portant in the virulence of streptococci for mice may be at the expense of streptolysin S production and other products.

Summary. (1) Streptolysin S was produced by streptococci of Lancefield groups A, C and G. (2) Streptococci of group A produced considerably more streptolysin S than did groups C and G. (3) Strains of group C streptococci of human origin produced more streptolysin S than did animal strains of group C. (4) In general, the passage of beta hemolytic streptococci through mice enhanced the production of streptolysin S but did not enhance their virulence. Nonhemolytic strains were not affected by animal passage.

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Phosphoglucomutase Activity in Skeletal Muscles of Vitamin E-Deficient Chicks.* (26494)

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In a previous study concerning changes in enzymatic activity in abnormal skeletal muscle, it was shown that a decrease in phosphoglucomutase (PGM) activity occurred in this tissue in mice with hereditary muscular dystrophy(1) a finding which was similar to that reported in man with muscular dystrophy(2). For purposes of comparison, this report is concerned with measurements of PGM activity in red and white chicken muscle rendered dystrophic by maintenance of the birds on a vit. E-deficient diet. PGM activity was measured without addition of the coenzyme, glucose-1-6-diphosphate, and with it in order to determine maximal enzymatic activity present.

Methods. Ten male white Plymouth Rock chickens were fed *ad lib* from hatching on a vit. E-deficient diet similar to one reported (3) except that the protein consisted of 10% Torula yeast (deficient in selenium), 5% casein and 5% isolated soy protein supplemented by selenium (0.1 mg/kg of diet).‡ To the diet of 5 of these birds was added 80 mg α-tocopheryl acetate/kg. Autopsy under Nembutal anesthesia was performed at 5 weeks of age. Samples of white (breast) and red (leg) muscles were frozen with dry ice immediately after excision and stored at −20° until assayed.

PGM activity was determined by the method of Najjar(4) as refined by Bodansky(5). Aqueous homogenates of muscle were prepared in the cold and adjusted to 7.5 mg white muscle/ml and 15 mg red muscle/ml. To the reaction tubes was added the following in 0.1 ml volumes; 5×10^{-2} M glucose-1-PO_4; 8×10^{-2} M cysteine, freshly prepared and adjusted to pH 7.5; 1.2×10^{-2} M Mg SO_4; 4×10^{-1} M Tris buffer, pH 7.5; and 0.4 ml of either water or coenzyme (prepared as described below) for maximum PGM activity. The reaction was started by adding 0.2 ml of muscle homogenate freshly pre-

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‡ Chickens were raised and their diet devised and supervised by Corlette C. Calvert, Dept. of Poultry Husbandry, Cornell Univ.

TABLE I. PGM Activity in Vit. E-deficient Chick Skeletal Muscle.

Exp. (10 chicks)	Without coenzyme		With coenzyme	
	Fresh tissue*	NCPN†	Fresh tissue*	NCPN†
White muscle				
Vit. E-deficient	26 ± 4.0	7.1 ± 1.4	66 ± 4.8	18 ± 1.9
Control	47 ± 1.3	13.6 ± .7	97 ± 3.0	28 ± 1.7
P value	<.01	<.01	<.001	<.01
Red muscle				
Vit. E-deficient	7.8 ± 1.2	2.7 ± .3	27 ± 2.1	9.3 ± .4
Control	9.1 ± 1.0	3.0 ± .4	29 ± 1.1	9.7 ± .7

* mg P/100 mg tissue/hr ± S.E.
† mg P/mg non-collagenous protein nitrogen/hr ± S.E.

pared. After 4 minutes of incubation at 37.5°, the reaction was stopped by adding 1 ml of 5 N H_2SO_4. The mixture was diluted to 5 ml. heated for 7 minutes at 100°, filtered and 1 ml aliquot taken for P determination(6). The results are reported as mg glucose-6-PO_4 P formed 100 mg fresh tissue hr. Non-collagenous protein nitrogen (NCPN) of the muscle was determined(7) and enzyme activity reported also on this basis.

Although pure α-D-glucose 1-6-diphosphate (coenzyme) is not available to permit saturation of the reaction mixture with coenzyme to determine maximum PGM activity, nevertheless it is possible to estimate maximum activity, as previously demonstrated in mouse muscle(1), by adding increasing amounts of a boiled muscle extract known to contain coenzyme, when prepared according to Cardini *et al.*(8), to a series of PGM reaction mixtures. By plotting the reciprocals of the concentration of added coenzyme (mg boiled muscle extract) *vs* velocity of the reaction (mg P/100 mg tissue/hr), a linear relationship obtains and extrapolation to infinite coenzyme concentration permits estimation of maximum PGM activity. In the present experiment, boiled extracts containing coenzymes were prepared from normal white chick muscles and added in amounts equivalent to 2-16 mg tissue in 0.4 ml volume. Linear increases in PGM activity occurred with addition of 2-8 mg equivalents of boiled extract but addition of more than 8 mg up to 16 mg failed to increase PGM activity further. Thus, a direct determination of maximum PGM activity was possible by adding 8-16 mg of the white muscle extract in these experiments which thereby increased the technical efficiency of the assay.

Results and discussion. The muscular lesions developed in the vit. E-deficient chicks varied from moderate to severe although the experimental and control birds consumed equal amounts of their diet and had similar average weight at autopsy (529 *vs* 534 g respectively). Grossly, white muscles exhibited more severe lesions than the red.

Results in Table I show that PGM activity in white muscle from vit. E-deficient chickens decreased by 48% when measured without added coenzyme and 36% with maximum coenzyme present, based on NCPN content of the tissues. In most instances, the decline in PGM paralleled the severity of the lesions as noted by gross inspection at autopsy. The red muscle, in contrast, failed to show a significant alteration in PGM activity. The activity of normal white muscle was 3-4 times that of red muscle when assayed either with or without maximum coenzyme additions.

The percent decline in PGM activity (calculated on the basis of NCPN and with added coenzyme) in white muscle of deficient chicks at 5 weeks of age is similar to that of dystrophic mouse muscle(1) at 13 weeks of age (36% *vs.* 39% for the latter). Ratios between PGM activity of vit. E-deficient and control chicken white muscles, when calculated from determinations made in absence and presence of added coenzyme are not markedly different (.52 *vs.* .64), thus permitting the inference that coenzyme content of the muscle differs by only a little more than does the PGM. In contrast, these ratios in the dystrophic mouse were .32 without

added coenzyme and .61 with coenzyme, from which it was inferred that a great loss in coenzyme accompanied the lesions in the mouse (1). Thus it appears that loss of coenzyme in the dystrophic chick muscle is proportionately not as large as in the dystrophic mouse muscle. Normal white chick muscle contains more G-1-6-diphosphate than rat or mouse muscle. Repeated experiments showed that saturation of the PGM system with this coenzyme was not possible with additions equivalent to 8-16 mg of boiled extract prepared from rodent muscle whereas saturation was readily achieved when white chick muscle was employed.

Phosphorylase activity, as well as PGM, is considerably greater in white than in red muscle of the normal chick(3). It is only in white muscle that phosphorylase is decreased in the vit. E-deficient chick. The sensitivity of white muscle to the vitamin deficiency might be correlated with the initially high levels of phosphorylase and PGM, and a longer period of feeding the deficient diet might possibly affect the red muscle as well. PGM activity in the back muscles of rabbits fed a vit. D-deficient diet was lower than normal but the results were not statistically significant(9).

Summary. Phosphoglucomutase activity determined with and without added coenzyme (glucose-1-6-diphosphate) was increased in white muscles of chicks fed a vit. E-deficient diet, but was unaffected in red muscle. Muscle lesions were more extensive in the white muscle. Normally white muscle contains more PGM than red muscle. The coenzyme for PGM is much more plentiful in white chick muscle than in rat or mouse muscle so that direct determination of maximum PGM activity by saturation of the system with added coenzyme is possible, thus eliminating the necessity of extrapolating to infinite coenzyme concentration.

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Estrogen Therapy in Immature Female Rats with Posterior Hypothalamic Lesions. (26495)

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Recent findings(1-5) support the concept of hypothalamic control of the hypophyseal-gonad axis. Corbin and Schottelius(6) have further implied a dual capacity of the hypothalamus, as regards puberal initiation and sexual maturation. Failure of the puberty-parturition continuum may be attributed to ovarian hypofunction, representing a secondary effect of posterior hypothalamic damage.

Gale(7) tested the effectiveness of replacement therapy in hypothalamically lesioned, pregnant rats, by administering progesterone and estradiol benzoate and preventing gestational impairment and abortion. Information is relatively scarce, however, relating estrogen therapy to reproductive organ maturation in immature female rats with discrete hypothalamic lesions. The response of the reproductive organs to estrogen replacement in prepuberal female rats with posterior hypothalamic lesions is the object of this study.