TISSUE CULTURE OF ADULT RABBIT AND HUMAN AORTA

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Tissue culture methods have frequently been used in studying the problem of atherosclerosis [2, 5, 11] and have enabled us to make a new approach towards the elucidation of the various pathogenetic mechanisms which lie behind atherogenesis [1]. Several research workers have shown that in the initial stages of atherosclerosis of the large vessels, the pathogenetic mechanism concerned is one of adipose infiltration of the intima involving the macrophages of the intima and that this mechanism, together with the effects of various chemicals and drugs on the process, can be studied successfully "in vitro" [2].

As a result of experiments carried out in this institute we have evolved an original method for culturing tissue derived from the intact ascending aorta of adult rabbits and from the aorta of rabbits with experimentally induced atherosclerosis. Our method employs exclusively homologous ingredients in the nutritive medium and can also be used for the culture of human aortic tissue removed during an operation for coarctation of the aorta.

EXPERIMENTAL METHOD

Cultures of Rabbit Aorta. Blood serum and heparizined plasma was prepared from rabbit blood obtained by aseptic bleeding of the animal through the carotid artery. The plasma was inactivated before use by heat treatment at 55° for 35 min. In order to prepare rabbit heart extract, the tissue was cut into small pieces with scissors and washed with medium 199, an equal volume of this medium was added and the whole maintained in a thermostatically controlled incubator at 37° [6] for 2 h. It was later kept for 24 h at 4°, the supernatant liquid sucked off and preserved at −20°. Before use, the extract was centrifuged for 20 min at 2000 rpm.

The ascending thoracic aorta was removed from the rabbit aseptically, freed from its adventitia and washed in medium 199 with plasma. Pieces of about 1.5 mm diameter were explanted onto a thin layer of medium in Carrel's flasks. This medium consisted of rabbit plasma diluted with 199 in proportions of 3 : 2, together with 1 drop of heart extract. The sealed flasks were placed for 24 h in an incubator at 37°. The following day each flask received 1-2 drops of fresh, diluted plasma and 3 h later liquid phase, consisting of 20% plasma, 10% heart extract and 70% medium 199. The liquid phase was changed at 2-day intervals after this. The pH of the medium was regulated by blowing expired air through the liquid in the flasks or by adding a 1.4% solution of sodium bicarbonate. The tissue was cultured in this way at 37° for a period of up to one month.

In some of our experiments the aortic cultures were maintained without using heart extract. This led to more rapid and intense liquifaction of the plasma coagulum and satisfactory results were obtained from such experiments only if fresh plasma were added to the flasks 2 days after explantation of the tissue into rabbit plasma diluted with medium 199. The liquid phase of cultures without extract consisted of 20% rabbit plasma and 80% medium 199; it was changed every three days.

In several experiments involving the culture of atherosclerotic aortic tissue from the rabbit we used hyperlipemic rabbit plasma, containing cholesterol (the cholesterol content of the plasma amounted to 545-940 mg%).

The cultures were stained with hematoxylin-eosin and also with a mixture of Sudan III and Sudan IV in Hexheimer's solution [10].

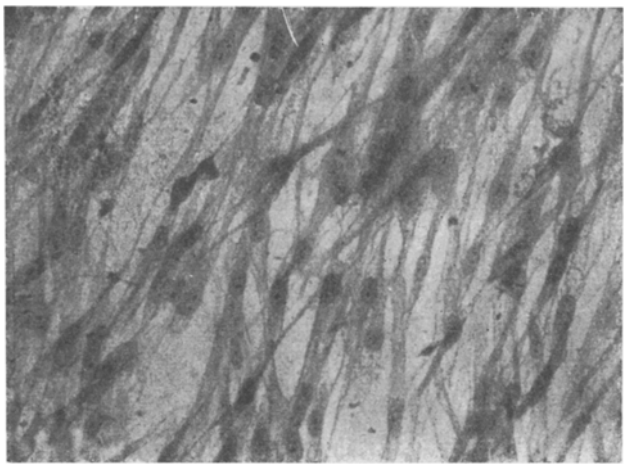

Fig. 1. Culture of intact aorta from a 6-year old male rabbit. Cultured without using extract (23rd day). Microphotograph. Delafield's hematoxylin-eosin, 40 × 7.

Fig. 2. Culture of rabbit aorta with experimental atherosclerosis. Cultured in nutritive medium with hyperlypemic plasma (14th day). Microphotograph. Sudan III and Sudan IV, 16 · 7.

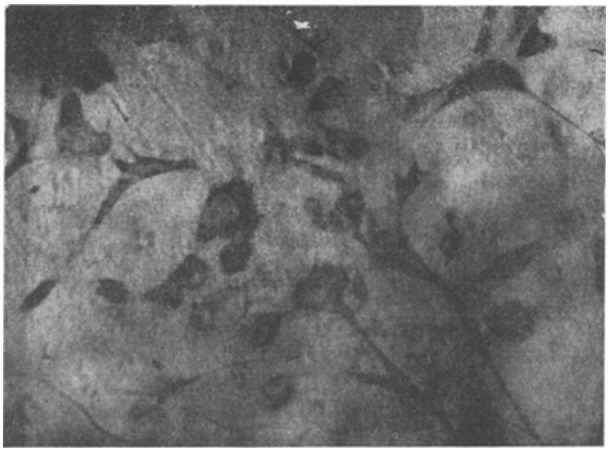

Fig. 3. Culture of human aorta (donor of aorta-patient R 31 years old). 14th day of culture. Microphotograph. Ehrlich's hematoxylin-eosin, 40 · 7.

Cultures of Human Aorta. The portion of aorta removed during an operation for aortic coarctation and freed from its adventitia was washed in medium 199. Pieces approximately 1.5 mm in diameter were then explanted into Carrel's flasks. The explantation was made onto a thin layer of mixed human and chick plasmas (1 : 1), diluted with medium 199 (3 : 2) and containing 1 drop of rabbit heart extract or chick embryo extract; it was maintained there for 10 days. Fresh mixed human/chick plasma (1-2 drops per flask) was added every day during the first 3 days following explantation. Liquid phase (30% inactivated human plasma, 10% extract and 60% medium 199) was added one day after explantation and changed three times a week. The pH of the nutritive medium was kept at a level of 7.2-7.4. Cultures were maintained for one month at 37°.

EXPERIMENTAL RESULTS

The latent period in culturing rabbit aorta tissue using heart extract is 2-3 days and in culturing the tissue without extract 3-5 days; in culturing tissue from the human aorta the latent period is 7-14 days.

Between the 8th and 10th day a connective tissue growth zone appears around the explant of rabbit aorta and this is characterized by the polymorphism of its cellular constituents. On staining with hematoxylin-eosin the growth zone is seen to consist mainly of fibroblast-like cells of variable size and shape (Fig. 1). Some of these cells resemble endothelial cells in their form and manner of distribution. Macrophage-like cells are encountered rarely. A zone of dense cytoplasm (endoplasm) occurs around the nucleus of many cells.

Cultures of rabbit aorta with experimentally induced atherosclerosis are distinguished by their more loosely aggregated cellular elements, among which predominate rounded or polygonal cells with sharply vacuolated cytoplasm and with the nucleus often occupying an eccentric position. This type of cell is particularly abundant in the initial stage of culture.

When rabbit aorta with experimentally induced atherosclerosis is cultured in media containing hyperlipemic plasma, vacuolation of the cells in the culture is more common and cellular degeneration is more marked.

When atherosclerotic aortic tissue is cultured in the presence of normal plasma and then stained for fat, the staining is less intense than in cultures of intact rabbit aorta maintained for the same period. This is possibly due to a tendency for normal plasma to remove fat from atherosclerotic aortic tissue. Among cells of cultures maintained in hyperlipemic plasma (Fig. 2), much greater amounts of fat appear than in cultures of the same aorta maintained in normal plasma.

The cells of the growth zone in cultures of human aorta (Fig. 3) are arranged loosely during the initial stages of their development. They are of rounded or polygonal form with 2-3 (or more) oval nuclei, each containing 1-2 nucleoli, and with a broad layer of finely vacuolated cytoplasm. Ultimately the tissue is characterized by the development of a number of fusiform cells.

The culturing of aortic tissue from the adult rabbit and the adult human has hitherto been successfully carried out on few occasions [8]. One of the first described attempts to culture rabbit aorta was that of Busse [3]. Haagen and Schlossberger [4] obtained better results by explanting rabbit aortic tissue on a medium consisting of rat plasma and mouse spleen extract. In Shibuiya's experiments the best results were obtained with homologous plasma and suprarenal gland extract. Kokubu and Pollak [5] cultured intact rabbit aortic tissue and rabbit aortic tissue with experimentally induced atherosclerosis in heterologous nutritive media. The method we have described differs in being comparatively simple and economical. All the procedures necessary for culturing and making histological preparations of the tissue may be carried out in the course of a normalworking day by two persons.

Cultures of the adult human aorta were first obtained by Peirce and coauthors [9]. These workers cultured aortas removed from cadavers during the first hour after death. Aortic tissue resected during the operation for coarctation of the aorta was successfully cultured by Ingenito and others. These authors explanted pieces of aorta on chick plasma, to which was added a mixture 40% human plasma, 20% chick embryo extract and 40% Hank's solution after the original chick plasma had coagulated. This nutritive medium was changed three times a week. Initial growth of the cultures occurred 3-4 weeks after explantation or even later.

SUMMARY

The author describes a method used for culturing the tissue of intact ascending rabbit aorta, as well as of ascending rabbit aorta with experimental atherosclerosis (without adventitia). Bits of these tissues were cultured in Carrel's flasks containing homologous nutritive ingredients. A method of culturing the tissue of human aorta, resected during the operation for coarctation of the aorta is also described. In culturing the rabbit aorta with experimental atherosclerosis in the presence of normal serum the degree of cellular adiposity was less marked than that observed in the cultures of intact aorta at the corresponding periods. In culturing the atherosclerotic rabbit aorta with hyperlipemic serum there was a much greater degree of lipoidosis and a greater intensity of cellular degeneration than in the cultures of the same aorta grown in the presence of normal serum.

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