Respiration and Anaerobic Glycolysis of Transplanted Cartilage.* (19416)

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Current knowledge concerning the viability of costal cartilage following transplantation has been derived primarily from static gross and histologic studies. Reports on the metabolism of these grafts are entirely lacking. The present investigation was therefore undertaken in order to study and compare the respiration and anaerobic glycolysis of fresh costal cartilage with that transplanted to another area in the same animal (autografts), as well as between different animals of the same species (homografts).

Material and methods. Non-littermate, female albino rabbits, approximately 3 months of age and 2 kg in weight, were selected for these studies. Costal cartilage was removed from the donor rabbits under aseptic conditions, carefully stripped of perichondrium and embedded subcutaneously in the abdominal wall of the recipient animals (53 autografts and 53 homografts). The grafts were removed at intervals of 7, 11, 15, 30, 60, 90, 120, and 150 days following transplantation. Generally, at least 3 transplants were tested at each of the specific time periods. A series of 20 determinations on costal cartilage immediately following its removal from donor animals served for the comparison of fresh with transplanted tissue. Respiration and anaerobic glycolysis were studied by standard manometric methods. After the removal of any adherent connective tissue, the cartilage was sliced in cross-section to a thickness of 0.3 mm by means of a modified microtome and approximately 250 mg was placed in each flask. The suspending medium for the tissue slices in the aerobic determinations consisted of 2.0 ml of Krebs-Ringer phosphate solution, 0.2 ml of 0.001 M aqueous methylene blue, and 0.2 ml of 10% glucose. For the absorption of carbon dioxide, 0.2 ml of 2 N KOH together with a small piece of fluted filter paper were placed in the center well of each flask. The manometers were gassed for 10 minutes with pure oxygen bubbled through water. The suspending medium for the anaerobic investigations consisted of 2.0 ml of Krebs-Ringer bicarbonate solution, 0.4 ml of distilled water, and 0.2 ml of 10% glucose. The manometers were gassed for 10 minutes with a water saturated mixture of 95% nitrogen and 5% carbon dioxide. The flasks were maintained at approximately 38°C. After the manometers were allowed to equilibrate for 15 minutes, readings were taken at 15-minute intervals over a 2-hour period. Oxygen consumption (Q_{O_2}) and anaerobic glycolysis (Q_G^N2) were expressed in terms of microliters per hour per mg of dry tissue.

Results. The results obtained from the study of respiration and anaerobic glycolysis of fresh costal cartilage appear in Table I. Because of the low rate of metabolic activity of the cartilage slices, it was found necessary to read the manometers at prolonged intervals so as to record significant variations. Moreover, since the oxygen uptake of the tissue was quite small, 0.2 ml of 0.001 M aqueous methylene blue was added to each aerobic system in order to increase further the magnitude of the manometric changes.

In the 20 determinations on fresh cartilage

TABLE I. Comparison of Respiration and Anaerobic Glycolysis of Fresh and Transplanted Rabbit Costal Cartilage.*

Costal cartilage	No. of specimens	Mean Q_{O_2}	No. of specimens	Mean Q_G^N2
Fresh	10	$-.41 \pm .04$	10	$+.70 \pm .08$
Autograft	25	$-.23 \pm .08$	28	$+.46 \pm .12$
Homograft	28	$-.22 \pm .07$	25	$+.40 \pm .13$

* The mean Q_{O_2} and Q_G^N2 values for autografts and homografts differed from the values of fresh cartilage at less than 1% level of probability.

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from different rabbits, the mean Qo_2 was found to be —0.41 ± 0.04 while the Q^N_G2 figure was +0.70 ± 0.08. The fresh cartilage slices were found to retain approximately 32% of their weight following drying. After implantation for 7 days, the mean rate of respiration for the autografts was —0.26 while the value for the homografts was —0.32. From 7 to 150 days after transplantation the mean Qo_2 values for the autografts varied between —0.13 and —0.29. In the homografts, the rates ranged from —0.16 to —0.28.

The mean Q^N_G2 values were consistently higher than the corresponding Qo_2 figures. After the 7-day implantation period, the mean rates of anaerobic glycolysis for the autografts and homografts were +0.50 and +0.54 respectively. During the interval from 7 to 150 days the values varied from +0.34 to +0.57 for the autografts and +0.29 to +0.50 for the homografts.

Upon drying, the autografts retained 32.3 ± 1.6% of their weight, while the homografts retained 32.9 ± 3.1%. Because of the similarity of the figures, 32% was used as the representative value for the entire series.

Discussion. Fresh cartilage possesses an extremely low rate of metabolism (1-7). The present study corroborated this finding. Following transplantation, the rates of respiration and anaerobic glycolysis were even lower than those of fresh cartilage. At the end of the first 7 days a marked decrease was noted in all of the grafts. From 7 to 150 days, however, statistical analysis revealed no significant difference between the values for consecutive time periods in either the autografts or homografts. The results in each series were therefore grouped irrespective of the time elapsed since implantation (Table I). Comparison of the collective values revealed that there was no salient difference between the metabolism of cartilage autografts and homografts in the rabbit (t value 0.50 for Qo_2, 1.75 for Q^N_G2; 53 degrees of freedom).

The fact that the autogenous and homogenous transplants gave well-defined values for both respiration and anaerobic glycolysis denoted the presence of living chondrocytes. This substantiated the previous gross and histologic evidence that both types of grafts remained in a viable state following transplantation (8-10). The relatively higher Q^N_G2 values recorded from these implants indicated, moreover, that the carbohydrate metabolism of both fresh and transplanted cartilage was predominantly anaerobic. This was in contrast to the findings reported for most other tissues.

The lowered Qo_2 and Q^N_G2 values displayed by these grafts could be partially attributed to a decline in the metabolism of the chondrocytes following transplantation. The decrease in the metabolic activity per cell, however, was not actually as great as the data would seem to indicate, because the Qo_2 and Q^N_G2 values were computed from the weight of the graft without consideration of its viable cell content. In a firm structure such as cartilage cellular necrosis can occur without significant weight loss since the matrix remains relatively intact. Thus, two grafts of equal weight could have entirely different viable cell counts and, while the metabolic quotients obtained from each would be different, the metabolism of any cell in either graft could be the same. In this investigation a decrease in the number of vital chondrocytes probably occurred in all of the grafts not only from the changes in metabolic activity during and after transplantation but also from the trauma incident to the surgical handling of the tissue. This decrease, therefore, would account for at least a portion of the diminished metabolism recorded from the cartilage grafts.

Summary. 1. The rate of respiration and anaerobic glycolysis of fresh rabbit costal cartilage is among the lowest of all tissues. Carbohydrate metabolism is predominantly anaerobic. 2. Transplantation resulted in approximately a 45% decline in the rates of both respiration and anaerobic glycolysis in both the autografts and homografts during the first 7 days. The major portion of the metabolic activity, however, remained on an anaerobic basis. 3. After 7 days no significant variations occurred in either the Qo_2 or Q^N_G2 values for any of the grafts. 4. No salient difference was observed between the metabolic activity of costal cartilage autografts and homografts in

the rabbit from 7 to 150 days after transplantation.

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Inhibition of Utilization of Glutamic Acid by *Lactobacillus arabinosus*.* (19417)

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Patterns of free amino acids determined by 2-dimensional paper chromatography were studied in a variety of mouse tumors, comparing the neoplastic with normal homologous tissues(1,2). It was found that each normal tissue examined had a distribution of free amino acids characteristic for that tissue, while all of the tumors, regardless of origin or source, showed very similar patterns. One of the most interesting findings was that the glutamine levels in the tumors were uniformly lower than in most of the normal tissues. It appeared feasible to attempt to disturb the protein synthetic mechanisms in tumors by interference with glutamine metabolism, since glutamine may play a strategic role in protein synthesis by virtue of its participation in amide exchange reactions(3-5). Most normal tissues, which have relatively large amounts of free glutamine, would presumably be more resistant to such an interference.

Glutamine metabolism could be disturbed by inhibiting the synthesis of glutamine or by blocking its subsequent utilization in further metabolic processes. *Lactobacillus arabinosus* 17-5 can serve as a good test organism for screening agents which can act in either manner, since this organism can be used both for glutamic acid and for glutamine assay. The present communication describes the results of the tests of several compounds in these assay systems. Methionine sulfoxide and β-hydroxyglutamic acid, previously reported antimetabolites for glutamic acid in *L. arabinosus*(6,7), and γ-glutamylhydrazide, a glutamine antagonist for some strains of hemolytic streptococci(8), have also been studied.

Experimental, assay methods. The essential microbiological procedures have all been described previously(9) and references to the original literature given. All glutamic acid assays were performed at pH 6.0 in a low aspartic acid medium(10) (Medium 1), conditions which minimize lag. In most instances the glutamine assays were performed at pH 7.0 in a high aspartic acid medium(11) (Medium 2), under which circumstances there is a sharp differentiation in response to glutamine and glutamic acid in the low concentration ranges. In other experiments the same conditions were used for glutamine as for glutamic acid. Solutions of all of the substances tested and glutamine were sterilized by Seitz filtration. When determination of growth was made at only one time interval, the turbidity readings were made at 24 hours. Several experiments were performed in which readings were made at numerous time intervals. All

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