as to the possibility of this mutation occurring *in vivo*. Preliminary attempts to isolate non-penicillinase-producing mutants from other penicillinase-producing organisms such as *E. coli* and *Shig. paradysenteriae* were unsuccessful. If non-enzyme-producing mutants are produced by these gram-negative bacilli, the frequency with which they occur is considerably lower than that of *M. pyogenes*.

Summary. Twelve of 13 strains of *M. pyogenes* which produce penicillinase yielded non-penicillinase-producing mutants which were susceptible to penicillin. This apparent spontaneous type of mutation occurs at a high rate of frequency. Cultivation of a strain of this organism at 45°C significantly increased the number of mutants. No evidence of reversibility of the mutation was obtained.

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Effect of Diet on Rate of Alcohol Oxidation by the Liver.* (20406)

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Many studies on the effects of alcohol have been conducted with little attention being given to other details of the diet. This is particularly true when relatively large amounts of alcohol are administered to animals who are otherwise consuming an adequate diet, for under these circumstances the total caloric intake is not increased, the amount of food eaten is decreased, and the consumption of essential nutrients may be reduced to a deficiency level. Effects which are attributed to alcohol may in fact be due to a concomitant nutritional deficiency (such as protein) produced by the substitution of alcohol for food. The present studies were conducted to determine how much the oxidation of alcohol itself by the liver might be influenced by protein depletion or inanition.

Experimental. The oxidation of alcohol was measured manometrically.† Livers were removed from decapitated rats, and weighed portions were homogenized with 4 volumes of 0.04M phosphate buffer, pH 7.4. Two pairs of Warburg flasks each contained 1 ml of the homogenate and 0.2 ml of 0.1M semicarbazide hydrochloride; one pair of flasks contained 0.2 ml of 15% ethyl alcohol (by volume) in the side arm. Water was added to all flasks to give a total volume of 2.0 ml, and an additional 0.2 ml of 10%KOH plus a filter paper were placed in the center wells. The flasks were equilibrated at 37° for 10 minutes, the zero reading was taken, and the alcohol was tipped in from the side arm. Readings were taken thereafter at 10-minute intervals for 80 minutes. The increased oxygen consumption in the alcohol-containing flasks was obtained by subtracting the endogenous respiration in the control flasks, and the net oxygen

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† This method is similar to the one used by Dr. Victor A. Najjar, to whom we are indebted for supplying the details prior to their publication.

TABLE I. Effect of Diet on Ability of Rat Liver to Oxidize Alcohol.

Rats	Diet	Days on diet	Body wt Start	Body wt End	No. of rats	Alcohol oxidation by liver homogenate*
Weanlings	As received	—	—	44	5	12 ± 2.0
	Chow	16	46	109	6	21 ± 1.2
	Purified 24% casein	16	49	80	5	18 ± 2.0
	Purified protein-free	16	43	31	7	3 ± 0.6
Adults	Chow	24	375	400	9	24 ± 1.2
	Purified 24% casein	24	369	407	10	19 ± 1.0
	" protein-free	24	378	302	10	4 ± 0.4
"	Starvation	7	216	156	12	11 ± 1.0
	50% restriction of chow	14	234	161	10	20 ± 0.9

* Alcohol oxidation values have been recorded as the net O_2 consumption in mm^3 per 10 min. per Warburg flask containing 200 mg fresh liver; mean ± stand. error of mean.

consumption due to alcohol was plotted against time. The rate of alcohol oxidation was taken from the straight-line portion of this curve, and has been recorded as cmm of oxygen uptake per 10 minutes per flask containing 200 mg fresh liver. The straight-line portion of the curve usually covered the first 2 to 6 readings, though with some livers of intermediate activity there was a slower rate or lag period during the 1st 10 or 20 minutes.

This procedure was designed to be a measure of the alcohol dehydrogenase in liver, but it has not been so labeled because there is no assurance that other components of the liver or other simultaneous reactions might not also affect the rate of oxygen uptake. Semicarbazide was added to the flask in order to bind the acetaldehyde and thereby limit the oxidation of the alcohol to its initial step; semicarbazide had no effect on the endogenous respiration and may have increased slightly the rate of oxygen consumption in the presence of alcohol. Since the transfer of hydrogen from the substrate, alcohol, to oxygen of the air involved numerous intermediate steps, and since any one of these steps could be limiting the rate of oxygen consumption, the effect of other additions was tested. Methylene blue, nicotinamide or DPN were without beneficial effect and may have been inhibitory. A washed heart muscle preparation as a source of the cytochromes was without effect. The simultaneous addition of DPN, cytochrome C and the washed heart muscle homogenate had no effect on the net rate of oxygen consumption with alcohol as the substrate, and this was true for the livers of low alcohol oxidative capacity obtained from protein-depleted rats as well as for normal livers. Hence there was no indication that the procedure measured anything but alcohol dehydrogenase, but there was also no positive evidence obtained on this point.

The effect of various diets on the ability of the liver homogenate to oxidize alcohol was studied in both weanling and adult rats. Some of the male weanling rats were analyzed upon receipt from Albino Farms; others were fed a diet of: 1) chow, 2) purified 24% casein, or 3) a protein-free diet for two weeks before analysis. Adult male hooded rats were divided into 3 groups and fed the same 3 diets for 24 days before analysis. In addition, the effect of caloric restriction was tested in the adult rats by completely starving one group for 7 days, and by allowing each rat in another group to eat only 10 g of chow daily; this was 50% of the *ad libitum* intake measured during the previous week, and was continued for 14 days before the rats were sacrificed.

The purified 24% casein diet contained (in g): Labco Casein 24, Crisco 4, Wesson oil 2, cod liver oil 1, Phillips and Hart salts 4, glucose 65, plus a vitamin supplement (in mg per 100 g of diet) of: choline 100, niacin 2.5, calcium pantothenate 1.0, riboflavin 0.4, thiamine 0.4 and pyridoxine 0.4. The protein-free diet had the same composition except that additional glucose replaced the casein.

The results have been summarized in Table I. The decrease in the alcohol oxidation system as a result of protein depletion or inanition (1) was similar to the changes previously reported(2) for xanthine oxidase, and was

somewhat greater than that found for many other liver enzymes(3).

Summary. 1. Weanling rat liver homogenate had about one-half the level of alcohol oxidation activity eventually achieved by adult rats on a chow diet (net oxygen uptake of 24 cmm per 10 minutes per 200 mg fresh liver for the adult normal rat). 85% or 75% respectively of this normal level of activity was reached by feeding chow or a purified 24% casein diet for 2 weeks. A protein-free diet removed about 85% of this enzymatic activity from the liver. 2. Adult rats responded similarly to these diets. Complete starvation for 7 days reduced this activity about 50%, while a 50% food restriction for 2 weeks gave less than a 20% decrease in the rate of alcohol oxidation.

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Occurrence of Hexose-6-phosphate Dehydrogenase in Chorioallantoic Membrane of the Chick Embryo.* (20407)

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Previous experiments on the *in vitro* metabolism of excised chorioallantoic membranes of chick embryos dealt with changes in aerobic glycolysis which occurred following inoculation with viruses(1). In connection with this problem it became necessary to investigate the metabolic pathways of this tissue in more detail. The investigation reported here was undertaken as a part of these studies, and showed that the chorioallantoic membrane contains, besides the Embden-Meyerhof-Warburg type of glycolytic enzyme system(1), a glucose-6-phosphate oxidase.

Materials and methods. The embryonated eggs of white Leghorn and Rhode Island Red hens were obtained from a local hatchery. The age of the eggs varied from 6 to 13 days. The membranes were carefully excised and thoroughly washed in ice-chilled homogenizing medium which consisted of 0.15 *M* KCl containing 0.05% ethylene-diamine tetraacetic acid. The pH of the KCl solution was brought to 7.1 with KOH, and then 2 ml of 0.5 *M* glycylglycine buffer (pH 7.3) per 500 ml were added. The membranes of 5 to 8 eggs were combined and quickly ground with ice-cold (4°C) KCl solution in a glass homogenizer. The homogenate was kept in an ice bath prior to enzyme assays. The protein content of the homogenate was determined colorimetrically according to Lowry *et al.*(2), with crystalline bovine albumen as reference standard. Triphosphopyridine nucleotide (TPN, 60% pure), diphosphopyridine nucleotide (DPN, 90% pure), and glucose-1-PO_4 K-salt were obtained from Sigma Chemical Company, Inc. The Ba-salt of glucose-6-phosphate was a purified sample received through the courtesy of Dr. D. Broida (Sigma Chemical Co., Lot 91-20) Fructose-6-phosphate (purchased as Ba-salt from Nutritional Biochemicals Corp.) and fructose-1-6-diphosphate (HDP, Ca-salt, Sigma) were purified by ion exchange(3). Both hexose monophosphates and HDP were made up to 0.1 *M* solutions as K-salts. Pyocyanine was recrystallized twice from hot water after chloroform extraction of a commercial sample (Delta Chemicals), as described by Wrede and Starck(4). Janus green B and methylene blue were commercial samples. Isatin-6-

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