TABLE I.
Oxygen Uptake and Cholinesterase Activity of Brain of Normal and DFP-Treated Rabbits.

DFP mg/kg i.v.		Cholinesterase activity cu mm CO/30 min/ 0.5 cc[2] homogenate	Q_{O_2}
0.0		233	11.2
0.0		299	10.9
0.0		246	9.6
0.0		222	10.2
0.0		244	9.9
0.0		233	10.3
0.0		239	10.5
0.0		221	9.9
	Avg	242	10.3
0.5		4	9.9
0.5		4	10.2
0.5		4	10.6
1.0		4	8.8
1.0		—0.7	10.4
1.0		—1.1	11.4
	Avg	2	10.2

All values are averages of duplicate or triplicate determinations.

effect on brain metabolism. It does show, however, that DFP has no appreciable effect on the normal oxygen uptake of cerebral cortical slices, and that cholinesterase activity is not essential for such oxygen uptake.

Discussion. Some related studies of the effect of DFP on enzymes other than esterases have been reported. Webb[9] found that the following enzyme systems were not affected by DFP at concentrations of M/500 to M/2000: cytochrome oxidase, succinic dehydrogenase, hexokinase, Robison ester glycolysis by muscle powder, choline, dehydrogenase, catalase, carboxylase, ptyalin, peroxidase, lactic dehydrogenase of muscle, diaphorase, liver alcohol dehydrogenase, and carbonic anhydrase. Hunt[10] has stated that the normal oxygen uptake by liver homogenate and the additional oxygen uptake due to added ethyl butyrate are not inhibited noticeably by DFP.

The increased respiration of rat brain slices observed by Levy[11] when the tissue was in concentrations of eserine salicylate between 1:500 and 1:1000 are attributable to the enormous concentration of the drug. It may be that the salicylate ion is being metabolised at a sufficient rate at this high concentration to produce the increased oxygen uptake attributed to eserine.

Summary. When injected into rabbits in lethal doses, diisopropyl fluorophosphate had no appreciable effect on the oxygen uptake of cerebral cortex slices of these animals even though cholinesterase was completely inhibited.

9 Webb, E. C., *Biochem. J.*, 1948, **42**, 96.

10 Hunt, C. C., personal communication.

11 Levy, J., *Bull. soc. chem. biol.*, 1946, **28**, 338.

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In vitro Studies of a Growth Inhibitory Fraction Obtained from Adult Sheep Cardiac Muscle.*

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In earlier papers[1,2] we have reported on the growth stimulating effects of a Tyrode's solution extract of animal tissue as demonstrated both *in vitro* and when applied topically to wounds in dog and man. In the course of attempts to fractionate adult animal tissue extracts in order to determine the factors responsible for this stimulation, one crude fraction has been of particular interest because it

* This work was supported by a grant furnished by the Committee on Growth of the National Research Council.

1 Hoffman, R. S., and Dingwall, J. A., *Surg., Gynec. and Obst.*, 1944, **79**, 103.

2 Hoffman, R. S., Dingwall, J. A., and Andrus, W. DeW., *Ann. Surg.*, 1946, **124**, 1125.

appeared to have inhibitory effects.

This fraction was prepared by extracting finely minced sheep cardiac muscle with acetone in the cold, evaporating the solvent, lyophilizing and dissolving the dry residue in 10 volumes of Tyrode's solution. From 660 g of fresh cardiac muscle the yield was approximately 10 g of residue. The following is a report on preliminary experiments dealing with the effects of this material.†

Effect on Normal Cells. Third passage hanging-drop cultures of chick heart fibroblasts were divided and one half of each culture was used as the test object, the other half as the control. Each was planted in a separate D-3.5 Carrel flask containing 0.5 cc of chicken plasma, 1 cc Tyrode's solution and one drop of chick embryonic extract. After 48 hours 0.5 cc of the test solution was added as a supernatant layer to the test culture and 0.5 cc of Tyrode's solution to the control. The pH of both control and test cultures was adjusted to from 6.5 to 7.0. The effect of the test solutions as compared to the controls was observed for from 2 to 4 days.

The residue of the acetone extract of the sheep heart, redissolved in Tyrode's solution, markedly inhibited the growth of chick fibroblasts as no increase whatsoever in surface area was observed after this material had been added in dilutions up to 1:500. The cells, however, were not killed, for when the cultures were washed with Tyrode's solution and transplanted into fresh medium they began to grow and to maintain a rate of growth eventually equaling that of the controls.

Effect on Tumor Cells. To test the effects of this extract on tumor cells, cultures of a mouse breast tumor were also studied. This neoplasm arose spontaneously in a C3H mouse 2 years ago, and while originally exhibiting all the features of a mammary adenocarcinoma, it has gradually become sarcomatous in character.‡ The tumor was cultivated in Carrel flasks in the same type of coagulum used for the normal cells, but 0.3 cc of rabbit serum and 0.4 cc of mouse embryo extract were added as a supernatant fluid phase. The latter was changed every 4 days and the cultures transplanted to other Carrel flasks every 10 days.

Second passage cultures were divided into equal parts and the halves were planted in a separate Carrel flask to be used either as test or control objects. All cultures were maintained in medium 4 or 5 days, during which time satisfactory growth of the tissue was observed. On the fourth or fifth day, the supernatant fluids were removed from all the cultures and replaced in the experimental series with the residue from the acetone solution that had been redissolved in Tyrode's solution to give a final dilution of 1:500. In the sister halves, or controls, 0.5 cc of Tyrode's solution was added. A complete cessation of growth was obtained in the tumor cultures treated with extract, whereas the sister cultures continued to show good growth.

Effect on Bacteria. The effectiveness of certain animal tissues in producing bacteriostasis and in converting *Staphylococcus aureus* R into an avirulent form has been reported by Nutini and coworkers in several publications;[3-6] and in view of these findings it is of interest to examine the effect of our acetone extract on certain bacteria. One gram of the crude residue was dissolved in 10 cc of Tyrode's solution and applied in serial dilution to standard cultures of organisms. It inhibited the growth of tubercle bacilli in the Tween albumin medium of Dubos and Davis[7] in further dilution of 1/40 and was effective against the same organism cultivated in oleic acid medium when the 10% solution of the crude material was further diluted 1/160.

Complete inhibition of growth of H Streptococcus C 203 occurred when the 10% Ty-

† The crude material was prepared by The Armour Laboratories.

‡ H. Toolan, personal communication.

3 Nutini, L. G., and Kreke, C. W., *J. Bact.*, 1942, **44**, 661.

4 Nutini, L. G., Kreke, C. W., and Scroeder, Gr. M. P., *J. Bact.*, 1943, **50**, 177.

5 Nutini, L. G., and Lynch, E. M., *J. Exp. Med.*, 1946, **84**, 247.

6 Nutini, L. G., and Lynch, E. M., *J. Bact.*, 1946, **52**, 681.

7 Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946. **83**. 409.

rode's solution was diluted 1/16. Staphylococcus was inhibited in dilution of 1/32 (crude material 1/320) and *E. coli* at 1/16.

Other investigators[8-11] have reported the extraction of substances inhibiting cell growth from liver and other sources by methods different from those reported here, and it is not yet clear whether the substance which we have used corresponds in its physical and chemical properties to any of those previously described.

Summary. Acetone extract of sheep cardiac muscle lyophilized and redissolved in Tyrode's solution inhibits the growth of chicken fibroblasts and mouse breast tumor cells *in vitro*. A similar inhibitory activity of varying degree is exhibited against standard strains of tubercle bacilli, hemolytic *Streptococcus aureus* and *E. coli*.

[8] Baker, L. E., and Carrel, A., *J. Exp. Med.*, 1925, **42**, 143.

[9] Heaton, T. B., *J. Path. and Bact.*, 1926, **29**, 293; 1929, **32**, 565.

[10] Shebruja, T., Inaba, M., and Kawaguchi, A., *Tr. Japan. Path. Soc.*, 1935, **25**, 412.

[11] Brues, A. M., Subbarow, Y., Jackson, E. B., and Aub, J. C., *J. Exp. Med.*, 1940, **71**, 423.

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Effect of Adrenal Cortex Extract Upon the Urinary Nitrogen of Rats Following Adrenalectomy.

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The adrenal cortex has been characterized as a regulator of protein metabolism. It is generally true that the adrenally insufficient animal excretes smaller than normal amounts of urinary nitrogen and that the administration of adrenal cortex extract or of the 11-oxy steroids favors an increased loss of nitrogen[1]. The present report concerns an exception to the above rule. Others are known.[2-5]

In studies on the force-fed rat it was noted[4,5] that following adrenalectomy the animals which were given saline to drink without additional treatment excreted more urinary nitrogen than did similar animals which were treated with adrenal cortex extract or than did sham-operated rats. The present study confirms and extends these preliminary observations.

Methods. Male rats of the Sprague-Dawley strain were maintained on stock diets of Purina Dog Chow (Experiment 1) and Archer Dog Pellets (Experiment 2). When the rats reached a weight of approximately 300 g they were adapted to the force-feeding of a medium carbohydrate diet by stomach tube each morning (8:30 to 9:15 A.M.) and afternoon (4:15 to 5:00 P.M.). The technic of force-feeding and the diet (Table I) used

TABLE I.
Medium Carbohydrate Diet.

Constituent	G
Cellu flour (Chicago Dietetic Supply)	120
Osborne & Mendel salt mixture	40
Dried yeast (Pabst)	100
Wheat germ oil	10
Cod liver oil	10
Vitamin K (2-methyl-1,4-naphthoquinone)	100 mg
Mazola oil	200
Casein (Labco)	160
Starch	200
Dextrin	190
Sucrose	200
Water to make total of	2000 cc

[1] Long, C. N. H., Katzin, B., and Fry, E. G., *Endocrinology*, 1940, **26**, 309.

[2] Koelsche, G. A., and Kendall, E. C., *Am. J. Physiol.*, 1935, **113**, 335.

[3] Berman, D., Sylvester, M., Hay, E. C., and Selye, H., *Endocrinology*, 1947, **41**, 258.

[4] Ingle, D. J., and Oberle, E. A., *Am. J. Physiol.*, 1946, **147**, 222.

[5] Ingle, D. J., Ward, E. O., and Kuizenga, M. H., *Am. J. Physiol.*, 1947, **149**, 510.