and vitamins, as described above, and the other received the regular stock diet. At the end of the 100-day period during which this experiment was continued, 15 of the treated animals survived as compared to only 8 of the control group.

The experiment just described seems to indicate that the decline in blood pressure associated with a low-sodium diet is not only not detrimental to animals with experimentally induced hypertension but appears to be beneficial insofar as duration of life is concerned.

Inhibitory Effect of Choline. Among the various vitamins which we have tested for their effect on the blood pressure, choline appeared to exert a slight but definite pressor action in animals with a mild degree of hypertension. When the substance was administered in high dosages (3% of the dried weight of the diet) together with a low sodium diet, the usual drop in blood pressure was not elicited. The mechanism of this inhibitory effect is not clear but unless one assumes that choline in large doses interferes with the excretion of sodium by the kidney of the hypertensive animal, we must consider the effect as possibly being due to the pharmacodynamic action of choline in such large doses.

Discussion. Many studies are available[6] on the effect of the restriction of salt on the blood pressure of human hypertensives but no previous study of this subject has been made on the experimental animal. Although the results on the human have been equivocal,[7] the present study can leave little doubt as to the possibility of reducing the blood pressure of the hypertensive rat by rigid sodium restriction. The mechanism whereby this effect is induced is not established. It is known that there is an accumulation of salt and water in the tissues of the hypertensive patient, which was designated by Ambard[8] as a "dry edema." It would appear most likely, therefore, that when subjected to a drastic sodium restriction the animal with experimental renal hypertension and certain (but by no means all) patients with hypertension rid themselves of the plethora of salt and water in their tissues and that this secondarily results in the observed reduction in blood pressure.

Summary. Drastic sodium restriction was shown to result in a marked drop in the blood pressure of rats with experimental hypertension. This drop in blood pressure was demonstrated to be accompanied by a prolongation of the survival of the animals. The drop was inhibited by the administration of large doses of choline.

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8 Ambard, L., and Beaujard, E., *La Semaine Med.*, 1905, **25**, 133.

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Penicillin Resistant Staphylococci.

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Cultures of staphylococci may readily be made resistant to penicillin *in vitro* as demonstrated by various workers.[1,2] Rammelkamp and Maxon[1] and others[3,4] have also shown that staphylococci may acquire a fastness to penicillin in the human body during the course of therapy with this agent. Further work is necessary before one can safely at-

1 Rammelkamp, C. H., and Maxon, T., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 386.

2 McKee, C. M., and Houck, C. L., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 33.

3 Gallardo, E., *War Medicine*, 1945, **7**, 100.

4 Lyons, C., *J. Am. Med. Assn.*, 1943, **123**, 1007.

tempt to evaluate the significance of acquired *in vivo* resistance to penicillin which bacteria may acquire. Investigation by Rake and collaborators[5] indicate that this phenomenon may not be of great consequence therapeutically due to a loss of virulence by organisms which undergo this change. This is in direct contrast to bacteria which develop sulfonamide fastness; the virulence of such organisms remains unimpaired.

Of much greater importance is the occurrence in nature of virulent staphylococci which are naturally resistant to penicillin. Kirby[6] recently reported the isolation of 7 strains of staphylococci which were naturally resistant to this antibiotic. From each of the 7 strains this worker succeeded in extracting penicillinase,[7,8,9] an enzyme capable of destroying penicillin. From a clinical point of view the occurrence of resistant staphylococci in large numbers could be of great importance. The study to be reported in this paper was undertaken to determine the prevalence and significance of naturally resistant staphylococci.

Materials and Method. Strains of staphylococci studied were isolated from patients in Temple University Hospital. Single colonies were picked from aerobic blood agar plates following incubation at 37° C for 24 hours. No more than one subculture was made from any one specimen or any one patient. Colonies of staphylococci were chosen without regard for hemolysis or pigmentation. The 115 strains studied were from a variety of infections. As indicated by the large number of coagulase-negative reactors many of the strains probably were not of pathognomonic significance and only part of the normal body flora. Subcultures made on infusion agar slants were stored at room temperature and studied in groups of 25 to 30.

The susceptibility of the strains to penicillin was determined by use of a series of beef extract agar plates containing three-fold concentrations of penicillin. Such plates were streaked with 18-hour broth cultures of the staphylococci under investigation. Plates were incubated at 37° C and readings were made at the end of 24 and 48 hours for inhibition of growth.

TABLE I.
Penicillin Resistance and Penicillinase Production of Staphylococci.

Least concentration of penicillin inhibiting growth of staphylococci		Penicillinase production
Units/ml	No. of strains	
0.05	90	—
0.15	9	—
0.45	0	—
1.35	7	+
4.05	7	+
>4.05	2	+

Penicillinase determinations were made on all 115 strains by adding penicillin to broth cultures and subsequently testing for destruction of penicillin by the Oxford cup method as previously reported.[8] Coagulase tests were carried out in the routine fashion. Plasma diluted with 4 parts of 0.85% NaCl in 0.5 ml volumes were inoculated with a loopful of a 24-hour broth culture. These tests were placed in the incubator at 37° C for 4 to 5 hours and left at room temperature over night. Any evidence of a coagulum was accepted as a positive test.

Results. In general the cultures of staphylococci studied fell into 2 distinct groups as to their sensitivity to penicillin. All of the susceptible strains were inhibited by 0.15 units of penicillin per ml of agar. Most of the strains were inhibited by 0.05 units. Resistant strains, on the other hand, required penicillin in concentrations 10 to 25 times greater for the same degree of inhibition. Many of these required as much as 4.0 units before inhibition took place. Table I summarizes the results of these tests. Sixteen strains or 13.9% of the staphylococci studied were resistant to penicillin as evidenced by the necessity for a concentration of penicillin greater than 0.15 units for complete inhibition.

Close correlation is observed between resistance of staphylococci to penicillin and

5 Rake, G., McKee, C. M., Hamre, D. M., and Houck, C. L., *J. Immunol.*, 1944, **48**, 271.

6 Kirby, W. M., *Science*, 1944, **99**, 452.

7 Abraham, E. P., and Chain, E., *Nature*, 1940, **146**, 837.

8 Bondi, A., and Dietz, C. C., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 132.

TABLE II.
Coagulase Activity and Penicillinase Production of Staphylococci.

Coagulase activity	Penicillinase production	
	Positive	Negative
Positive	12	54
Negative	4	45
Totals	16	99

their ability to produce penicillinase. Each strain requiring a concentration greater than 0.15 units for inhibition was found to produce penicillinase. On the other hand not a single susceptible strain produced penicillinase. It is apparent from Table II that the ability to produce coagulase is rather common among the penicillinase-positive staphylococci.

Discussion. It is clinically significant that such a large number of staphylococci are naturally resistant to penicillin. The incidence of 13.9% observed in this study is in general agreement with that of Spink and collaborators,[10] 12.0%, and that of Gallardo,[3] 12.9%. Infections caused by such staphylococci are not likely to respond to penicillin in dosages generally prescribed.[11] It remains to be seen whether increased dosage might be effective against such infections. No doubt certain of the therapeutic failures which have been reported[12] were infections caused by these naturally resistant staphylococci. The routine *in vitro* testing of staphylococci to penicillin prior to treatment would be of definite value in the prediction of such failures.

The perfect correlation which exists between resistance of staphylococci to penicillin and their ability to produce penicillinase is worthy of note. It is apparent that their resistance is the result of their ability to produce penicillinase. This is not true of other penicillin resistant bacteria.[13] Organisms such as *E. typhosa* and *Salmonellas* are resistant to penicillin but they do not produce penicillinase. Members of the genus *Shigella* and other organisms produce this enzyme but whether this is the only factor in their resistance to penicillin remains to be seen. As far as staphylococci are concerned one could run a penicillinase test in order to determine their susceptibility to penicillin. Ability to produce penicillinase may be accepted as *a priori* evidence of resistance to penicillin.

There seems to be no general relationship between coagulase production and penicillinase production. Of the 16 strains producing penicillinase 12 or 75.0% were coagulase positive. Inasmuch as the ability to produce coagulase is generally accepted as an important criterion of the vriulence of staphylococci, it is significant that so large a number of resistant staphylococci are coagulase positive.

There is but little doubt in the opinion of the authors that the resistance of the 16 strains of staphylococci to penicillin is a natural rather than an acquired resistance. This opinion is based upon previous work of the authors[13] as well as that of other workers, namely, that organisms made resistant to penicillin either *in vitro* or *in vivo* do not acquire the ability to produce penicillinase. The mechanism involved in such induced resistance of bacteria to penicillin is as yet unknown, but it is quite definite that the development of such resistance is not accompanied by an acquisition of the ability to produce penicillinase. That all 16 of the staphylococci produced penicillinase appears to the authors to be sufficient evidence for the claim that the resistance is a natural one. In the case of staphylococci the penicillinase test should prove to be an important tool in the study and differentiation between natural and acquired resistance to penicillin.

Summary. (1) In a study of the susceptibility of 115 strains of staphylococci to penicillin 16 or 13.9% were found to be resistant. (2) All strains resistant to penicillin pro-

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10 Spink, W. W., Ferris, V., and Vivino, J. J., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 207.

11 Kolmer, J. A., *Penicillin Therapy*, D. Appleton-Century Co., 1945.

12 Bloomfield, A. L., Kirby, W. M., and Armstrong, C. D., *J. Am. Med. Assn.*, 1944, **126**, 685.

13 Bondi, A., and Dietz, C. C., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 135.

duced penicillinase; none of the susceptible strains had this property. (3) Many of the penicillinase-producing staphylococci produce coagulase indicating the importance of the former in infection. (4) Staphylococci naturally resistant to penicillin appear to be resistant as a result of their ability to produce penicillinase.

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Relationship Between Precipitative and Protective Antibodies of Type III *Shigella paradysenteriae* (Flexner) Immune Serum.

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The isolation and properties of the specific somatic antigens of several types of *Shigella paradysenteriae* (Flexner) have been described in detail in a previous publication.[1] These substances appear to be single chemical entities constituted from a phospholipid, a protein and a polysaccharide. The antigens are toxic and as far as has been determined, account for all of the immunological properties of the microorganisms from which they are derived. Thus, when small quantities of antigen are injected into experimental animals they give rise to bacterial agglutinins and precipitins which are similar in all respects to those evoked by the microorganisms themselves. In addition, they elicit antibodies in mice which protect them against lethal infections with homologous bacilli. The polysaccharide portion of the complex is neither toxic nor is it antigenic in rabbits; it is this constituent, however, which is responsible for most of the serological properties of the somatic antigen.

The present study was undertaken to determine whether the specific lipocarbohydrate-protein complex of Flexner dysentery bacilli is the only antigen which stimulates the production of protective antibodies or whether the microorganisms contain additional constituents which are important in the immune response. Experiments designed to ascertain the immunological role of the polysaccharide hapten are included as well.

In the following experimental account we shall compare the protective antibody present in the sera of rabbits immunized with Type III Flexner bacilli with that remaining after absorption with the homologous specific antigen and its polysaccharide hapten.

Materials and Methods. The Type III specific antigen used in this study was isolated as described previously.[1] The polysaccharide hapten was obtained by dissociation of the complex with acetic acid.[1] The immune sera were prepared by subjecting rabbits to 3 prolonged courses of injections with formol-killed *Sh. paradysenteriae* (Flexner) Type III (Andrewes and Inman,[3] Type Z, Boyd,[2] and Weil *et al.*,[4] Type III). The rabbits were bled after each course of injections and the sera obtained were pooled. Passive protection tests were performed on Swiss mice weighing 18-20 g. 0.1 ml of 5-fold dilutions of the sera to be tested were injected intra-

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2 Boyd, J. S. K., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1940, **33**, 553.

3 Andrewes, F. W., and Inman, A. C., *Med. Research Council*, Special Report Series, No. 42, London, 1919.

4 Weil, A. J., Black, J., and Farsetta, K., *J. Immunol.*, 1944, **49**, 321.