METHODS OF ESTIMATING THE DEGREE OF EXPERIMENTAL ATHEROSCLEROSIS

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The estimation of the degree of development of experimental atherosclerosis has until recently been highly unsatisfactory. The use of descriptive words (slight, moderate, severe) or the designation of the severity of the process by means of a number of plus signs are totally inadequate for research workers. A great advance was made by K. G. Volkova's suggestion [1] that the degree of atherosclerosis should be judged by the amount of lipids infiltrating the aorta. This biochemical method, notwithstanding its accuracy, has the drawback that it does not permit the area of the lesion to be judged, and it gives no idea of the character of the atherosclerotic process. It becomes necessary to supplement this method by a planimetric device, permitting the distribution of the process to be expressed as a percentage of the total surface area of the aorta. Several authors have suggested that the "atherosclerotic profile" of the aorta be deduced in relation to human postmortem material [2].

The methods which we suggest are as follows.

Gravimetric planimetric method. The aorta, extracted in toto from the semilunar valves to the bifurcation is stretched on glass and, after passing through formalin, is stained in toto with Sudan (Fig. 1a).

The stained aorta is stretched on glass, placed on a board and covered with transparent x-ray film, washed free from emulsion, and is fixed to the board with drawing pins. The contours of the aorta are traced in ink with a fine pen, after which the contours of all the atheromatous elements of the aorta are superimposed. It is especially complicated to trace the area of lesions represented by multiple small plaques and lipoid patches. In these cases it is necessary, to preserve some degree of accuracy, to "unite" the small elements into larger ones, and to outline the postulated contour of the "united" plaques (Fig. 1b).

When the contours of the atherosclerotic elements have been transferred to the film, it is essential to insert a conventional sign inside the contour immediately (as has been done in Fig. 1b), so that during the subsequent treatment of the film the contours of the elements outlined on it may be recognized.

After the aortogram has been obtained, i.e. the contours of the atheromatous elements have been transferred

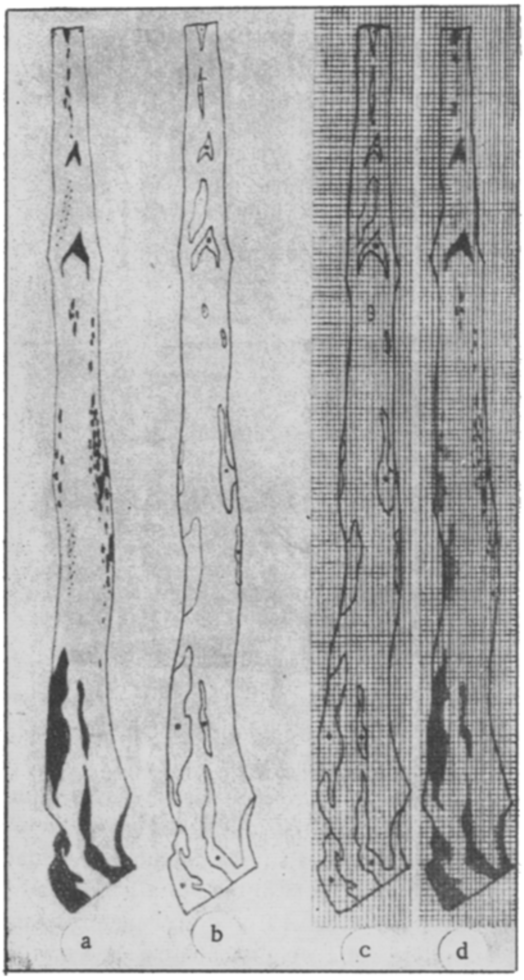

Fig. 1. Methods of planimetric investigation of the rabbit's aorta affected by atheroma. a) aorta of rabbit with cholesterol atherosclerosis (stained with Sudan III); b) aortogram of the same aorta. The large dots inside the contours correspond to large atheromatous plaques, the small dots to thin atheromatous plaques, and absence of sign inside the contour corresponds to lipoid stains; c) aortogram traced on squared paper; d) planimetric grid placed on an aorta stains; with Sudan III.

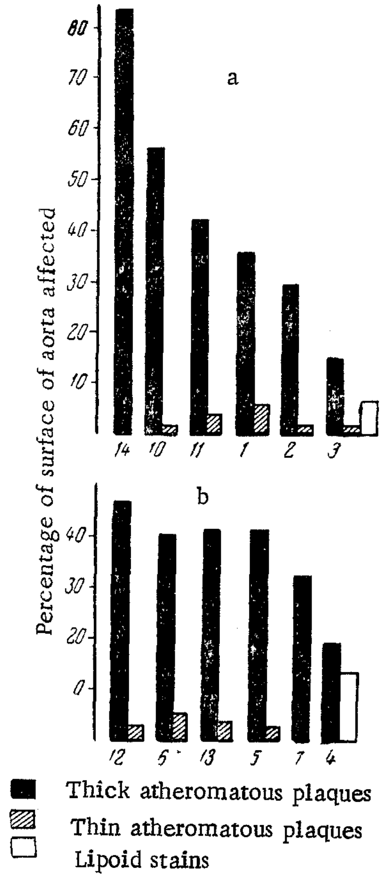

Fig. 2. Graphic illustration of the atherosclerotic formula of the aorta in rabbits. a, b) two groups of animals with comparable degrees of development of atheroma. The figures below the columns designate the number of the experimental animals.

to the film, a piece of the film, which has been removed from the aorta, is cut out corresponding in area to the whole aorta, and weighed on torsion scales. Next, pieces of film corresponding to areas of thick plaques, thin plaques and lipoid stains are cut out in succession.

After the weighing, a simple calculation is made and the atherosclerotic formula of the aorta is deduced. Let us assume that the piece of film corresponding to the surface of the whole aorta weighed 500 mg, and that the pieces of film denoting the area of the thick plaques, the thin plaques and the lipoid stains weighed 50, 100 and 25 mg respectively. Since 500 mg is equivalent to 100% of the surface of the aorta, the atheromatous elements are respectively equivalent to 10, 25 and 5% of the total surface area of the aorta.

For the sake of clarity it is best to give the "atherosclerotic formula of the aorta" in the form of a fraction, in which the numerator is the total percentage of the area of the aorta occupied by atherosclerotic elements, and the denominator gives successively the percentage of the area of the aorta occupied by thick plaques, by thin plaques and by lipoid stains. The atherosclerotic formula of the aorta for the case chosen for the illustration would be of the form: $\frac{40}{10 - 25 - 5}$. Where any of the atherosclerotic elements were absent, their place would be occupied by zero or a minus sign.

For the sake of clarity and to enable comparison of the degree of atherosclerosis in groups of experimental animals to be made, the values of the "atherosclerotic formula of the aorta" obtained may be shown graphically (Fig. 2) and in the form of a table.

Method of indirect planimetry. As in the gravimetric method, an aortogram is prepared on x-ray film. This is placed on squared paper (Fib. 1c), after which the number of square millimeters occupied by the whole aorta, and then the number of these on the various atheromatous elements, are counted.

Method of direct planimetry. This method is more precise and more complicated than the preceding method. A planimetric grid is placed beforehand on the transparent film, and through it is counted the number of square millimeters occupied by atheromatous elements, without any transfer of their contours to the film (Fig. 1d).

The use of a planimeter does not give a more accurate estimation of the degree of atherosclerosis than by means of the planimetric grid, because in this case too, the difficulty remains of determining separately the area of each of the smaller atheromatous plaques, on account of the inertia of the apparatus.

Experience of the use of all these methods in practice, in several tens of experiments, enables us to recommend them, although none of them is absolutely accurate. The gravimetric method is less accurate but quicker; the method of direct planimetry is more accurate and less rapid.

SUMMARY

Several planimetric methods are presented by means of which the degree of atherosclerosis may be represented by atherosclerotic formulae. This facilitates the objective evaluation of experimental atherosclerosis.

LITERATURE CITED

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