preparations utilized. Synthetic Angiotensin II† was administered either into the arterial inflow to the recipient's head or via the femoral vein to the recipient's peripheral circulation.

Results. The effects of Angiotensin II on donor and recipient blood pressures in the dog cross circulation preparation are summarized in Table I. Angiotensin II administered into the arterial inflow to the recipient's head in doses varying from 0.2 to 4 μg/kg produced consistent pressor responses in both recipient and donor animals with pressure increments ranging between 12% and 50% above normal levels in recipient and 30% to 100% above pre-drug level in donor. Central hypertensive response was shorter in duration (0.5 to 5.0 min.) than pressor response in the donor animal (2.0 to 5.0 min.). Intravenous administration of Angiotensin II into the peripheral circulation of the recipient produced marked hypertensive responses of relatively long duration (2.5 to 6.0 min.), in the recipient only. In 2 preparations, 2 doses of Angiotensin II, 1 μg/kg, were injected into the head of the recipient and the hypertensive effect in both animals recorded. Subsequently, 1.0 mg/kg of piperoxan was slowly infused into the recipient's femoral vein. Angiotensin, 1 μg/kg, was then administered into the arterial inflow to the recipient's head and produced pressor responses in the donor only. Intravenous administration of Angiotensin into the peripheral circulation of the recipient produced the usual pressor response. Vasopressin (0.1 μ/kg) administered *via* the arterial inflow to the untreated recipient's head produced hypertensive effects in the donor animal only.

Summary. Angiotensin II appears to produce an increase in blood pressure by 2 mechanisms: 1. A direct peripheral action on the vascular smooth musculature producing a marked increase in peripheral resistance, which is not blocked by piperoxan. 2. A central hypertensive effect, probably due to stimulation of central sympathetic structures and evoking peripheral sympathetic discharges, which are blocked by administration of a sympatholytic agent into the peripheral circulation.

† Kindly supplied as Hypertensin by Ciba Pharmaceutical Products, Inc., Summit, N. J.

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Production of Streptolysin S by Streptococci Before and After Mouse Passage.* (26493)

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No difference was found by Stollerman and Bernheimer (1) in streptolysin S production by strains of group A beta hemolytic streptococci isolated from patients with streptococcal pharyngitis or with rheumatic fever. That streptolysin S was produced in presence of yeast nucleic acid by strains of Lancefield's group A as well as by certain strains of groups D, E, G, H, and L was reported by

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Bernheimer(2).

The experiments reported here were performed (a) to determine quantitatively the amount of streptolysin S produced by streptococci isolated from clinical sources and grown in presence of yeast nucleic acid and (b) to study the effect of animal passage on level of production of this hemolysin by streptococci.

Materials and methods. A. *Preparation of Yeast Nucleic Acid Medium.* The medium utilized for growth of streptococci was prepared according to the method of Bernheimer and Rodbart(3). Beef heart for infusion (Difco) was substituted for the fresh beef heart. One ml of a Seitz-filtered solution of yeast ribonucleate (70 mg/ml) adjusted to pH 8.0 was added to the medium after it was sterilized by autoclaving. Control tubes received one ml of distilled water adjusted to pH 8.0. Before inoculation of the medium, freshly neutralized sodium thioglycollate was added to give a final concentration of 0.01%.

Seventy-five strains of streptococci isolated from clinical sources were used. Broth cultures of these organisms were kept frozen at −70°C until needed. Heart infusion medium was inoculated with the streptococci and incubated at 37°C for 6 hours. The yeast nucleic acid medium was inoculated with 0.1 ml of this suspension. Heart infusion medium lacking yeast nucleic acid was also inoculated. After 16 hours incubation at 37°C, the cultures were centrifuged and supernatant fluids were assayed for content of streptolysin S.

B. *Assay of Streptolysin S.* Serial 2-fold dilutions of each of the culture supernates, in phosphate buffer, were prepared in triplicate. To each tube of the first set of dilutions containing 1 ml of the diluted supernate was added 0.5 ml of a phosphate buffer pH 7.0 and 0.5 ml of 2% suspension of rabbit red blood cells. Because streptolysin O is inhibited by cholesterol and streptolysin S is inhibited by lecithin, a second set of serially diluted culture received 0.5 ml of a suspension of lecithin (5 mg/ml), while the third set of tubes containing serially diluted supernatant fluids received 0.5 ml of a suspension of cholesterol (50 μg/ml) in the place of the phosphate buffer. This procedure enabled the investigators to ascertain whether the hemolysin was due to streptolysin O, S, or both. After incubation for 45 minutes at 37°C, the tubes which showed hemolysis were removed from the water bath and centrifuged. The supernatant fluids were decanted, diluted 1:6 and optical density was determined at a wavelength of 5500 Angstroms, using a Coleman Universal spectrophotometer. The 50% hemolytic endpoint was calculated by use of conversion factors established by Von Krogh (4).

The unit of streptolysin S, as used in these experiments, is defined as that amount of streptolysin S dissolved in one ml of phosphate buffer which will lyse 50% of the rabbit red blood cells in 0.5 ml of a 2% suspension.

C. *Preparation of Lecithin and Cholesterol Suspensions.* Ten ml of 95% ethyl alcohol were added to 0.3 g of ovolecithin (Nutritional Biochemical Corp.). Solution was effected by stirring with a glass rod. Fifty ml of phosphate buffered saline were added to give a final concentration of 5 mg per ml.

Five mg of cholesterol (Difco) were suspended in one ml of absolute ethyl alcohol. This suspension was slowly poured into 100 ml of boiling water which then was filtered through paper.

Procedure for Passage of Streptococci through Mice. Mice were inoculated intraperitoneally with 0.5 ml of an 18 to 24 hour culture of streptococci. Between each animal passage the organisms were isolated from the mouse spleen or heart's blood and grown in Todd-Hewitt broth (Difco) for 18 to 24 hours.

Streptococci from such broth cultures were passed to mice by intraperitoneal inoculation; aliquots were frozen at −70°C for later evaluation of virulence and of production of streptolysin S. Virulence of the streptococci for mice was measured by intraperitoneal inoculation of 0.5 ml of 10-fold dilutions of an 18 hour culture (prepared from the frozen broth culture) into groups of 6 mice. The mice were observed for 12 days and LD_{50} determined by the method of Reed and Muench(5). For assay of streptolysin S, 0.1 ml of each streptococcal broth culture was inoculated into nu-

TABLE I. Production of Streptolysin S According to Group of Streptococcus.

Group	No. of strains studied	No. strains producing streptolysin S	Streptolysin S production (units/ml) Range	Mean
A	43	42	10–128	52
B	6	0	—	
C Human	4	4	2–32	18
Animal	7	3	3–5	2
D	4	0	—	
G	11	10	2–18	7.5

cleic acid medium and incubated for 18 hours.

Results. Addition of cholesterol to the tubes did not inhibit the hemolysin while the addition of lecithin completely prevented hemolysis of the rabbit red blood cells. This indicated that streptolysin O was not present in sufficient amounts to be detectable and that the observed hemolysis was due to streptolysin S. Partial inhibition by lecithin and or cholesterol was not observed.

Amounts of streptolysin S produced by 75 strains of streptococci of Lancefield groups A, B, C. D, and G are given in Table I.

The group A streptococci produced more streptolysin S than any of the other groups. Only one strain of group A streptococci produced streptolysin S in absence of yeast ribonucleate and this production was only 1.5 units per ml of culture supernate. However, one strain of group A failed to produce streptolysin S even in presence of yeast RNA. None of the group B or group D strains produced the hemolysin. A considerable difference is also apparent in production of streptolysin S by human and animal strains of group C.

Because considerable difference was noted between the groups with respect to streptolysin S production and also between members of the same group, a second experiment was performed to determine the effect of animal passage or adaptation to the host on production of streptolysin S. These results are shown in Table II.

This experiment indicated that production of streptolysin S can be modified by animal passage. In all strains which produced streptolysin S before animal passage, with one exception, streptolysin S production was augmented by such passage. The animal strain of group C, which did not produce streptolysin S before animal passage, did produce a limited amount after 8 animal passages. However, production of streptolysin S by strains of group D was not demonstrated at any time.

No increase in virulence of the streptococci for mice was obtained by animal passage.

Discussion. The significance of the production of streptolysin S by streptococci of Lancefield group A, C and G is not understood. However, some importance may be attached to the fact that most streptococcal infections in man are caused by members of these groups. It is similarly of considerable interest that streptolysin O(6) and streptokinase(7) also are produced only by members of these groups. That the largest amounts of streptolysin S are produced by streptococci of group A suggests that this streptolysin may have a significant role in human infections. The fact that this hemolytic toxin (S) is serum extractable has appealed to investigators relative to possible wide diffusion in tissues.

Leedom and Barkulis(8) showed that the enhanced virulence of streptococci after mouse passage was associated with decreased streptolysin S production. Although no enhancement of virulence of streptococci of groups A and C was obtained in our experiments after 8 to 10 mouse passages, the ability of the organism, with one exception, to produce streptolysin S was enhanced. However, these results do not preclude the possibility that the production of substances im-

TABLE II. Effect of Mouse Passage on Production of Streptolysin S by Strains of Streptococci of Groups A, C, and D.

Lancefield Group	Mouse passage level 0	2	4	6	8	10
A	10*	22	26	32	32	72
A	16	16	24	32	64	—
A	1	6	8	16	16	32
A	128	128	100	75	64	64
C	<1	5	5	6	8	10
D	<1	<1	<1	<1	<1	<1
D	<1	<1	<1	<1	<1	<1

* Units of streptolysin S/ml of culture supernate.

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.