itory action of sulfonamides on flavoprotein has been abundantly reported(13).

The results of the parallel aerobic and anaerobic experiments therefore show that oxygen, tetrazolium and amino acids, and tetrazolium, sulfathiazole and pyruvate compete for the same enzyme site.§

In the absence of evidence showing utilization of the amino acids, the observed effects of these substances appear to be contingent upon the inhibition of reactions competing for the available hydrogen. As to the nature of these competing reactions it is of interest to note that Meyerhof and Schulz(14) found that only upon addition of certain nitrogenous substances (certain amino acids, amines or ammonium sulfate) to fermentation mixtures did yeast completely convert hexoses to CO_2, because under these conditions assimilation of sugar was restricted. In the absence of added nitrogenous substances only 75 to 80% of the theoretical yield of CO_2 was obtained.

Summary. Amino acids, aerobically and anaerobically, cause an acceleration of the reduction of tetrazolium by *Micrococcus pyogenes* var. *aureus* in the presence of glucose, and can be shown to shift glucose metabolism towards increased lactate production. This effect of amino acids appears to be related to the inhibition of normally occurring reactions resulting in an increased availability of hydrogen for the reduction of tetrazolium or pyruvate.

Oxygen inhibits the reduction of tetrazolium in the absence of added amino acids, while sulfathiazole inhibits the amino acid accelerated reduction of tetrazolium. Since flavoprotein is reported to mediate tetrazolium reduction and to be susceptible to inhibition by sulfathiazole, the accelerative effect of amino acids on the reduction of tetrazolium could be related to an interaction of the amino acids with the flavoprotein enzyme. Competition for this enzyme site between tetrazolium, oxygen and pyruvate, modified by the presence of amino acids and sulfathiazole is suggested as an explanation consistent with the observed accelerations and inhibitions of the reduction of tetrazolium.

13. Sevag, M. G., Gots, J. S., and Steers, E., *The Enzymes,* Vol. I, New York, Academic Press, 1950, pp115-186.

§ The alternative postulate that the amino acid acts by lowering the oxidation-reduction potential of the medium to a level more favorable for tetrazolium reduction does not appear to find support in the fact that all the amino acids and other nitrogenous substances tested are active in low concentrations and under strictly aerobic and anaerobic conditions under which O/R potentials would widely vary.

14. Meyerhof, O. and Schulz, W., *Biochem. Z.,* 1936, v287, 206.

Received May 3, 1951. P.S.E.B.M., 1951, v77.

Protein Depletion Enhances Pancreatic Damage Caused by Ethionine. (18853)

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Destruction of pancreatic tissue by the administration of ethionine in the rat has been recently reported by Farber and Popper(1) and by Goldberg, Chaikoff, and Dodge(2). In an attempt to reproduce the results of these investigators pancreatic damage was found to be slight and seen only irregularly in rats on a complete diet. It is the purpose of this communication to describe a procedure by which pancreatic damage can be regularly produced by simply putting the rats on a protein depletion regime prior to the administration of the drug.

1. Farber, E. and Popper, H., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 838.

2. Goldberg, R. C., Chaikoff, I. L., and Dodge, A. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 869.

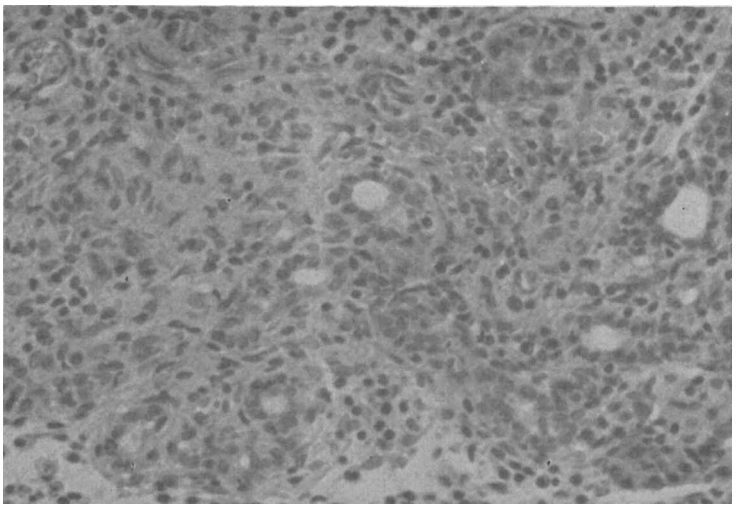

FIG. 1. Photomicrograph of pancreas. Hematoxylin-eosin stain × 250. Rat was kept for 12 days on the protein depletion diet prior to ethionine administration; sacrificed 6 days later. There is extensive replacement by proliferating fibrous tissue present. Only few acini are preserved. Small ducts show dilated lumina.

Experiments and results. Young male albino rats of the Wistar strain were used. Of 11 rats, on a complete Rockland diet, weighing between 120 and 250 g, 8 received 1 mg of ethionine (α-amino-γ-ethylthiolbutyric acid) (Nutritional Biochemical Corporation, Cleveland, Ohio) per g body weight given in 3 divided doses intraperitoneally after a fast of 16 hours as suggested by Farber and Popper(1). In 3 animals this procedure was repeated on 3 consecutive days and the rats were killed 3 to 5 days later. In all instances there was infiltration of the peri-pancreatic fat tissue by mononuclear cells and occasional polymorphonuclear leucocytes. Degenerative changes within the pancreas, however, were either entirely absent, or only of moderate degree often restrictd to focal isolated areas. It was considered possible that thorough protein depletion prior to the administration of the drug might lead to more consistent results. Rats were therefore put on a protein depletion diet consisting of 77.6 parts corn starch, 15 parts hydrogenated cotton seed oil (Crisco), 4 parts salt mixture No. 2 U.S.P. XII, 4 parts Ruffex, and 0.4 parts Brewers yeast powder. In addition the animals received twice weekly one drop of cod liver oil. Within 10 to 14 days these rats lost an average of 22% of their initial body weight. In all experiments 1 mg of ethionine per g body weight was given in the same manner as previously mentioned, however, without preceding fast. Altogether 56 rats weighing between 140 and 215 g were used of which 3 died within 72 hours following the administration of ethionine.

It was found in preliminary experiments that the rats had to be kept on the protein deficient diet for a minimum period of 5 days in order to increase the pancreas damaging action of the drug. Thirty-three rats were therefore given the protein depletion diet for a period of 7 to 19 days and killed 1 to 7 days later.

The microscopic appearance of the damaged pancreas was essentially similar to that described by Farber and Popper(1), and Goldberg, Chaikoff, and Dodge(2). After 24 hours there was loss of basophilia and marked swelling of the cytoplasm. In the subsequent days there was prominent vacuolization of the cytoplasm while coagulation necrosis was only occasionally noticed in our material. Nuclei were little changed. Infiltration by polymorphonuclear leucocytes, eosinophiles and lymphocytic elements became pronounced on the third day. There was considerable proliferation of fibroblasts commencing on the third to fourth day. Strands of proliferating fibro-

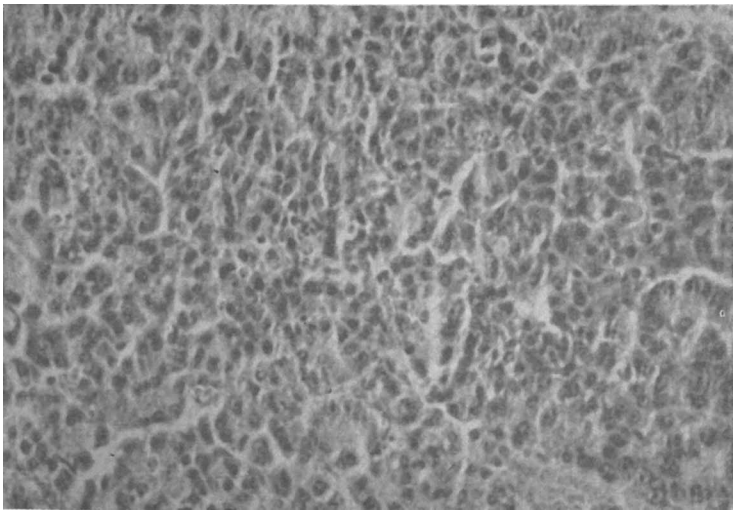

FIG. 2. Photomicrograph of pancreas. Hematoxylin-eosin stain × 250. Rat was sacrificed after 17 days on the protein depletion diet. There is focal depletion of zymogen granules present.

blasts separated remaining pancreatic acini. These acini showed basophilia of the cytoplasm but very few zymogen granules. Langerhans islands remained intact but pancreatic ducts were often dilated. While in some instances the changes were only focal, in most they were diffuse and were estimated to involve up to ¾ of the acinar tissue on representative sections (see Fig. 1). In sections of the pancreas of 6 control rats kept for 11 to 18 days on the protein depletion diet, focal moderate zymogen degranulation was seen(2). There was no loss of basal and perinuclear basophilia. No comparable destructive or inflammatory changes were observed. (See Fig. 2).

The livers of some of the rats which had been given ethionine showed the histological picture of protein depletion as described by Elman, Smith, and Sachar(3). In these instances the liver cells appeared enlarged with prominent cell walls and rarified cytoplasm with no or very little stainable fat. In other livers, however, the cells were small and showed diffuse fatty infiltration(4).

Comment. The decrease in resistance to the pancreas damaging action of ethionine in young male rats after protein depletion is very striking. It is interesting to note that the susceptibility of the pancreatic islands to the toxic action of alloxan is influenced by age and dosage level(5) as well as by the preceding diet. Houssay and Martinez(6) have shown that the toxic and diabetogenic action of alloxan increases in rats fed a low protein diet and even more in the case of a high lard or ox-fat diet. Starvation for a period of 48 to 60 hours made rats likewise considerably more susceptible(7).

High activity of radioactive sulphur found in the pancreas of normal rats after the administration of labelled methionine is thought to represent principally the metabolic activity of the exocrine portion of the pancreas(8). Since ethionine presumably interferes with the utilization of methionine(1) the increased susceptibility of the rat pancreas under the experimental conditions described can be satisfactorily explained by the depletion of tissue

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4. Jaffe, E. R., Humphreys, E. A., Benditt, E. P., and Wissler, R. W., *Arch. Path.*, 1949, v47, 411.

5. Charalampous, F. C., and Hegsted, D. M., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 207.

6. Houssay, B., and Martinez, C., *Science,* 1947, v105, 548.

7. Kass, E. H., and Waisbren, B. A., PROC. SOC. EXP. BIOL. AND MED., 1945, v60, 303.

8. Wheeler, J. F., Lukens, F. D. W., and György, P., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 187.

protein prior to the administration of the drug.

Summary. Ethionine given to young male rats of the Wistar strain on a complete diet caused only inconsistent and moderate pancreatic damage. Rats that had been on a protein depletion diet for a period of at least 6 days showed extensive destruction of the pancreas following the administration of equal amounts of the drug.

Received May 11, 1951. P.S.E.B.M., 1951, v77.

Successful Subcutaneous Growth and Transplantation of Human Tumors in X-Irradiated Laboratory Animals.* (18854)

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Neoplasms fail to grow in heterologous hosts unless one or both of two conditions are met. Either the tumor is one which has been transplanted for many generations or the implantation is made in sites at which the host offers minimal resistance to foreign tissue. Examples of these sites are the anterior chamber of the eye, brain, testes and immature tissues such as avian embryos and newborn animals. Spontaneous neoplasms, such as those of man, have been propagated heterologously only when implanted in the anterior chamber of the eye(1). The percentage of failures has been high, even under these circumstances, the rate of growth of the tumors slow, and the transplantability poor(2). To achieve an improved method for growing human neoplasms in animals, the work here reported was undertaken

Murphy showed(3) that rats which had been X-irradiated would support the growth of subcutaneously implanted mouse tumors for the duration of the lymphopenia produced by the radiation. A modification of this procedure has been employed in our experiments.

Methods and materials. Young adult female albino rats (Carworth Farms) and mice (Banks strain of Swiss) were employed. The rats received 3 doses each of 200 r, total body X-irradiation, on alternate days and the mice 4 doses of 150 r on successive days. Two 180 kv. tubes were employed (one above, one below the animal).

Previously, it had been established that this procedure would reduce the total white count in the particular strains of animals used, to approximately 400-800 mm^3. Several extra animals were included in each group irradiated to check the degree of lymphopenia produced. Counts were *not* made on the animals to receive implants since too great a variable is introduced by the cardiac puncture required to obtain a true picture of the total white count of rodents; tail blood is not usable because of its hemoconcentration.

The divided irradiation doses caused no symptoms of toxicity in the animals whereas a single dose large enough to produce the same reduction of white cells often caused diarrhea and even an occasional death. It must be em-

* We wish to acknowledge support of this study by funds from the National Cancer Institute, Public Health Service, Institution of Research Grants, American Cancer Society and the Damon Runyon Memorial Fund for Cancer Research.

Our gratitude is expressed to Dr. C. P. Rhoads for his faith and encouragement; to Drs. Stewart, Foote and Sunderland of the Pathology Department, Memorial Hospital, for their cooperation in the procurement of human tumors; to Miss Priscilla Goettler who grew the original tumors and transplants in tissue culture; and to Dr. M. Harland for the considerable histological material prepared.

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