CHANGES IN ACTIVITY OF SOME ENZYME SYSTEMS IN EXPERIMENTAL ATHEROSCLEROSIS INDUCED BY CHOLESTEROL

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The development of experimental cholesterol atherosclerosis is associated with disturbance of a number of protein metabolic processes, such as lowering of the rate of protein anabolism in the organs and tissues of the body [1], intensification of catabolic processes in these tissues [2], as well as changes in the plasma proteins, involving fall in serum albumin content and rise in the β-and γ-globulin contents [3].

N. N. Anichkov, Gofman, and others have expressed the view that cholesterol and lipoproteins play a specific role in the development of atherosclerosis. In accordance with this view, and since cholesterol forms complexes with proteins and probably with enzymes, it is to be expected that significant changes might be found in this condition, not only in the activity of proteolytic enzymes [2] but also of other enzyme systems involved in other aspects of metabolism.

We have, in connection with this, examined the activity of a number of different enzyme systems in experimental cholesterol atherosclerosis. These were succinic dehydrase, which takes part in cell respiration, glutamic acid dehydrase, and the enzymes catalyzing the processes of reductive amination.

This paper presents comparative data on the activity of succinic and glutamic acid dehydrases and of the enzyme system responsible for amination of pyruvic acid in the liver, in healthy animals and experimental cholesterol atherosclerosis. The effect of complex compounds of cholesterol and proteins on the activity of these enzyme systems was also studied.

EXPERIMENTAL METHODS

The experiments were performed on two groups of half-grown male chinchilla rabbits, of which one was atherosclerotic. Atherosclerosis was induced by the procedure of N. N. Anichkov, as described in an earlier paper [3]. The animals were killed by decapitation after 100-120 days, the liver was quickly removed, and was made into a 30% homogenate, with cooling, with Ringer-phosphate buffer at pH 7.3. Respiration was measured in a Warburg apparatus, in which the flasks contained 1.3 ml of Ringer-phosphate solution at pH 7.3 and 0.5 ml of 0.05% methylene blue in the main compartment, filter paper moistened with 0.2 ml of 10% caustic potash in the well, 0.5 ml of liver homogenate in one side-tube, and 0.5 ml of respiration substrate in the other. The concentration of these substrates (succinic and glutamic acids) was such as to give a final concentration in the systems of 0.01-0.012 mol.

The flasks were saturated with oxygen, and were incubated for 1 hour at 37.8°. The oxygen uptake was read every 10 minutes, and the Q_{O_2} values were calculated, on the basis of the dry content of the tissue.

The Q_{O_2} values were determined at the same time for control animals of the same age, weight, and sex, which had been maintained under the same conditions as the test animals since the beginning of the experiment, but which did not receive cholesterol supplement in their diet.

EXPERIMENTAL RESULTS

It is evident from the data presented in Table 1 that the Q_{O_2} values for liver homogenates from atherosclerotic animals with succinic acid and glutamic acid as substrates are much lower than for healthy animals. This effect is found not only for each pair of experiments, but also for the whole series. Only in one case (Rabbit No. 14) did the Q_{O_2} for succinic acid approach that found for the healthy controls; this was the highest Q_{O_2} found for any atherosclerotic animal, whereas it was the lowest found for any healthy animal.

TABLE 1

Changes in Activity of Succinic and Glutamic Acid Dehydrases

Serial No. of rabbit	Description of animal	Degree of atheromatosis of the aorta (macroscopic)	Q_{O_2} succinic acid	Q_{O_2} glutamic acid
1	Healthy	—	3.01	0.66
2	Atherosclerotic	++++	0.91	0.12
3	Healthy	—	2.11	0.72
4	Atherosclerotic	+++	1.44	0.32
5	Healthy	—	2.38	0.81
6	Atherosclerotic	+++	1.80	0.48
7	Healthy	—	2.04	
8	Atherosclerotic	++	1.66	
9	Healthy	—	2.35	0.59
10	Atherosclerotic	++	1.83	0.47
11	Healthy		2.44	
12	Atherosclerotic	++	1.42	0.63
13	Healthy	—	2.22	0.56
14	Atherosclerotic	++	2.04	0.90
15	Healthy	—	2.75	1.38
16	Atherosclerotic	+++	1.74	0.53
17	Healthy	—	2.36	
18	Atherosclerotic	++++	1.36	
19	Healthy	—	2.45	0.85
20	Atherosclerotic	++	1.72	0.57
21	Healthy	—	2.14	0.52
22	Atherosclerotic	+++	1.60	0.39

It appears from the glutamic acid data that the homogenate from Rabbit No. 14 was exceptional, in giving a higher dehydrase activity than its normal control. In all other cases both succinic and glutamic acid dehydrases were much lower for the atherosclerotic group than for the controls. This difference cannot be due to a fall in protein content per unit weight of liver tissue of atherosclerotic animals, since determinations of protein content showed this did not exceed 20% (due to increase in fat content).

Our experiments therefore allow us to draw the conclusion that the development of experimental cholesterol atherosclerosis is associated with lowered succinic and glutamic acid dehydrase activity.

Our experiments on synthesis of aminoacids from ammonia and pyruvic acid were done on liver slices [4]. The amount of aminoacids formed was derived from measurements of amino-mitrogen, determined gasometrically by the Van Slyke nitrite method. The results are presented in Table 2. It appears from this table that there are only small differences in increase of amino-nitrogen when material from atherosclerotic and healthy animals is incubated with ammonia alone. With both ammonia and pyruvic acid, however, the rise in amino-nitrogen is substantially greater for liver slices from atherosclerotic than from normal animals.

The data presented in Tables 1 and 2 show that the deveolpment of experimental cholesterol atherosclerosis is associated with significant changes in the activity of the hepatic enzyme systems studied. These changes consisted in lowered activity of succinic and glutamic acid dehydrases, which catalyze cell respiration, and in raised activity of the enzyme systems involved in synthesis of aminoacids by reductive amination.

Changes in other enzyme systems have been reported in atherosclerosis; as has been mentioned, we found an activation of proteolytic enzymes [2]. It has also been found that elastase activity is substantially reduced;

this enzyme system takes part in the synthesis of the elastic fibers of the arteries [5]. There is also a very marked lowering of activity of the enzyme system responsible for synthesis of cholesterol [6], a considerable fall in adenosinephyrophosphatase [7], carbonic anhydrase [8], and hyaluronidase [9] activities.

TABLE 2

Rate of Formation of Amino Groups From Ammonia and Pyruvic Acid in Liver Tissue of Normal and Atherosclerotic Animals.
The systems consist of 500 mg of liver slices in 6 ml of 0.05 mol sodium pyruvate and 0.02 mol ammonium carbonate in bicarbonate buffer at pH 7.5; time of incubation 2 hours.

No.	Experiments with ammonia alone		Expts. with both ammonia and sodium pyruvate	
	Healthy rabbits	atherosclerotic rabbits *	healthy rabbits	atherosclerotic rabbits
1	75	69	82 **	119 **
2	66	63	82	123
4	84	64	116	132
5	96	54	70	135
6	62	98	91	184
7	73	53	96	86
8	76	67	71	149
9	81	67	72	128

*The experiments were performed on the same animals as in Table 1, and in the same order.

**The figures represent increase in NH_2—N content, in mM per g of tissue.

All these data show conslusively that devlopment of the atherosclerotic process is associated with changes in the activities of a number of enzyme systems responsible for a number of metabolic processes. In order, however, to understand the pathogenesis of this disease it is necessary to study the mechanism whereby these changes are effected.

In view of the importance attached to complex compounds of cholesterol and proteins as factors in the development of atherosclerosis it was thought to be of interest to study the effect of such compounds on various enzyme systems.

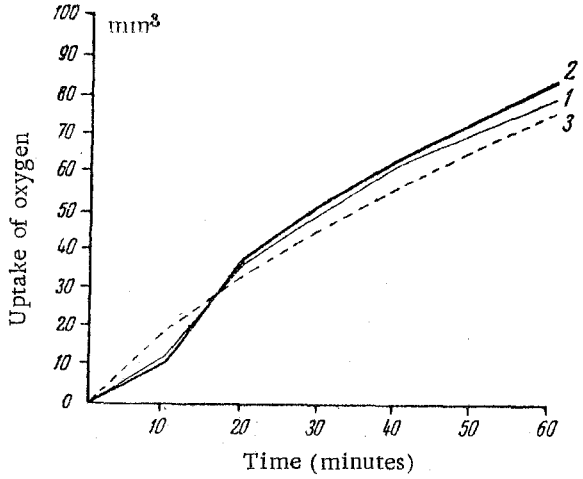
With this object we examined the effect of stable emulsions of cholesterol in blood plasma, prepared according to Pollak and Wadler [10], and of blood plasma of atherosclerotic rabbits, which has a high content of cholesterol compounds, on the activity of succinic and glutamic acid dehydrases, proteolytic enzymes, and aminoacid-synthesizing enzymes.

The experiments were carried out as described above, with the difference that 0.5 ml of stable cholesterol emulsion or of atherosclerotic rabbit serum, containing 700-900 mg-% of cholesterol, were added to the systems. Corresponding amounts of healthy rabbit serum or of the protein solution used for emulsifying cholesterol were added to the control flasks.

The graph illustrates the results obtained for succinic acid dehydrase activity in such systems. It is evident that addition of cholesterol-protein complexes has no effect on dehydrase activity. Similar results were obtained with glutamic acid dehydrase, glucose dehydrase, proteolytic enzymes, and reductive amination enzymes.

It thus appears that the changes in activity of different enzyme systems found in atherosclerosis are due not to excessive amounts of cholesterol compounds present in the organism, but rather to binding by cholesterol of enzymes and their inhibitors, leading to the inactivation of some enzyme systems, and to the activation of others.

Our experimental results are capable of being interpreted in this way.



Effect of stable emulsions of cholesterol in protein solutions on the activity of succinic acid dehydrase. 1) Uptake of oxygen by liver homogenates from healthy rabbits in presence of succinic acid; 2) uptake after adding cholesterol emulsion; 3) uptake after adding protein solutions.

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