to that observed for hypoxia. While the venous and tissue oxygen tensions in moderate hypoxia are quite normal, however, this is not the case after exposure to moderate levels of carbon monoxide, as was shown by Campbell (1929-30) in England. This effect is due to the lack of a cardiorespiratory compensating adjustment, and to the displacement of the oxyhaemoglobin dissociation curve to the left. The displacement was first investigated by J. B. S. Haldane (1912) and later in more detail by Roughton and Darling (1944) and by others, but has never attained any physiological interest.

An important breakthrough in carbon monoxide physiology was made by the Swede T. Sjöstrand (1951), 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 so long as it did not interfere seriously with the oxygen transport of the blood.

Effects of Exposure

During the past 10 years the interest in the physiological and pathological effects of moderate carbon monoxide exposure has increased considerably, mainly owing to the concern about the risks of the growing air pollution, especially that due to car exhaust.

It should be stressed, however, that non-smokers do not run the risk of getting significantly raised 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

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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 first shown by McFarland and his associates about 40 years ago by demonstrating impaired discrimination of small differences in light intensity at 2 and 4% carboxyhaemoglobin (McFarland, 1970). Also various performances in tests—for instance, the estimation of time intervals without having a clock, and the duration of auditory signals—are found to be decreased by some investigators at carboxyhaemoglobin levels around 5% (Beard and Grandstaff, 1970).

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 about three times higher carboxymyoglobin concentrations (Coburn, 1970), which, of course, to a considerable extent interferes with the oxygen transport function of myoglobin. It has been shown that 5-10% carboxyhaemoglobin in man leads to an increased coronary blood flow and to a decreased coronary arteriovenous difference (Ayres *et al.*, 1970). Similar carboxyhaemoglobin levels have been shown to intensify myocardial ischaemia 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 two weeks to carbon monoxide, leading to a carboxyhaemoglobin concentration of about 18% (Kjeldsen *et al.*, 1972). The changes are are very similar to changes after severe hypoxaemia.

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.

Present Investigations

Today I am going to give the main results of our clinical and experimental work, which in our opinion definitely prove that carbon monoxide had 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.

For our animal experiments air-tight 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 18 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 are not described in detail. In the first experiment cholesterol-fed rabbits were continuously exposed to 0·017% carbon monoxide for 10 weeks giving carboxyhaemoglobin concentrations around 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 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 18 rabbits each to carbon monoxide for 12 or four hours a day, we obtained respectively three and five times higher cholesterol accumulation than by continuous exposure, as shown in Fig. 1.

In another series of experiments (Fig. 2) we exposed groups of 18 rabbits to various degrees of hypoxia (16 and 10% oxygen respectively for eight weeks) and found that the cholesterol concentration in the aortic walls from the experimental groups were three to three and a half 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% oxygen respectively) the accumulation of cholesterol in the 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.

Differentiation of Lesions

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 noticeable 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 basement membrane and

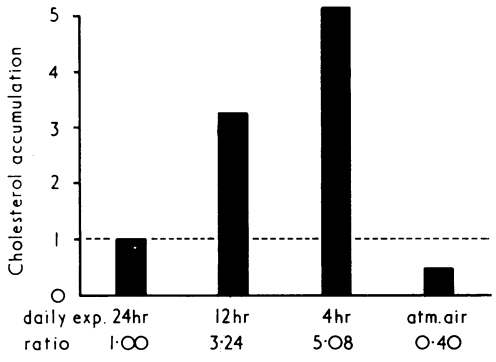

FIG. 1—Relative values of aortic cholesterol in cholesterol-fed rabbits exposed to 0·018% carbon monoxide for 24, 12, or 4 hours daily, or to atmospheric air for 10 weeks. Each experiment comprised 18 rabbits.

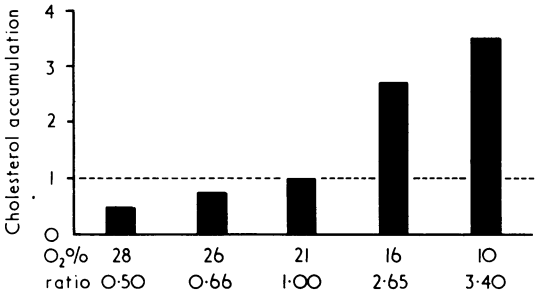

FIG. 2.—Relative values of aortic cholesterol in cholesterol-fed rabbits breathing air with varying oxygen content for 10 weeks. Each experiment comprised 18 rabbits.

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 0·018% of carbon monoxide for only two weeks, giving about 16-18% carboxyhaemoglobin, a pronounced change occurred. It was shown 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 two weeks to atmospheric air with 5% nitrogen, giving an oxygen percentage of 16, corresponding to oxygen tensions occurring at an altitude of around 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.

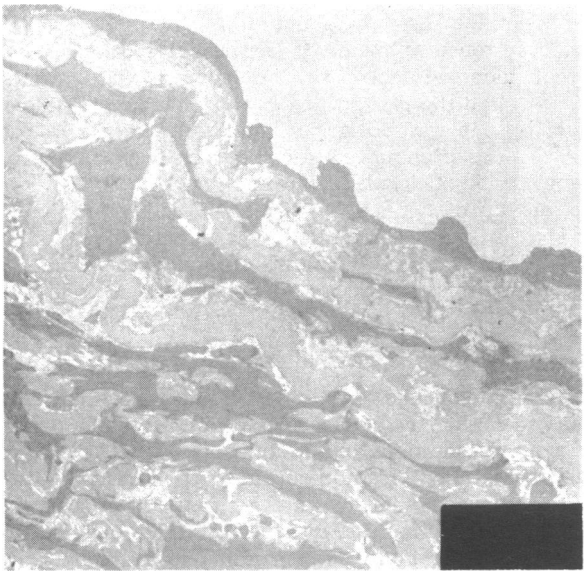

FIG. 3—Luminal part of thoracic aorta from normal rabbit. (Primary magnification ×3,300.)

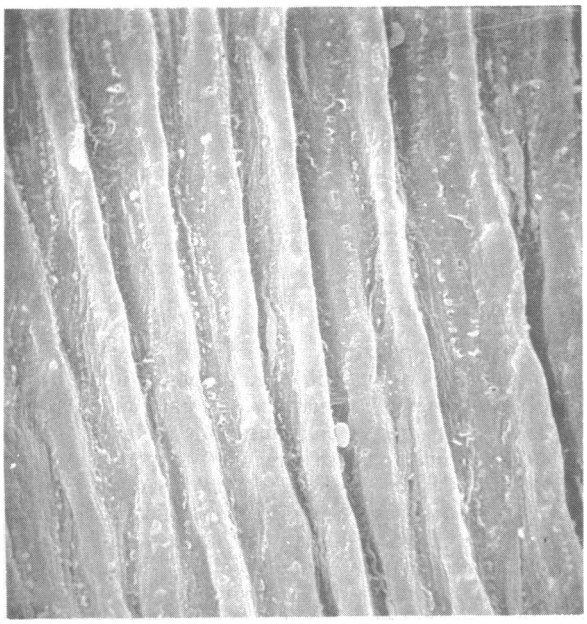

FIG. 4—Surface of the distal part of thoracic aorta from normal rabbit showing regular endothelial folds arranged longitudinally. (Primary magnification ×1,100.)

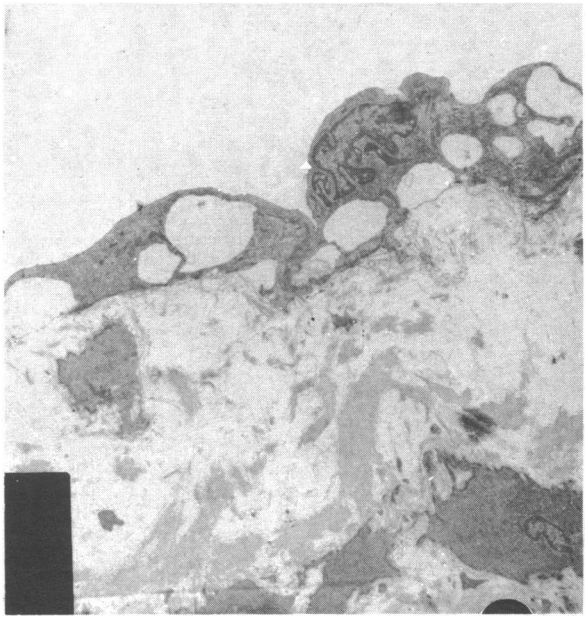

FIG. 5—Luminal part of distal thoracic aorta from carbon monoxide exposed rabbit. Note the protruding endothelial cells and subendothelial blisters separating the endothelial cells from the basement membrane. Note also the considerable widening of the oedematous subendothelial space. (Primary magnification ×8,200.)

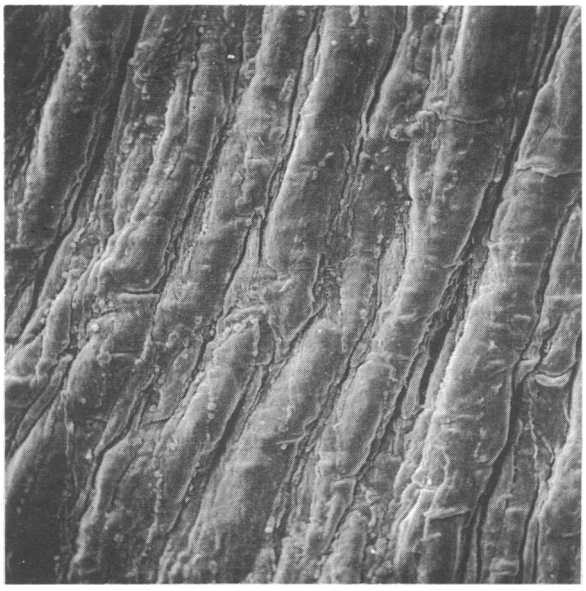

FIG 6—Surface of the distal part of thoracic aorta from carbon monoxide exposed rabbit. Note swollen and irregular endothelial folds. Width of endothelial folds is about twice that of controls. (Primary magnification ×1,130.)

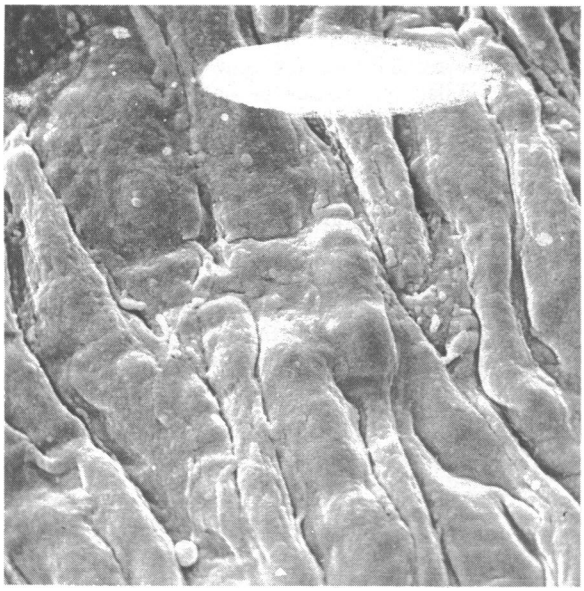

FIG. 7—Surface of distal part of aortic arch from carbon monoxide exposed rabbit. Normal arrangement of endothelium in folds tends to disappear. Instead, surface structure exhibits a highly irregular picture with extreme swelling of endothelial cells. (Primary magnification ×1,130.)

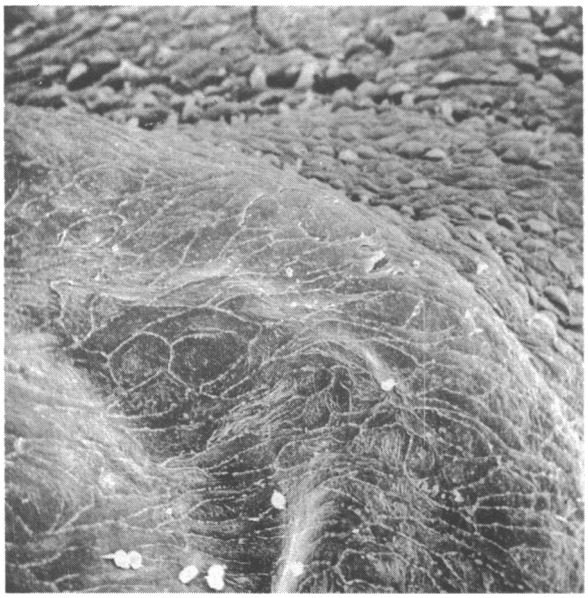

FIG. 8—Surface of slightly raised aortic plaque from carbon monoxide exposed rabbit. Plaque is situated between arch and thoracic part. Endothelium covering plaque is quite smooth and shows a characteristic network of fibrin-like material, possibly depicting intercellular junctions. Plaque is surrounded by cobblestone-structured endothelium. (Primary magnification ×212.)

Pathogenesis of Arteriosclerosis

The problem we now faced was the pathophysiological explanation of the experimental findings and 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 of 3 to 4% in the serous cavities—that is, 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 shown by the microscopical and electron microscopical pictures. To evaluate this hypothesis a comparison was made between changes in some physiological and biochemical parameters measured in human subjects 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 and Pauli, 1968).

It was shown 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 ^{131}I 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 been studied in more detail in my department (Parving, 1972, and Parving *et al.*, 1972) by measuring in human subjects the disappearance rate of ^{131}I albumin injected intravenously, and by following the protein flux in lymph in dogs. It was confirmed that the disappearance rate of ^{131}I albumin after exposure to carbon monoxide (20-25% carboxyhaemoglobin) for three hours is increased about 50%.

The occurrence of increased permeability during exposure to carbon monoxide could also be shown 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 more pronounced for the high molecular proteins than for the low molecular ones (Parving *et al.*, 1972). For example, the flux of α_2-macroglobulin (MW 830000) in the lymph could increase two to three times more than the flux of albumin (MW 69000). We do not know why this happens. Some might say it may be due to a carbon monoxide induced widening of the gaps between the cells, since we have not observed increased pinocytosis in our ultrastructural studies of arterial walls in carbon monoxide exposed rabbits.

When discussing these results, it should be emphasized that proteins and other macromolecules penetrate the vascular wall under normal conditions 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 ^{131}I albumin is about 5-6% per hour, corresponding to the penetration of the total plasma albumin pool through the vascular walls over 16 to 20 hours. This means that the transport probably cannot be looked on 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 mechanism controlling the transport, which probably is influenced also in other ways, which I am not going to discuss here.

Similarity of Results

The results mentioned are consistent with the few quite old experimental studies along similar lines reported in the literature. It has also been known for many years that acute exposure of animals and men to higher concentrations of carbon monoxide (30-40% carboxyhaemoglobin) leads to congestion of tissues. This also occurs after 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 oedema. The pathogenesis seems to involve increased permeability of various endothelial and 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 the new-born, may have a similar pathogenesis. In some diseases without arterial hypoxaemia increased transvascular protein permeability has also been found—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. It is my feeling that this might lead to a deeper understanding of some fundamental biological processes and could give a new pathogenetic approach to some diseases.

Filtration Theory

Let us now look at the relation of the development of atheromatosis to a more or less chronically increased vascular permeability. I am not going to discuss in detail the pathogenesis of atheromatosis, 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 filtrate into the arterial wall from the luminal side 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 probable 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 correlation between serum cholesterol values in middle-aged men and their risk of developing obliterating arterial diseases.

The infiltration 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 well known 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 show a correlation between carboxyhaemoglobin levels in smokers and the incidence of atherosclerotic disease (Kjeldsen, 1969). This is shown in Table I. The individuals with atherosclerosis (early coronary thrombosis, angina pectoris, or peripheral arterial obliterations) have significantly 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 we feel justified in concluding that carbon monoxide is the atherogenic agent in the smoke. This is also shown by preliminary results of a current investigation in a steel mill, where about 300 people have been exposed daily for years to a certain amount of carbon monoxide. The incidence of atherosclerotic diseases in these workers seems to be higher than in a non-exposed control group.

TABLE I—*Average Carboxyhaemoglobin Values in Atherosclerotic and Non-atherosclerotic Smokers Chosen at Random*

Age Groups	Atherosclerotic Smokers		Non-atherosclerotic Smokers	
	No.	COHb (%)	No.	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

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 seems likely that the massage of 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.

Metabolic Concept

In my opinion the physicomechanical 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. I will not 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 be an increased formation of lipids during hypoxia, and the removal of already accumulated lipids seems to be especially influenced by the oxygen supply. This is shown by the results of the following experiment where rabbits breathing normal atmospheric air were fed cholesterol for 10 weeks. They were then fed normal rabbit food, and half of them were exposed to hyperoxia (26% oxygen) for a further 10 weeks. There was no difference at all between the mean serum cholesterol values in the two groups, but the aortic cholesterol was much less in the hyperoxic than in the normoxic group. 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 thought to promote injuries and atherosclerosis (Hueper, 1944) supposed to occur locally when the blood flow may be turbulent.

Fetal Development

I should now like to turn to another important aspect of the toxicity of small amounts of carboxyhaemoglobin: the effect on fetal development (Astrup *et al.*, 1972). It is well known from several studies that babies delivered of mothers who smoke weigh about 200 grammes less than babies delivered of non-smoking mothers. We thought that this might be due to inhaled carbon monoxide and correlated to carboxyhaemoglobin levels in the smoking and non-smoking women during pregnancy. The average birth weight of the babies delivered of 176 smokers was 2,999 g, while it was 3,235 g in 177 non-smoking mothers. These figures correspond well with published data. There was a negative correlation at the 0·05 level between the mean carboxyhaemoglobin concentrations and the birth weight of the babies, thus indicating that the carbon monoxide in the tobacco smoke might be responsible for the weight-diminishing effect.

However, there might also be something else in the tobacco, so we decided to expose pregnant rabbits to carbon monoxide to see if carbon monoxide by itself would have an effect. The experiment involved 54 carefully selected rabbits. They were observed for three months. The animals were divided into three groups according to the average number of babies of their mothers and of their own first pregnancy. They were made pregnant for the second time and placed in our exposure chambers. Group 1 was exposed to atmospheric air, groups 2 and 3 to 0·009% carbon monoxide (9-10% carboxyhaemoglobin) and 0·018% carbon monoxide (16-18% carboxyhaemoglobin) respectively. The day before expected delivery they were moved from the exposure chambers to ordinary breeding cages in a normal atmosphere.

The average birth weight of the babies was significantly lower in the carbon monoxide exposed groups (Table II), and

TABLE II—*Effect of Carbon Monoxide Exposure of Pregnant Rabbits on Birth Weight and Neonatal Mortality*

	Group 1 (0% COHb)	Group 2 (9–10% COHb)	Group 3 (16–18% COHb)
No. of pregnant rabbits ..	17	14	17
Total No. of babies	116	81	123
Average weight of babies (g)	53·7	51·0	44·7
Stillborn and babies died within first 24 hr	1	8	44

there was a dramatic increase in the number of stillborn and babies that died during the first 24 hours. The mortality after six and after 21 days of babies that survived the first 24 hours was, however, the same in the three groups. Some of the babies of the exposed mothers were born without a leg, thus showing also a teratogenic effect of carbon monoxide.

Hypoxia may be expected to have the same effect on fetal development as carbon monoxide, which is supported by the fact that women living at a high altitude (about 3,500 m) deliver babies with a birth weight of about 200 g lower than babies born at sea level, while the weight of the placenta is higher (McClung, 1969).

Further details concerning the many interesting aspects of these pregnancy investigations are not discussed here, but we feel justified in concluding that moderate carbon monoxide exposure has a severe effect on the fetal development in rabbits, which is exemplified by reduced birth weight and by an increase in the number of stillborn and of babies dying within 24 hours. Increased carboxyhaemoglobin concentrations in pregnant women who smoke may have a similar effect.

Conclusions

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 hope that our experimental results have convinced you that this assumption is wrong, and I should like to finish by summarizing our results and conclusions.

In man intermittent exposure to carbon monoxide, rather than to nicotine, due to tobacco smoking may be regarded as the real cause of the much higher risk for smokers to develop arterial diseases compared with non-smokers. There is no evidence from animal experiments that nicotine has an atherogenic effect.

The increase in permeability of the endothelium induced by carbon monoxide or hypoxia leads to the formation of subendothelial oedema, lipid accumulation, and other arterial injuries in experimental animals. This agrees well with the filtration theory for the pathogenesis of atherosclerosis and emphasizes the importance of this theory. The molecular processes involved in the changed permeability should be identified and a possible relation to a hypothetical oxygen-dependent control system for endothelial permeability should be evaluated. Carbon monoxide or hypoxia probably also delay the elimination of lipids already deposited in the arterial walls, since an enhanced elimination is seen in hyperoxia.

Carbon monoxide does not only hasten the development of atherosclerosis but it also has a damaging effect on the myocardium, thus worsening the consequences of coronary obliterations.

Low concentrations of carboxyhaemoglobin in pregnant rabbits have a profound influence on fetal development. This effect of carbon monoxide and a postulated similar effect of hypoxia should be studied in more detail, and the consequences for pregnancies in tobacco smoking women should be evaluated.

Carboxyhaemoglobin levels measured after smoking may indicate the risk for the smoker of developing atherosclerosis and might help to discourage smoking.

The demonstrated toxicity of low concentrations of carboxyhaemoglobin should be acknowledged when discussing air pollution and threshold limit values for carbon monoxide.

The investigations leading to the results presented here have proved fascinating and challenging to us, not only because of the interesting theoretical problems but also because of their important 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 importance for the theory and practice of medicine.

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