TABLE I. Concentration of Aureomycin in Embryo Tissues.*

Time (hr)	Allantoic fluid† (γ/ml) 9 day	11 day	Allantoic membrane† (γ/ml) 9 day	11 day	Plasma‡ (γ/ml) 11 day			Organs (γ/ml) Embryo	Liver 11 day	Brain 11 day	Heart
0	150	75			6.2	.2	6.2				
1	125		5.2				0				
2		45		1.7	1.5	.1	12.5	0	.2	0	0
3	50						12.5				
4				1.0	12.5	<.1	0				
6	150	20	4.0	1.7		<.1	1.5	1.5	.2	0	0
8	100	12				<.1					
20		7			0						
25	50	7	6.3	1.5	0	<.1		0.37	.2	0	0
48	40	2	7.0	.5				0	0	0	0
72	12		4.0								
96		2		.37				0	0	0	0
120	7		1.5								
144		0.75		.2					0	0	0

Sensitivity varied from 0.05 to 0.1 γ/ml.

* In most instances, crystalline aureomycin + glycinate buffer was used (unbuffered crystalline aureomycin was used in two instances with essentially the same results).

† Determinations on fluids and membranes were carried out in several experiments, and most figures represent the average of two or three results.

‡ Because of irregularities, three individual experiments (corrected for dilution factor) are shown.

the exact amount of the drug which is associated with the tissue. However, it can be seen that a continuous decrease in amount of aureomycin occurred with the increase in time.

The results of assays on plasma appear somewhat irregular. It would seem, however, that drug was present in the blood stream but for a short time.

Of the organs tested, the liver was the only one which showed any detectable amount of aureomycin. This was an exceedingly small quantity. No organs other than the brain, heart and liver were harvested because of the difficulty in distinguishing them in these young embryos.

Summary. Data were presented on the distribution and persistence of aureomycin in the allantoic fluids and tissues of 9- and 11-day-old embryos following injection of the drug into allantoic cavity. The drug was found associated with allantoic fluid, membrane, blood and liver, but not with brain or heart. The amount of drug found following injection decreased with an increase in time.

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Influence of Oxygen Tension on Tetrazolium Reduction by Epiphyseal Cartilage of Rachitic Rats *in vitro*.* (20174)

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Little is known concerning oxidative processes in cartilage. Oxygen uptake has been demonstrated(1-3) but in amounts so small as to be comparable only with the oxygen consumption of mammalian erythrocytes. Articular cartilage of the horse and of the rabbit has been shown to reduce methylene

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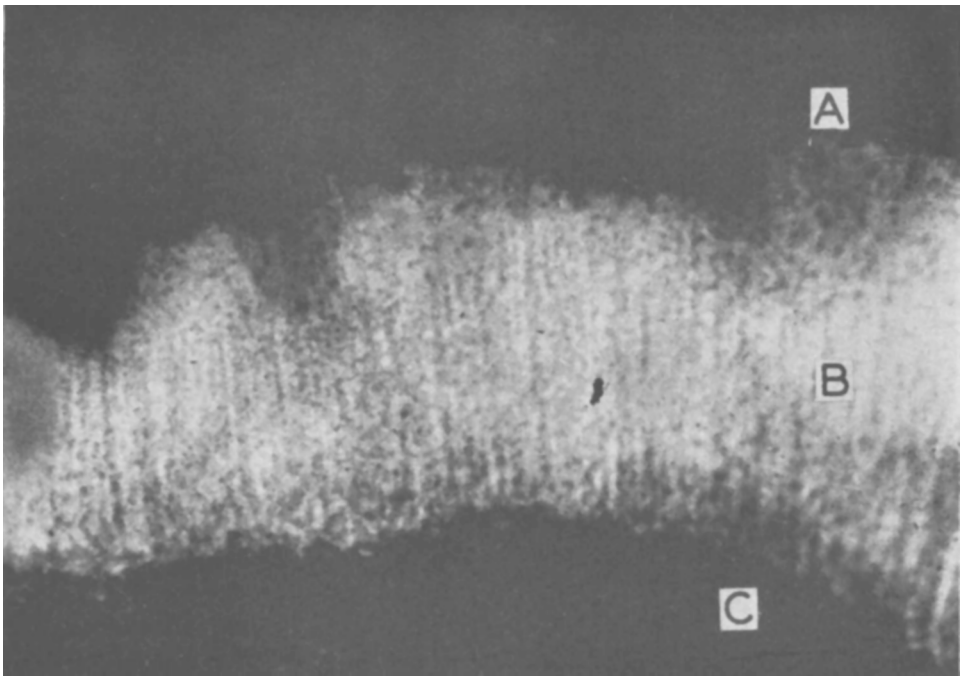

FIG. 1. Reduced triphenyltetrazolium chloride (TTC) deposition in epiphyseal cartilage cells of a rachitic rat bone slice after 1 hour incubation at 37°C in 0.5% TTC-Ringer-phosphate in an atmosphere of 100% nitrogen. A—Epiphysis. B—Epiphyseal cartilage. C—Metaphysis.

blue, with a concomitant increase in oxygen consumption(2). Only traces of cytochrome have been seen on spectroscopic examination (3) and none was demonstrable histochemically(4). Follis(5) has shown both succinic and citric dehydrogenase activity in cartilage. Calcification of rachitic rat epiphyseal cartilage slices *in vitro* has been shown to proceed equally well in both oxygen and nitrogen(6). It has been shown(7,8) that 2,3,5,-triphenyl tetrazolium chloride (TTC), a colorless, water-soluble reagent, when incubated with viable tissue slices, is connected to a deep red, water-insoluble formazan which is deposited within the cytoplasm of the cell. This process is thought to occur as a result of its reduction by intracellular dehydrogenase activity. The reduction of ditetrazolium salts by liver homogenates(9) and of TTC by liver, kidney and adrenal slices(10) has been found to take place more actively under anaerobic than aerobic conditions. This phenomenon has been interpreted as evidence of a competition between the tetrazolium salt as a hydrogen acceptor and the cytochrome-cytochrome oxidase system(9).

In the present study TTC has been incubated *in vitro* with rat epiphyseal cartilage slices under anaerobic and aerobic conditions. A profound difference has been noted in the extent of TTC reduction by cartilage cells under these conditions, comparable to that observed with other tissues.

Methods. Thirty-day-old male rats (Carworth Farms), mildly rachitic after 7 to 10 days on the Schneider-Steenbock low-phosphorus diet(11), were sacrificed by a blow on the head, and contiguous thin slices of the upper ends of the tibiae were cut free-hand. These were placed in Erlenmeyer flasks containing 10 cc of Ringer-phosphate (pH 7.4) and 0.5% TTC. Following equilibration for 5 minutes with 100% oxygen or 100% nitrogen, incubation was carried out in a closed system for one hour at 37°C with constant slow shaking in the Warburg bath. Since the colored formazan fades on exposure to light, the bone slices were examined immediately

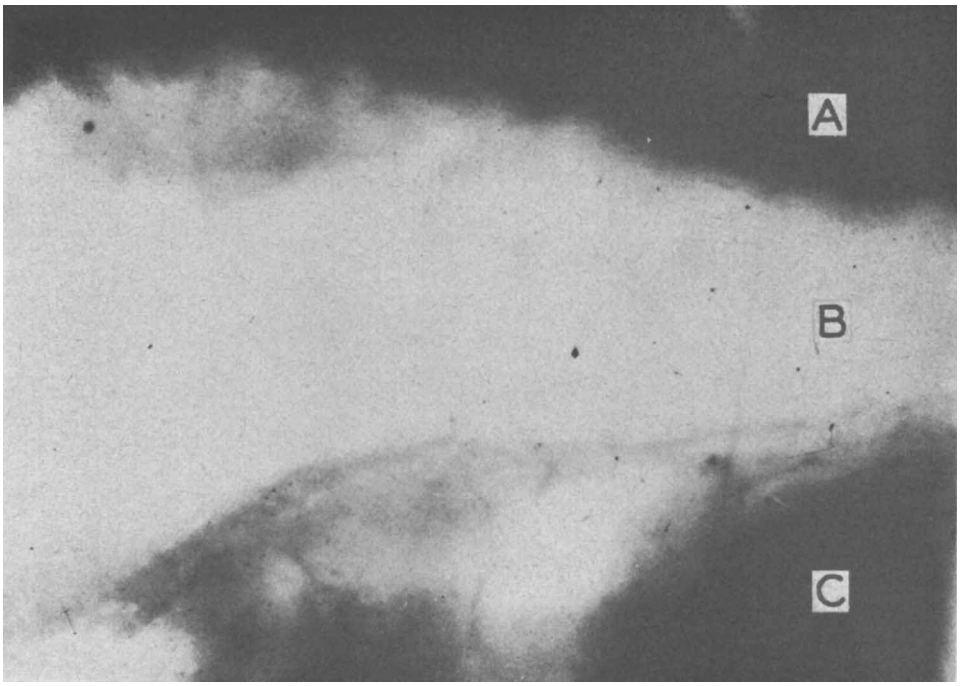

FIG. 2. Absence of reduced triphenyltetrazolium chloride (TTC) deposition in epiphyseal cartilage cells of a rachitic rat bone slice after 1 hour incubation at 37°C in 0.5% TTC-Ringer-phosphate in an atmosphere of 100% oxygen. A—Epiphysis. B—Epiphyseal cartilage. C—Metaphysis.

after incubation under 80x magnification for evidence of formazan deposition and photographed to obtain a permanent record.

Results. In slices equilibrated with an atmosphere of nitrogen during incubation a regular pattern of formazan deposition could be seen within the cartilage cells throughout the epiphyseal cartilage plate (Fig. 1). In contrast, there was virtually no deposition of formazan in the cartilage cells exposed to an atmosphere of 100% oxygen during incubation (Fig. 2). The difference was consistently noted in all 24 experiments (Table I).

The adjacent epiphyseal and metaphyseal bone was diffusely stained by the formazan, assuming a pink color during aerobic and a deep red color during anaerobic incubation. That this formazan deposition was attributable to the activity of bone marrow elements was evident from the absence of any TTC reduction on either aerobic or anaerobic incubation of bone slices from which the marrow had been removed.

TABLE I. Reduction of TTC by Epiphyseal Cartilage in Oxygen and in Nitrogen, 24 Exp.

Formazan deposition	Gas atmosphere	
	100% O_2	100% N_2
4+	0	24
3+	0	0
2+	0	0
+	8	0
0	16	0

Summary and conclusions. 2,3,5-triphenyl tetrazolium chloride when incubated with slices of epiphyseal cartilage obtained from rachitic rats is much more intensively reduced to its formazan in nitrogen than in oxygen. The reduced formazan is deposited within the cartilage cell. Based on the current interpretation of a similar phenomenon in other tissues, this observation provides additional evidence for the existence of oxidative enzyme systems in cartilage cells.

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