and the durations of the hindleg components of the "first" seizure recorded. At various time intervals after the "first" seizure, a "second" supramaximal electroshock was given and the durations of the hindleg components of the "second" seizure recorded. This procedure was repeated, with longer time intervals between seizures, until the observed durations of the "second" seizure components were not significantly different from their "first" seizure values. In addition, the time (RT_{50}) required for 50% of animals to recover ability to exhibit a second tonic-clonic seizure after a first was determined. The data obtained may be summarized as follows: The order in which the durations of the various components and the total duration of the "second" seizure returned to their "first" seizure values were the same in both groups: total seizure duration, terminal clonus, tonic extension, and tonic flexion. The total duration of a maximal seizure is relatively independent of marked changes in duration of the individual seizure components. Dilantin prolonged the recovery time of terminal clonus, tonic extension, and tonic flexion by approximately 300%, 150%, and 170%, respectively. In addition, Dilantin prolonged RT_{50} by 325%. Evidence is presented which suggests that Dilantin acts to prolong the latent period before recovery of the tonic-extensor component starts, and that the recovery process *per se*, once initiated, is relatively unaffected by the drug.

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Dehydrogenase Activity of Rat Epiphyseal Cartilage.* (22763)

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Some years ago dehydrogenase activity was demonstrated in epiphyseal cartilage, using succinate and citrate as substrates and the decolorization of methylene blue as an indication of enzyme action(1). This technic does not allow for any precise localization of the enzymes in the cell. Hence, the application of newer technics utilizing tetrazolium compounds was undertaken, since so little is known about the metabolic activities of growing cartilage.

Materials and methods. Young (40-60 g) rats were killed by a blow on the head. After the lower extremity was dissected of muscle, free hand sections, as thin as possible, were made with a razor through the epiphyseal cartilages of the upper tibia or lower femur. Such slices were washed briefly in saline to remove any blood and were placed in depression slides containing 4 drops of .2M phosphate

TABLE I. Degree of Tetrazolium Reduction in One Hour with Different Substrates.

Control	0
Succinate	+++
Succinate and malonate	0
Citrate	++
Isocitrate	+++
Lactate	+++
Malate	+++
Pyruvate	0
Glucose	0
Glutamate	0
Oxalacetate	+
Xanthine	0

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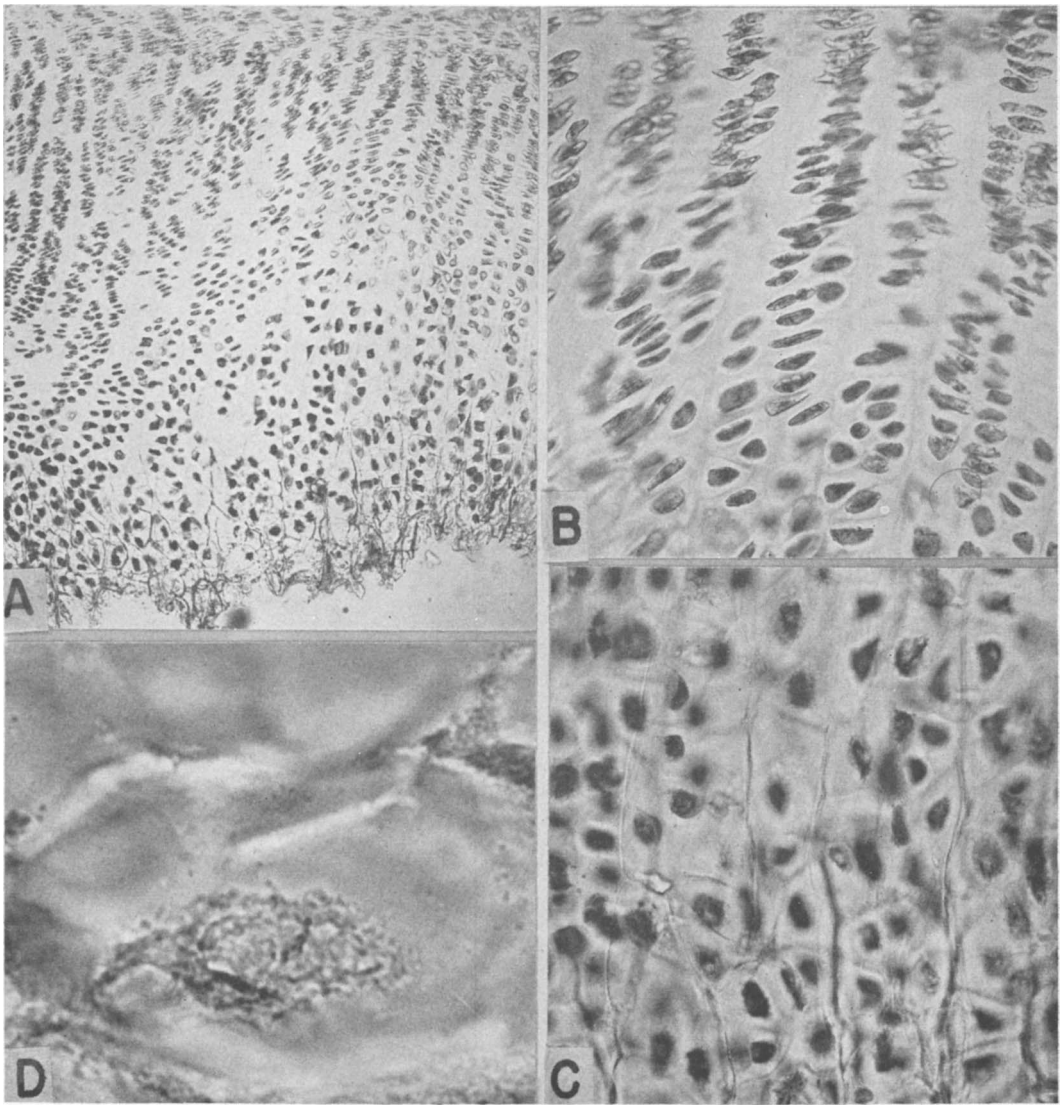

FIG. 1. Microphotographs of rat epiphyseal cartilage slices incubated with buffered sodium succinate and neotetrazolium chloride. A. Entire width of epiphyseal cartilage. ×75 (A.F.I.P. neg. 56-5005). B. Higher power of zone of proliferating cartilage cells. ×260 (A.F.I.P. neg. 56-5006). C. Higher power of zone of hypertrophic cells. ×260 (A.F.I.P. neg. 56-5007). D. High power of single cartilage cell to show rods and granules of formazan about non-staining nucleus. ×1400 (A.F.I.P. neg. 56-15407).

buffer of pH 7.4, 2 drops of .2M substrate in phosphate buffer and 3 drops of neotetrazolium chloride (0.1%) in phosphate buffer. Coverslips were applied, care being taken to exclude air; the preparations were incubated at 37°C. The following substrates were employed; glucose, succinate, malonate, malate, isocitrate, oxalactate, pyruvate, lactate, glutamate, xanthine, alpha-ketoglutarate and alanine. Control slices were incubated with 2 drops of buffer substituting for substrate. The preparations were observed under the microscope every half hour, generally for at least 2 hours.

Results. The distribution of enzyme activity with succinate as a substrate will first be described. Purplish granules of formazan pigment began to appear after one half hour of

incubation, reaching a maximum in 2 hours. Under the low power of the microscope such pigment deposits seemed to delineate the nuclei of the cartilage cells. Under higher power, however, these structures were seen to remain unstained; the granules and short rods of pigment were grouped around an unstained area (the nucleus) in the center of the cell. A wide clear zone was present between this pigment and cell membrane. Formazan pigment was found in the cartilage cells at all stages of development. During the time of observation no formazan pigment developed in the slices incubated with malonate or without substrate.

Variations in degree of intensity of the strain depending on the substrate used are shown in Table I. Those leading to the most pigment formation in the shortest period of time were succinate, isocitrate, citrate, lactate, and malate.

Discussion. These histochemical observations confirm the presence of dehydrogenase activity of epiphyseal cartilage described earlier(1) and amplify the few biochemical studies which have been carried out on articular(2) and epiphyseal(3,4) cartilages.

The epiphyseal and costal cartilages are unique in that they display all stages of differentiation and growth at a time when other tissues in the organism have matured. The needs of the epiphyseal cartilages for energy to grow must be great when it is realized that a rat's lower femur increases .18 mm in length per day(5). Hence, it is not surprising to find evidence for the active metabolism of certain compounds involved in the Krebs cycle. Exactly how such data may fit into the total metabolism of cartilage remains to be elucidated. These observations would appear to have no bearing on the calcification mechanism since activity was found in all strata of the epiphyseal cartilage. However, they provide some understanding for the presence of citric acid in cartilage(6) though they do not indicate what proportion may be utilized in the citric acid cycle and what amounts may be purely fortuitous, as in bone(7).

Summary. Utilizing the tetrazolium technic, intracellular dehydrogenase activity has been localized in epiphyseal cartilage cells. The most favorabe substrates appear to be succinate, isocitrate, citrate, lactate and malate.

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Effect of Aminonucleoside on Ribonucleic Acid Content of Mouse Mammary Adenocarcinoma *in vivo*. (22764)

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Puromycin (6-dimethylamino-9-[3'-deoxy-3' (p-methoxy-L-phenylalanylamino)-β-D-ribofuranosyl]purine), an antibiotic isolated by Porter *et al.*(1), and structurally characterized by Waller *et al.*(2) has been shown by Troy *et al.*(3) to inhibit the growth of certain experimental tumors. It has further been shown by Oleson *et al.*(4) that the carcinostatic activity for mouse mammary adenocarcinoma resides in the aminonucleoside portion of puromycin. The structure of the aminonucleoside suggests that its antitumor activity could involve nucleic acid metabolism. To test this hypothesis, C3H mammary adenocarcinomas were analyzed for RNA content and composition, and compared to the same tumor