

IMMUNOLOGIC AND BIOCHEMICAL INVESTIGATION OF CARDIAC AND AORTIC TISSUES OF DOGS WITH EXPERIMENTAL ATHEROSCLEROSIS

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It was shown by the complement fixation reaction (50% titer) that cardiac and aortic tissues of dogs with experimental atherosclerosis are indistinguishable immunologically from homologous tissues in healthy animals. The content of electrophoretic fractions of the blood serum, heart muscle, and aorta is significantly changed in dogs with atherosclerosis.

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Previous investigations [1, 2] showed that cardiac and aortic tissues of patients with atherosclerosis differ antigenetically from homologous tissues of healthy persons.

The object of the present investigation was to determine whether immunologic changes take place in the cardiac and aortic tissues of animals with experimental atherosclerosis. Parallel to the immunologic study of cardiac and aortic tissues of dogs with atherosclerosis, the proteins of these tissues were studied electrophoretically.

EXPERIMENTAL METHOD

Atherosclerosis was produced in three dogs by the method of Steiner and Kendall [8] and T.A. Sinit-syna [3] over a period of six months; during the first month the dogs were fed with 6-methylthiouracil in a dose of 0.075 g/kg body weight, after which cholesterol was added in a dose of 0.5 g/kg.

The serum cholesterol concentration of the dogs was determined by the Engel'gardt-Smirnova method, phosphatides by the Fiske-Subbarow method, and the relative content of serum protein fractions and protein fractions of cardiac [7, 9] and aortic [5] tissues by electrophoresis on paper.

Rabbits were immunized by intraperitoneal injection of homogenate of cardiac and aortic tissues of the dogs in physiological saline (1:10) in doses of 2 ml every other day for 8 days. Cardiac and aortic tissues of dogs with atherosclerosis and homologous tissues of healthy dogs were used as antigens. Antisera were obtained on the 8th day.

Test antigens for the complement fixation reaction (50% titer) [4] consisted of saline extracts of cardiac and aortic tissues of the dogs (1:10). The test antigens were equalized in protein content, which was estimated by Lowry's method [6].

EXPERIMENTAL RESULTS

Investigation of the blood serum from dogs with atherosclerosis revealed an increase in the cholesterol concentration to between 355 and 1288 mg% from a normal value of 136 ± 7.11 and an increase in phosphatides to between 223.9 and 360.2 mg% from a normal value of 209.7 ± 16.39 ; phosphatide-cholesterol ratio falling from a normal value of 1.54 to 0.36 in atherosclerosis. A considerable increase in the β - and γ -globulins and decrease in the fraction of albumins was observed (Table 1).

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TABLE 1. Relative Content of Protein Fractions in Blood Serum, Heart Muscle, and Aorta of Dogs under Normal Conditions and with Experimental Atherosclerosis ($M \pm m$; in percent)

| Series of experiments | No. of animals | Serum protein fractions | | | | | |
|------------------------------|----------------|---------------------------------|--------------------------------|--------------------------------|---------------------------------|---------------------------------|--------------------------------|
| | | A | α_1 | α_2 | β | γ | |
| Normal | 15 | 44,20 \pm 1,04 | 8,80 \pm 0,70 | 10,29 \pm 0,70 | 22,31 \pm 0,90 | 13,39 \pm 0,96 | |
| Atherosclerosis | 3 | 31,34 \pm 3,27 $P < 0,002$ | 6,65 \pm 0,48 $P < 0,05$ | 7,63 \pm 1,62 $P > 0,05$ | 32,32 \pm 1,52 $P < 0,001$ | 22,06 \pm 0,07 $P < 0,001$ | |
| Aortic protein fractions | | | | | | | |
| | | I | II | III | IV | V | VI |
| Normal | 7 | 16,72 \pm 1,18 | 11,81 \pm 0,23 | 24,09 \pm 1,24 | 20,91 \pm 1,57 | 15,79 \pm 1,38 | 10,68 \pm 0,31 |
| Atherosclerosis | 4 | 25,40 \pm 1,91 $P > 0,01$ | 8,98 \pm 0,21 $P < 0,001$ | 14,16 \pm 1,68 $P < 0,05$ | 19,29 \pm 1,44 $P > 0,05$ | 16,41 \pm 1,07 $P > 0,05$ | 15,76 \pm 1,49 $P < 0,02$ |
| Myocardial protein fractions | | | | | | | |
| | | I | II | III | IV | V | VI |
| Normal | 7 | 7,59 \pm 0,49 | 5,36 \pm 0,51 | 12,78 \pm 0,39 | 20,39 \pm 1,09 | 29,33 \pm 0,80 | 24,55 \pm 1,63 |
| Atherosclerosis | 3 | 13,76 \pm 2,22 $P < 0,05$ | 6,60 \pm 0,50 $P > 0,1$ | 15,81 \pm 0,91 $P < 0,02$ | 20,77 \pm 0,25 $P > 0,5$ | 28,29 \pm 0,59 $P > 0,25$ | 14,77 \pm 1,72 $P < 0,01$ |

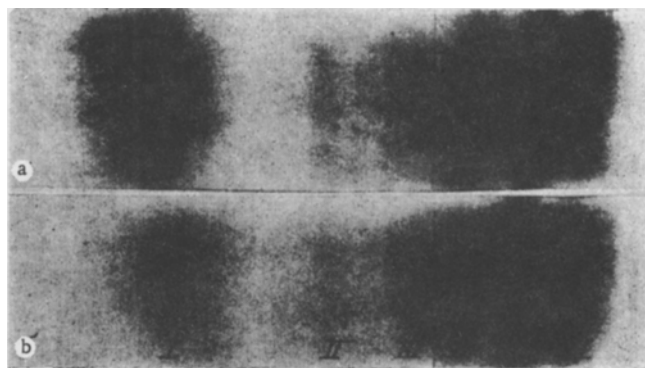


Fig. 1. Electrophoresis of heart muscle proteins of normal dog (a) and of dogs with atherosclerosis (b). I-VI fractions (see text).

Table 1 shows that the content of some fractions of the heart muscle and aorta of dogs with atherosclerosis also differed significantly from normal.

Typical results of electrophoresis of the heart muscle proteins of the dogs are shown in Fig. 1. Data concerning identification of heart muscle proteins of rabbits [7, 9], extracted by buffer of low ionic strength, have been published in the literature. Comparison of the results of electrophoresis of heart muscle proteins of dogs and rabbits showed a definite similarity in the arrangement of the protein fractions. During electrophoresis of dogs' heart muscle proteins our fraction I corresponded to myoalbumins, fractions III and IV to protein possessing phosphorylase activity, and fractions V and VI to myogens identified by electrophoresis in rabbits' muscle. An additional fraction II, absent in rabbits' heart muscle, was also found by electrophoresis in dogs' heart muscle.

Typical results of electrophoresis of dogs' aortas are given in Fig. 2. During electrophoresis the aortic proteins of healthy and experimental dogs were separated into six fractions (I-VI). The fractions of aortic proteins were compared with serum proteins in electrophoresis mobility: fraction I of aortic proteins corresponded to albumins, fractions II and III to α -globulins, fraction IV to β -globulins, and fractions V and VI to serum γ -globulins.

The content of fractions I and VI of soluble aortic proteins was increased while that of fraction III was decreased. The contents of the other fractions were practically indistinguishable from normal (Table 1).

In atherosclerosis an increase in the content of cholesterol and phosphatides and significant changes in the relative proportions of the proteins fractions of the serum, heart muscle, and aorta of the dogs compared with normal were observed.

Analysis of results of the complement fixation reaction showed that serum against atherosclerotic heart tissues reacts with homologous test antigen in the same titer as with heterologous. Sera against heart tissues of healthy dogs react in equal titers with cardiac tissues of healthy dogs and of atherosclerotic dogs. A similar pattern was observed when serum against aortic tissues of dogs with experimental atherosclerosis and serum against aortic tissues of healthy dogs reacted with homologous and heterologous test antigens: the investigated antisera reacted with both homologous and heterologous test antigen in the same titer.

The results thus show that, despite considerable biochemical changes in the investigated tissues, no immunologic differences could be found between the cardiac and aortic tissues of dogs after development of atherosclerosis for 6 months by the quantitative complement fixation reaction to 50% titer. Investigations in this direction are proceeding.

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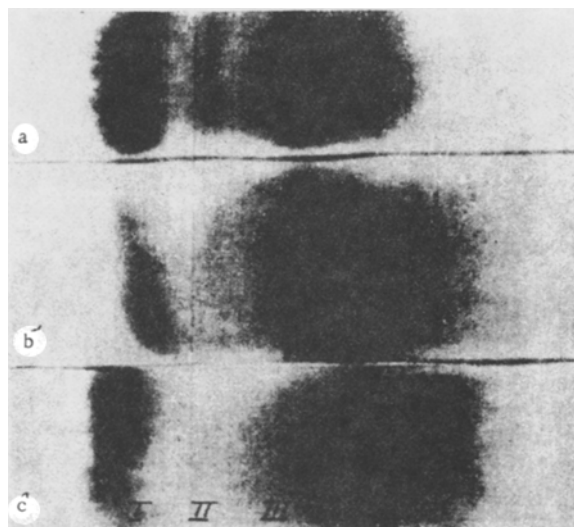


Fig. 2. Electrophoresis of serum proteins (a) and aorta of a normal dog (b) and of a dog with atherosclerosis (c). I-VI) fractions (see text).

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