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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Received September 10, 1956. P.S.E.B.M., 1956, v93.

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

TABLE I. Purine-Bound Ribose Content of C3H Mammary Adenocarcinomas.

Group	Treatment	Mean tumor wt, mg*	μM PBR per g tissue†
1	0.2 ml 0.9% NaCl in-	143	5.0
2	traper. twice daily	352	6.2
3	for 2½ days	675	6.8
4		1800	6.3
5		2500	5.9
	Mean	1094	5.84‡
6	3 mg 2484L in 0.9%	220	2.8
7	NaCl intraper. twice	362	3.5
8	per day for 2½ days	625	3.8
9		1400	4.7
10		2320	4.0
	Mean	985	3.76‡

* Mean wt of similar sized tumors from 2 to 15 backcrossed C3H mice depending on tumor size.

† Micromoles of purine-bound ribose/g wet tissue.

‡ Difference between means of purine-bound ribose of the 2 treatments is statistically significant since $P < 0.008$ by analysis of variance.

from mice which had been treated with 6-dimethylamino-9- [3′-deoxy-3′-amino-β-D-ribofuranosyl]purine (2484L).

Methods. In order that an appropriate comparison might be made, the established tumors on backcrossed C3H mice were so selected that the control and 2484L treated tumors would be of approximately equal size at the end of the treatment period. Mice bearing much larger tumors were selected for 2484L treatment than those intended for no treatment. The aminonucleoside (2484L), pre pared by the method of Baker *et al.*(5) was injected intraperitoneally into the mice in five 1.5 mg doses in 0.9% NaCl at intervals of about 12 hours. At the end of the treatment period, the mice were decapitated and the tumors rapidly excised and frozen in dry ice. The frozen tumors were homogenized in cold 6% $HClO_4$ and the ribonucleic acid extracted by a modified Schmidt-Thannhauser-Schneider procedure(6). The RNA was estimated as purine-bound ribose by the orcinol reaction (7). A portion of the ribonucleotide containing extract was paper chromatographed and the component monoribonucleotides estimated by the method of Elson *et al.*(8).

Results. The RNA (purine-bound ribose) content of 2484L treated tumors was significantly less than that of untreated controls

TABLE II. Ribonucleotide Distribution in C3H Mammary Adenocarcinomas.

Group*	Treatment	Mean tumor wt, mg	Adenylic acid		Cytidylic acid		Guanylic acid		Uridylic acid		Total μM mononucleotide/g	Ratio, μM purine to μM pyrimidine
			μM/g†	Base ratio‡	μM/g†	Base ratio‡	μM/g†	Base ratio‡	μM/g†	Base ratio‡		
1	.9% NaCl	143	2.30	1.00	3.33	1.45	4.00	1.74	1.59	.69	11.11	1.29
4		1800	2.63	1.00	3.92	1.50	4.34	1.65	2.36	.90	13.25	1.11
6	3 mg 2484L	220	1.20	1.00	1.64	1.37	2.08	1.73	.79	.66	5.71	1.36
9		1400	1.92	1.00	2.84	1.48	3.40	1.77	1.72	.90	9.90	1.17

* Group No. corresponds to tumor group in Table I.

† Micromoles/g wet tissue. All values are means of 3 determinations.

‡ Ratio of μM of base to that of adenine.

(Table I). The nucleotide composition of the RNA fraction of the tumors revealed no gross difference in base ratios (Table II). A comparison of the total ribonucleotide contents of these groups confirms the lower RNA concentration of the 2484L treated tumors. The effect of 2484L treatment on tumor RNA cannot be ascribed to hydration, since it was found that the total nitrogen per gram wet weight of similarly treated and control groups did not differ significantly. These results suggest that 2484L causes an inhibition of synthesis (or an acceleration of degradation) of RNA in C3H mammary carcinoma. Ledoux and Revell(9) have shown that the decrease in growth rate of an ascites tumor (Landschütz) is associated with a parallel decrease in RNA content under normal conditions of growth. Thus the inhibition of growth of this tumor by 2484L may be associated with the decrease in RNA content.

Summary. (1) The RNA content of 2484L treated C3H mammary adinocarcinomas was shown to be lower than that of untreated controls. (2) The nucleotide composition of both treated and control tumors appeared to be similar.

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Received September 11, 1956. P.S.E.B.M., 1956, v93.

Anaerobic Glycolysis of Brain Cortex Slices in Hyperthyroid and Control Rats.* (22765)

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The primary site of action of the thyroid hormone in the intermediary metabolic scheme cannot yet be considered as definitely established. An early theory first enunciated by Haffner shortly after the advent of the Warburg metabolic techniques, was that the thyroid hormone primarily affects a step involved in anaerobic glycolysis(1). This was disputed by McEachern who claimed that anaerobic glycolysis was not increased in the livers of rats made hyperthyroid, altho slices of such livers invariably showed increased oxygen consumption when compared with normal control liver-slice values(2). His data confirmed similar earlier work of Dresel(3). Furthermore Anselmino *et al.* had early claimed that spleen slices from hyperthyroid rats failed to show a rise in either oxygen consumption or anaerobic glycolysis in a comparison with normal controls(4). However, in view of a recent statement by Goldstein that anaerobic glycolysis is increased in hyperthyroid rats(5), it was decided to reinvestigate the problem of the relationship of thyroxin to glycolysis beginning with the anaerobic type and brain cortex slices for a reason to be discussed later.

Methods. L-thyroxine is dissolved in a

* Supported by research grants from Public Health Service, No. A-580, Amer. Medical Assn., Council on Pharmacy and Chemistry, William Volker Grant No. 4 and Am. Philosophical Soc.