have Essential Familial Hypercholesterolemia[3] and to represent the heterozygous abnormal of this condition.

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17289. Synthesis of Nucleic Acid and Phosphoprotein in Normal and Cancer Tissue Slices Studied with Radio Phosphorus.*

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Experiments have been performed using radioactive isotopes to study the synthesis of phospholipids and proteins in tissue slices and homogenates.[1-6] The synthesis of large molecules, *in vitro*, can be studied by the tracer technic even though they undergo a net degradation under the conditions of the tissue slice technic.[1]

In experiments reported here, radioactive phosphorus in the form of phosphate was used to study the synthesis of total nucleic acid and phosphoprotein in both normal and tumor tissue slices, by measuring the incorporation of radioactive phosphorus, under aerobic and anaerobic conditions and in the presence and absence of added glucose. Briefly, the experiments indicate the dependence of the incorporation on the presence of oxygen, hence presumably on energy yielding reactions. The inhibition of phosphate incorporation in the absence of oxygen is partially prevented in tumor tissue, by the addition of glucose, thus suggesting that anerobic glycolysis can also provide the necessary energy.

Experimental. White rats were decapitated and the liver or kidneys removed. The slices cut were about 0.3 mm in thickness[1] and weighed about 25-30 mg when dry. They were placed in 50 cc Erlenmeyer flasks in 5 cc of Krebs-Ringer bicarbonate solution[1] containing radiophosphate. The solution had previously been equilibrated with the same gas mixture, either 95% O_2:5% CO_2 or 95% N_2:5% CO_2, as was used in the flasks during the 2 hours of incubation with shaking at 37°C. The inhibition by N_2 was determined, in each case, on slices originating from the same organ or tumor.

The concentration of radioactive phosphorus in the Krebs-Ringer bicarbonate was approximately 1 microcurie per ml. In the experiments in which glucose was added to the Krebs-Ringer bicarbonate solution, its concentration was 0.2%.

Eleven normal rats and 9 rats bearing the transplantable carcinoma 256 (Walker tumor) were utilized. Care was exercised in sampling to avoid including any of the central necrotic regions of the tumor. The body weights of the normal rats employed were about 200 g, and of the tumor bearing rats, 120 g.

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[1] Taurog, A., Chaikoff, I. L., and Perlman, I., *J. Biol. Chem.*, 1942, **145**, 281.

[2] Melchior, J., and Tarver, H., *Arch. Biochem.*, 1947, **12**, 309.

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[5] Frantz, I. D., Jr., Zameenik, P. C., Reese, J. W., and Stephenson, M. L., *J. Biol. Chem.*, 1948, **174**, 773.

[6] Friedkin, M., and Lehninger, A. L., *J. Biol. Chem.*, 1949, **177**, 775.

After incubation, the nucleic acid and phosphoprotein were separated from all other phosphorus-containing substances, essentially according to the method of Schmidt and Thannhauser,[7] before counts were made. The contents of each flask were first repeatedly extracted with trichloracetic acid until the supernatant solution did not count above background, and the supernatants were discarded. In a similar manner the residue was then extracted with a mixture of alcohol and ether and finally with a mixture of chloroform and methanol; the chloroform-methanol supernatant never did count above background.

The residue, containing only total nucleic acid and phosphoprotein phosphorus, was dried, weighed, and dissolved in alkali; an aliquot of this alkaline solution was then counted. Complete solution by alkali did not uniformly occur—in some samples a small part of the residue remained finely suspended. However, since duplicates which varied in degree of solubility showed no significant discrepancy in counts, the phenomenon was ignored.

Results and discussion. The results obtained are listed in Table I. Each result is the average of 2 determinations. Each sample was counted until a total of at least 2000 counts was obtained; thus the standard error of counting in each case is less than 3%.

The results show, in the case of liver and kidney tissue, that the incorporation of radiophosphorus into nucleic acid and phosphoprotein was inhibited in nitrogen, the magnitude of the inhibition averaging about 50-70%.

The fact that no significant difference between the percentage inhibition in normal tissue as a function of the presence or absence of glucose was observed indicates that under the conditions of the experiments the absence of added substrate was not a limiting factor. In other words, perhaps more than enough substrate for anerobic glycolysis was already present in liver and kidney.

On the other hand in tumor tissue a considerable decrease in the percentage inhibition was observed when the results with

TABLE I.
Radiophosphate Uptake in Nucleic Acid and Phosphoprotein of Tissue Slices.

Tissue	Tumor			Kidney			Liver		
	% P^{32} uptake in O_2	% P^{32} uptake in N_2	% inhibition	% P^{32} uptake in O_2	% P^{32} uptake in N_2	% inhibition	% P^{32} uptake in O_2	% P^{32} uptake in N_2	% inhibition
With glucose	4.7	3.6	24	2.1	0.76	75	2.6	0.83	69
	4.0	3.4	15	2.4	0.93	62	3.2	2.1	40
	8.4	8.3	1.5				—	—	61
	4.2	2.8	12				—	—	32
	1.6	1.2	22						
			—			—			—
			Avg 15			Avg 69			Avg 51
Without glucose	3.1	0.37	94	1.1	0.42	60	3.1	1.7	46
	3.0	0.73	77	1.7	0.71	58	—	—	87
	2.0	0.85	59				3.6	0.85	75
	2.1	0.20	91						
			—			—			—
			Avg 80			Avg 59			Avg 69

[7] Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.*, 1945, **161**, 83.

added glucose were compared with those without added glucose. This indicates, therefore, that at least in tumor tissue under anerobic conditions and in the presence of glucose, incorporation of the major portion of the radiophosphate may depend upon glycolysis. However, the evidence presented does not permit one to rule out the possibility that the remaining radiophosphate incorporation in liver and kidney slices under anerobic conditions is an "exchange" phenomenon independent of oxidative energy.

Summary. Phosphate incorporation into nucleic acid and phosphoprotein of liver, kidney and tumor tissue slices shows some dependence upon the presence of oxygen.

The inhibition of radiophosphate incorporation into nucleic acid and phosphoprotein of tumor slices by nitrogen and its partial reversal by the addition of glucose suggest that, at least in tumor tissue, glycolysis can also serve as a source of energy for the incorporation.

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17290. Low Speed Microtomy for the Electron Microscope.

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The beam of a 50-kv electron microscope does not penetrate specimens that are thicker than 0.1 μ. Thus, in order to study biological specimens with this type of microscope we have further developed the technic described by Pease and Baker,[1] who altered a Spencer Rotary Microtome (model 820) by adding a wedge to the mechanism for forward movement. We reduced the angle of the inclined plane surface by a factor of 10 to 1 so that each step is 0.1 μ rather than 1.0 μ.

Since forward movement results from a pin in sliding contact with an inclined plane surface, it is important that the surface of the plane be as nearly perfectly flat as possible. The accompanying photograph, Fig. 1, illustrates the means by which the problem of a flat surface for even forward movement was solved. At the left of the picture (A) the original plane can be seen. The reduced angle is shown by D. The light gray triangular area (B) is an angle-reducing block of steel, to which is fastened an optical flat (C). A brass disc (E) instead of a pin is used for the feed screw tip. Horizontal movement of the disc is responsible for forward movement of the specimen. Vertical movement of the flat and the mechanism to which it is attached causes the slicing action of the microtome. Since the disc and flat are held firmly in contact by a spring, any irregularities in the surface of the plane will be evident on the sliced section.

Since the ordinary microtome knife cannot

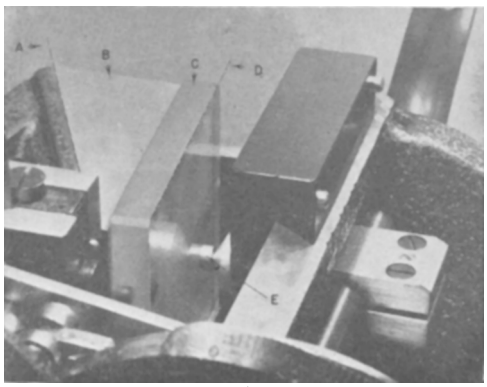

FIG. 1.
Microtome.
A. Original inclined plane surface of Spencer Microtome.
B. Steel wedge reducing from 25° to 2.5° the angle of the original plane surface with respect to direction of the feed screw movement.
C. Optical flat upon which cross feed disc rides.
D. Altered inclined plane surface, making an angle of 2.5° with direction of cross feed movement.
E. Cross feed disc.

[1] Pease, D., and Baker, R., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 470.