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Viability Assessment of Allograft Valves by Autoradiography

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Autoradiography using [^{3}H]thymidine is a quantitative method to assess tissue viability. It has been found to be very useful in the organization of fresh aortic valve allograft banks. A brief description of the technique, as well as the marked difference in the active metabolism of fibroblasts of valves placed in two storing solutions, is outlined.

INTRODUCTION

Major problems confronting the field of transplantation surgery, apart from immunological concerns, are organ procurement and organ preservation. The full benefits of tissue transplantation can only be realized if long-term storage of viable organs can be accomplished. This objective has been attained in the case of cellular elements such as blood corpuscles and sperm. However, in whole organ transplantation the results of techniques to prolong preservation are far from successful. A related problem in this regard is the difficulty of designing reliable methods to assess tissue viability. The importance of using viable renal and cardiac allografts needs no emphasis. Methods to assess viability in these situations have been summarized by Abouna (1). In cardiac valve replacement using biological tissue, the importance of viability has not been sufficiently emphasized, even though there is ample evidence that viable allograft valves give superior results (3,8). Therefore, it becomes necessary to devise useful methods which give reproducible results for assessing the viability of stored tissue valves, since use of fresh and immediately harvested human allograft valves is logistically almost impossible. We have used autoradiography in the assessment of tissue valve viability (2,4), and this article summarizes our results and emphasizes the relevant importance of the method.

MATERIALS AND METHODS

The use of autoradiography for tissue valve viability assessment has previously been described (2) and is a modification of the method proposed by Kopriwa and Leblond (7). Viability of 126 human allograft aortic valves preserved in Hanks' solution, and 90 valves stored in nutrient medium have been assessed. The valves were obtained from routine postmortem material 24 to 48 hr after death and without any attempt at sterility. They were then preserved in Hanks' solution or nutrient medium 199 with 10% calf serum. All valves were stored at 4° C. Both solutions contained antibiotics for valve sterilization. Viability was assessed by autoradiography prior to and at regular intervals after sterilization and preservation.

At the time of autoradiography, pieces of aortic arterial wall and leaflets from the allograft valve are incubated at 37° C for 48 hr in nutrient solution 199. To this solution 10% calf serum and 5% methyl-tritiated thymidine are added. After the period of incubation, the tissues are washed twice with unlabeled nutrient medium at 2-hr

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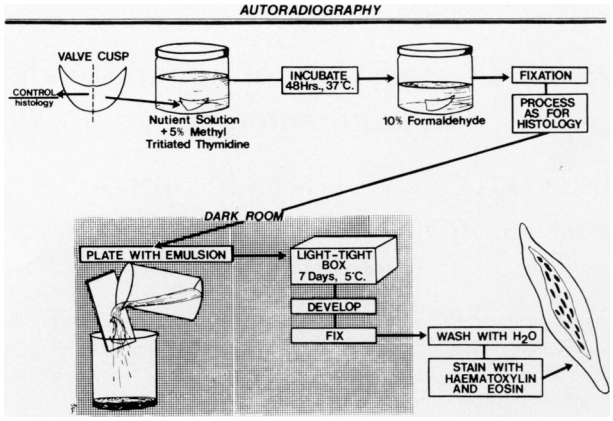

FIGURE 1

intervals to remove excess tritiated labeled medium. Next, the tissues are fixed in 10% formalin and subjected to routine histological procedures. A thin film of emulsion (Ilford K2 nuclear emulsion)[2] is plated over the tissue sections, allowed to dry, and then kept in light-tight boxes at 5°C for 7 days. The emulsion-covered sections are then developed with Ilford 1-19[2] and Ilford 1-F2[2] solution. Sections are then stained with hematoxylin and eosin and Giemsa. The above steps in the technique are shown in Fig. 1.

Thymidine, a component of deoxyribonucleic acid, can be traced readily when it has been labeled with a radioactive ion (Fig. 2). It becomes firmly bound in the nucleus of metabolically active fibroblasts, while the unincorporated tritiated thymidine is rapidly catabolized and removed during the histologic procedures. Rays emitted by

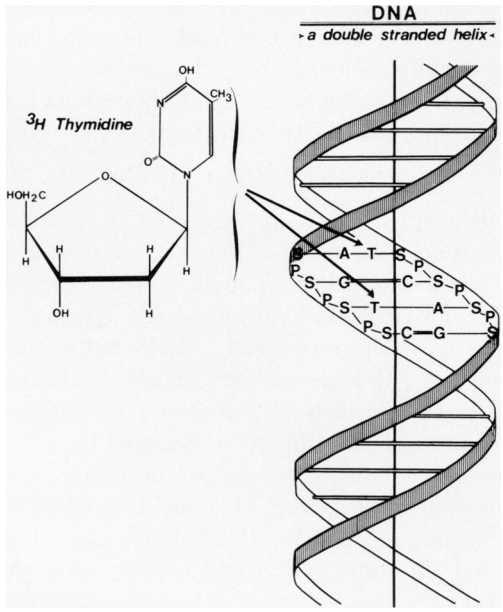

FIGURE 2

[2]Ilford, Ltd., Ilford, Essex, England.

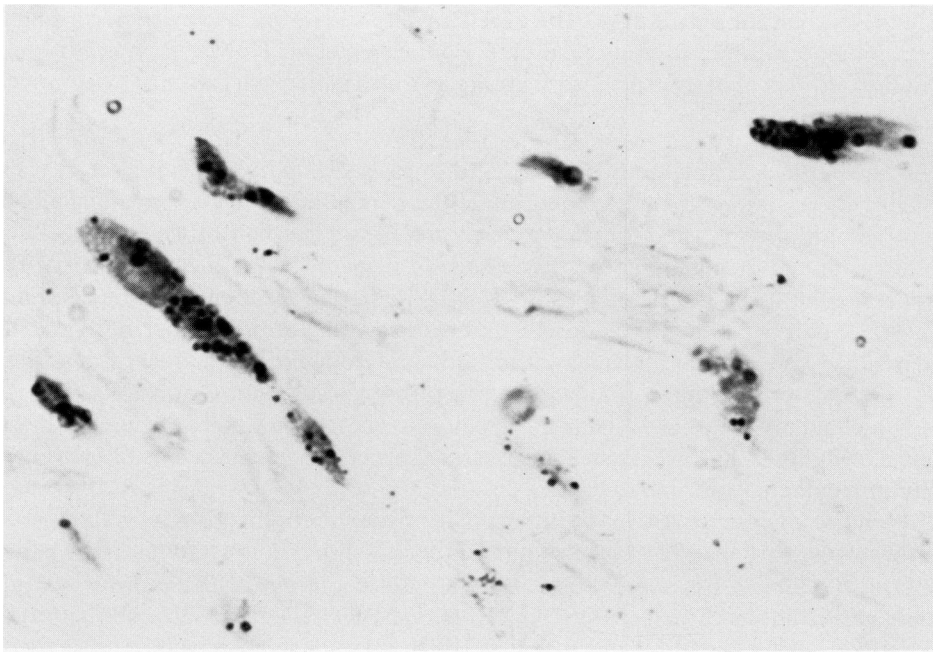

FIGURE 3

the incorporated radioactive thymidine in the nuclei of metabolically active fibroblasts strike the overlying emulsion and become visible as black granules on light microscopy (Fig. 3). The percentage of viability for a given section is calculated by relating the number of labeled fibroblasts to the total number of fibroblasts in that field. The mean value of 20 random fields determines the percentage of viability for a given section.

RESULTS

The results are summarized in Fig. 4. About 70% of the fibroblasts are viable at 24 hr of storage in either medium, but thereafter the percentage of viable cells in Hanks' solution starts diminishing markedly as compared to those valves stored in nutrient medium. At 8 weeks, the percentage of viable cells in allograft valves stored in nutrient medium is comparable to the percent viability of valves preserved in

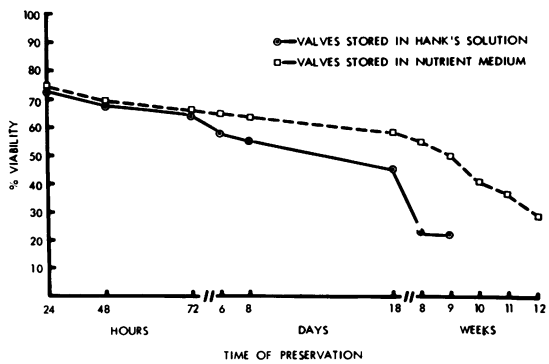

FIG. 4. Comparison of thymidine uptake in homograft valves stored by the two methods.

Hanks' solution for only 8 days. The percentage of sterile valves varied according to the antibiotic formula used in each preservation medium. However, the pattern of viability outlined above was true with sterile as well as unsterile valves.

COMMENT

Tissue culture techniques have been used to assess viability (9). However, it is not possible by this method alone to determine the percentage of cells remaining viable. Autoradiography, on the other hand, is an important method of assessing cellular metabolism and quantitating the percentage of living cells in a given tissue (6). The importance of viability assessment is twofold. First, the technique can be helpful in the evaluation of various methods of tissue storage in terms of their potential for maintaining viability and, thus, can lead to the development of an ideal or near ideal way of tissue preservation. It should be remembered, however, that viability assessment techniques for whole organs may require a greater and more stringent physiological evaluation than are needed for assessing parts of organs, such as tissue valves, cellular elements, bone marrow, etc.

Second, it can demonstrate the importance of viability in the long-term function of tissue valves. Although there are no hard data on long-term function of tissue valves in terms of viability assessment by autoradiography, there is sufficient evidence that viable valves do much better than nonviable ones. For allograft valve implantation, all methods of preservation which render the tissues nonviable have been discarded. After a review of 311 patients undergoing allograft valve replacement and followed for up to 10 years, Ross and associates (8) concluded that fresh or fresh-frozen valves are superior to freeze-dried (nonviable) valves. Barratt-Boyes and colleagues (3) have drawn the same conclusion as far as use of viable allografts is concerned, and this is substantiated by the less satisfactory results with valves in an earlier series which were sterilized by ethylene oxide and preserved by freeze-drying (5).

In conclusion, autoradiography, using tritiated thymidine, is a useful and reliable method for assessing the percentage of viable cells in a given allograft valve. The application of this technique should help the selection of high quality valves for clinical use.

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