THE EFFECT OF OXYGEN DEFICIENCY ON THE DEVELOPMENT EXPERIMENTAL ATHEROSCLEROSIS OF THE CORONARY ARTERIES

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The role of oxygen deficiency in the development of atherosclerosis and cardiosclerosis has received inadequate attention from the point of view of experimental research.

In experiments on rabbits, R. L. Al'tshul' found that administration of cholesterol to animals kept in chambers with an increased oxygen content (up to 60-65%) greatly inhibits the development of atherosclerosis. In this author's opinion an increased supply of oxygen stimulates oxidation of cholesterol, and the oxycholesterol is either excreted from the body more readily or, passing with the bile into the intestine, does not undergo reabsorption as in the case of cholesterol.

The aim of the present research was to study the effect of prolonged oxygen deficiency on the degree and character of the atherosclerotic changes occurring in the coronary vessels during experimental cholesterol atherosclerosis.

EXPERIMENTAL METHOD

Experiments were carried out on 27 rabbits of the same sex, weighing 2000-2400 g, and kept under identical conditions on a standard diet.

The animals were subdivided into three groups.

The first group (10 rabbits) acted as controls, and received cholesterol (300 mg/ kg body weight) dissolved in vegetable oil, daily for 6 months.

The second group, like the first, received cholesterol in the same daily dosage, but at the end of the second month, in addition to feeding on cholesterol, the animals were kept each day for 3-6 hours in a chamber with a lowered oxygen concentration. The oxygen content of the chamber, whose volume was 250 cm^3, was brought down to 12% (by addition of nitrogen to the outside air). Air and nitrogen entered the chamber simultaneously and were mixed by an electric fan. Entry of the air and nitrogen was controlled by means of a kerosene rheometer. The concentration of oxygen and carbon dioxide in the chamber was determined by means of a Haldane apparatus.

The third group (5 rabbits) received no cholesterol, but at the end of the second month, like the rabbits of the second group, they were placed for 3-6 hours every day for 4 months in the chamber with the lowered oxygen concentration.

Every 15 days, before the rabbits were placed in the chamber and immediately after exposure to the low oxygen concentration for 3-6 hours, the blood cholesterol of all the animals was estimated by Grigaud's method.

TABLE 1

The Blood Cholesterol Concentration of the Rabbits of the First Control Group Receiving Cholesterol Alone

Rabbit No.	Weight		Blood cholesterol concentration (in mg %)												Degree of atherosclerosis	
				day of the experiment												
	before experiment	at the end of the experiment	before experiment	15th	30th	45th	60th	75th	90th	105th	120th	135th	150th	165th	180th	of the aorta	of the coronary arteries
95	2300	3100	84	260	326	482	642	814	940	1070	846	900	812	700	770	+++	++
96	2200	3500	76	350	474	530	620	780	830	930	760	822	750	680	610	+++	+
97	2300	2500	58	280	370	522	730	880	1060	1140	840	912	890	710	780	+++	++
98	2100	2800	42	212	286	336	400	450	512	420	450	516	536	580	470	+++	++
99	2200	2600	56	228	320	420	500	580	476	550	382	412	322	360	330	++	+
102	2100	3200	92	262	322	510	742	810	780	800	770	518	600	650	562	+++	++
103	2300	3500	68	312	440	622	864	928	1180	1280	1160	1050	870	840	810	+++	++
104	2000	3300	72	346	412	540	650	740	850	920	876	720	670	616	576	+++	++
105	2200	2800	88	210	356	410	542	710	890	850	740	580	550	630	680	+++	++
106	2000	3000	64	180	260	300	386	448	542	456	400	376	340	320	308	++	+
Average . . .			70	264	357,6	466,2	607,6	714	806	841,6	721,8	670,6	630	608,6	549,6		

TABLE 2

The Blood Cholesterol Concentration of the Rabbits of the Second Group, Receiving Cholesterol and Kept in a Chamber with a Low Oxygen Concentration

Rabbit No.	Weight		Blood cholesterol concentration (in mg %)												Degree of atherosclerosis	
				day of the experiment												
	before experiment	at the end of the experiment	before experiment	15th	30th	45th	60th	75th	90th	105th	120th	135th	150th	165th	180th	of the aorta	of the coronary arteries
68	2200	3100	76	328	600	772	856	1110	1420	1560	1680	1756	1610	1580	1558	++++	+++
69	2000	3200	94	344	428	660	780	1150	1530	1618	1534	1588	1670	1490	1350	++++	+++
78	2100	2900	82	270	362	448	534	700	946	1150	1390	1350	1440	1220	1146	++++	+++
79	2200	3250	68	242	326	400	580	770	884	970	854	920	784	850	986	++++	+++
84	2200	3300	76	226	412	430	380	562	684	642	560	620	462	570	462	++	+
86	2000	3500	58	300	390	380	458	760	810	872	990	960	916	924	844	+++	++
88	2300	3600	48	410	548	484	550	860	1160	1018	940	1084	1250	1176	1060	++++	+++
101	2200	3100	96	196	310	550	744	1026	1380	1260	1278	1210	1260	1320	1280	++++	+++
113	2100	3450	66	230	380	526	618	942	1000	1210	1324	1500	1540	1400	1312	++++	+++
118	2000	3200	74	310	486	622	826	1310	1450	1780	1600	1520	1470	1430	1500		
Average . . .			73,8	285,6	429,6	527,2	682,6	919,0	1126,4	1208	1215	1251	1240,2	1196	1149,8		

At the end of the sixth month all the animals were killed by air embolism and their aorta and heart were examined. The aorta was stained by Sudan III in toto, and serial sections of the heart were cut and stained by the usual methods.

The degree and character of the atherosclerosis of the aorta and coronary arteries of the heart were studied. The degree of atherosclerosis was designated conventionally by corsses: + alight changes; ++ moderate; +++ severe; ++++ most pronounced.

TABLE 3

The Blood Cholesterol Concentration of the Rabbits of the Third Group (Not Receiving Cholesterol), Kept in a Chamber with a Low Oxygen Concentration

Rabbit No.	Weight		Blood cholesterol concentration (in mg%)								
				day of the experiment							
	Before experiment	At the end of the experiment	Before experiment	15th	30th	45th	60th	75th	90th	105th	120th
183	2200	2900	82	98	74	102	96	86	88	100	96
184	2300	3000	76	88	96	90	94	78	96	92	88
186	2000	2800	54	78	92	86	98	94	88	96	76
187	2200	3000	92	104	96	110	94	108	102	94	100
201	2200	3200	66	84	92	102	86	100	98	100	94
Average			74	91	90	98	93	93	94	98	90

EXPERIMENTAL RESULTS

In the course of the experiment, for reasons unconnected with the experimental conditions, 2 rabbits of the second group died (in the 3rd and 4th months).

On the 15th day after the beginning of cholesterol feeding, the blood cholesterol level in the rabbits of the first, control group rose to 3-5 times its initial value, to reach its maximum in the 3rd-4th month of feeding, after which it gradually fell until the end of the experiment (Table 1).

In the experimental rabbits of the second group, cholesterol feeding plus keeping the animals in the chamber with a low oxygen concentration led to a sharp increase in the blood cholesterol, in individual cases up to 1780 mg%, which was maintained at a high level throughout the whole experiment (Table 2).

Thus, as a result of anoxia, the rise in the cholesterol concentration in the rabbits of the second group was significantly higher than that in the rabbits of the first, control group (see Table 1).

In the rabbits of the third group, which received no cholesterol but which were kept in the chamber with the low oxygen concentration, the blood cholesterol level rose insignificantly (Table 3).

Prolonged anoxia thus led to an increase in the blood cholesterol concentration, which was evidently due to a reduction in the processes of assimilation and synthesis of cholesterol and of the power to excrete it from the body.

In the rabbits of the first, control group the atherosclerotic plaques were situated mainly in the origin and the arch of the aorta, in the form of continuous outgrowths, markedly raised above the surface of the aorta. In the lower segments of the aorta isolated plaques were present, and where these became confluent, they formed separate areas of lipoidosis. The degree of atherosclerosis in the rabbits of the first, control group was assessed in the majority of cases as +++ (see Table 1).

In the rabbits of the second group atherosclerotic plaques were situated throughout the whole length of the aorta, including its abdominal portion. Merging with each other, they formed continuous zones of atherosclerosis, markedly elevated above the surface of the aorta in the form of outgrowths. The degree of atherosclerosis was assessed in the majority of cases as ++++ (see Table 2).

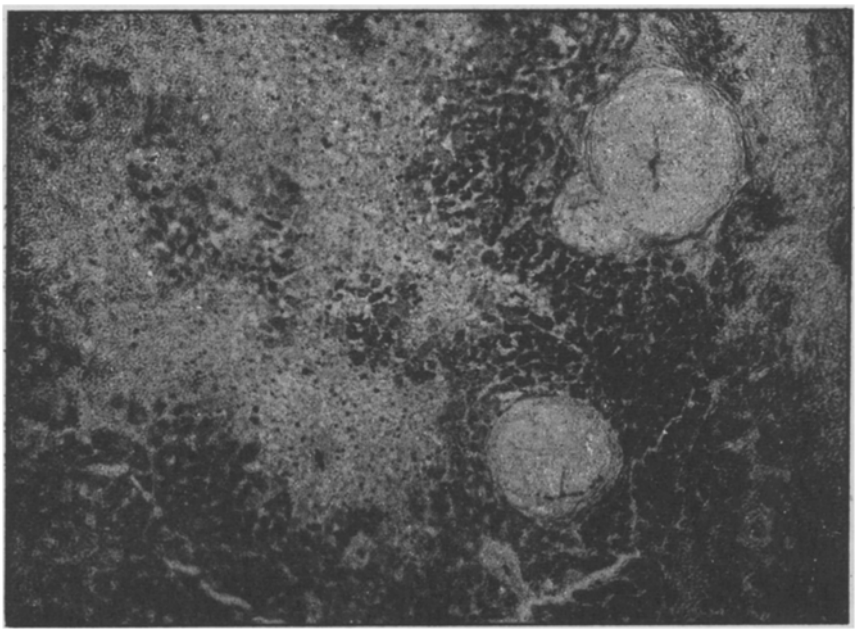

Fig. 1. Large atherosclerotic plaques in the intramuscular branches of the left descending coronary artery. Extensive area of severe destructive changes in the fibers of the myocardium.
Stained by Mallory's method. Objective 6×, ocular 7×. Rabbit No.69, Group – fed on cholesterol and kept in a chamber with a low oxygen concentration (12%). Duration of experiment 6 months.

In the third group no atherosclerotic changes could be found either macroscopically or microscopically.

Examination of the coronary arteries of the rabbits of the first, control group showed in the majority of cases a moderate atherosclerosis (++). Atherosclerotic plaques were situated mainly in the intramuscular branches of the left coronary artery. These atherosclerotic plaques greatly narrowed the lumen of the vessel, in some cases almost completely closing it.

Sometimes the atherosclerotic process mainly affected the subepicardial branches of the left coronary ar - tery, but here they were mainly infiltrative in character; large plaques projecting into the lumen of the vessel were not found. From time to time the branches of the right coronary artery were affected also.

In the second group of rabbits the atherosclerosis of the coronary arteries was severe in the majority of cases (+++, see Table 2). Atherosclerotic plaques were found both in the intramuscular branches of the left coronary artery and in the main subepicardial trunks, projecting considerably into the lumen of the vessel.

In contrast to the control group, in the rabbits of the second group atherosclerosis affected almost all the subepicardial trunks of the left and, to some extent of the right coronary artery too. In the branches of the right coronary artery in the rabbits of the second group atherosclerotic plaques could be found, grossly narrowing the lumen of the vessel. The plaques consisted of large quantities of lipoid deposits, homogeneous or in places granular in structure. In the plaques there were strongly developed elastic fibers, and in places the internal elastic membrane was absent.

In the rabbits of the third group no atherosclerosis was present.

From our findings we concluded that oxygen deficiency in conjunction with cholesterol feeding caused a more pronounced hypercholesteremia and a severer degree of atherosclerosis of the aorta and the coronary arteries of the heart than feeding with cholesterol alone.

Oxygen deficiency evidently causes a disturbance of oxidative processes in the body, which in turn is reflected in the assimilation and synthesis of exogenous cholesterol. Without cholesterol feeding, oxygen deficiency raised the blood cholesterol concentration only insignificantly, and did not cause the development of atherosclerosis.

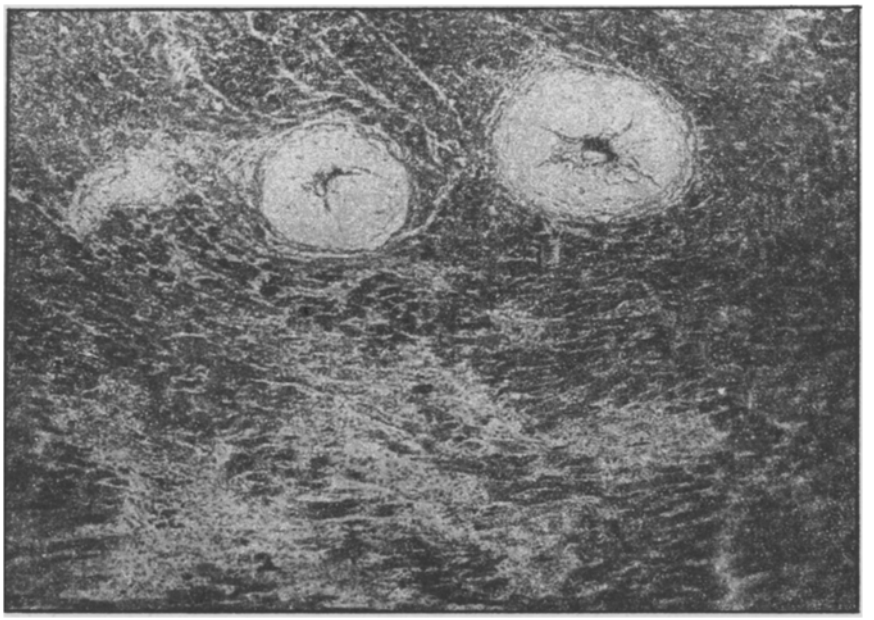

Fig. 2. Atherosclerotic plaques causing gross narrowing of the lumen of the intramuscular branches of the left coronary artery, near which can be seen well marked fibrotic lesions in the muscle of the left ventricle.
Stained by Mallory's stain. Objective 6×, ocular 7×. Rabbit No.79, the same as in Fig. 1.

So far as myocardial changes were concerned, in the first, control group the atherosclerosis of the coronary arteries led in the majority of cases to moderate cardiosclerosis. In the heart muscle were observed small connective tissue scars, in places firm, in others friable, and mainly situated close to intramuscular arteries affected by atherosclerosis, with gross narrowing of the lumen. Areas in which fibers of connective tissue had developed were most often found in the anterior and anterolateral walls of the left ventricle.

In the second group, on examination of the heart muscle, extensive focal collections of cells with large number of lipoid inclusions in their protoplasm were discovered in the interstitial tissue of the mycoardium. These collections of cells were most often found in the papillary muscles. In places in the muscle of the left ventricle there was a diffuse lipoid infiltration of whole groups of muscle fibers.

Close to the arteries with atherosclerotic plaques and in other areas of the myocardium were observed gross lesions of a dystrophic or fibrotic character.

The dystrophic lesions consisted of destruction and necrosis of whole areas of muscle fibers. Many muscle fibers were severely vacuolated. The fibrotic lesions in the heart muscle of the rabbits consisted of multiple and extensive reticular scars.

Alongside areas of loose connective tissue were observed extensive, dense connective tissue scars, composed of coarse collagen fibers, staining intensively with picrofuchsin a crimson color. Some muscle fibers were separated by a large quantity of interstitial myocardial tissue, containing many cells. Among the areas of cellular infiltration were seen isolated cells undergoing destruction. The blood vessels were well filled and many capillaries and veins were dilated. The muscle of the ventricles, especially of the right ventricle, was hypertrophied.

The changes observed in the heart muscle of the rabbits of the second group could be regarded as a manifestation of diffuse cardiosclerosis with the formation of extensive areas of necrosis of whole groups of muscle fibers, as in the case of an infarct of the myocardium (Fig. 1). Here could be traced all stages of development of

sclerotic changes in the heart muscle, from areas of recent hemorrhage, destruction and lysis of muscle fibers up to scar tissue formation (Fig. 2).

Evidently in consequence of gross stenosing atherosclerosis of the coronary arteries and the action of anoxia, a severe disturbance of the normal blood supply of the heart muscle took place, with the development of extensive areas of necrosis. The diffuse atherosclerotic cardiosclerosis was severe (+++) in the rabbits of the second group.

In the third group of rabbits slight dilatation of individual capillaries and veins was observed in the heart muscle. In some areas of the left ventricle, close to its cavity, and in the papillary muscles solitary muscles fibers were found in a state of destruction. Small areas of proliferation of histiocytes were seen there also. No other abnormalities were found.

SUMMARY

Experiments were performed on rabbits. The author studied the effect of prolonged hypoxia on the development of aortic and coronary atherosclerosis in experimentally induced cholesterol atherosclerosis. Oxygen deficiency was attained by placing the animals daily into a chamber with decreased oxygen content (down to 12%) for 3-6 hours, for 4 months.

It was shown that prolonged hypoxia brings about a high hypercholesteremia and greatly intensifies the development of aortic and coronary atherosclerosis.

Prolonged hypoxia considerable increases stenosing atherosclerosis of the coronary arteries and causes severe dystrophic changes in the cardiac muscle in the form of numerous necroses of certain groups of muscular fibers and also always results in the development of diffuse cardiosclerosis in all cases.