developed late. These lesions were smaller than is usual. A suspension of the popliteal lymph nodes of one of these rabbits was used to inoculate the testes of two other rabbits, one of which later developed an orchitis less severe than that ordinarily observed with the Nichols strain. The testes were ground and an *in vitro* test was performed to determine whether any alteration had occurred in the resistance of the spirochetes to penicillin. Using this suspension another intratesticular passage was made in rabbits and the action of penicillin on the spirochetes again determined. The mean of the results of both tests is shown by the broken line in Fig. 1. These clearly show that the strain obtained from the insufficiently treated rabbit was more resistant to the action of penicillin than was the original Nichols strain. The tests in which 1600 O.U. were present in 0.8 ml of final mixture showed that 62 to 81% of the spirochetes of the unaltered Nichols strain were immobilized, while only 6 to 18% of the penicillin-fast spirochetes were rendered immotile. This finding indicates that in the treatment of human syphilis, care must be exercised to administer a sufficient quantity of penicillin to cure the patient rapidly. Otherwise, there will be the possibility of producing a penicillin resistant strain.

Summary. The test for spirocheticidal action of drugs *in vitro* (Eagle[2]) has been used with certain antibiotic substances. Penicillin, originally presumed to be inactive *in vitro*, has proved to be active under these conditions in the relatively high concentration of 800 to 1600 Oxford units per 0.8 ml. Inadequate therapy of syphilis in a rabbit resulted in the production of a more resistant strain of *Treponema pallidum* and emphasizes the importance of adequate initial therapy.

14501 P

Influence of Vitamin K Preparations on Blood Pressure in Hypertensive Rats.*

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While we have been occupied with the preparation of kidney extracts capable of lowering the blood pressure, it has become increasingly clear that the hypertension resulting from inadequate blood supply to the kidney might be due to faulty deamination of certain amino acids. Holtz *et al.*[1] found that in anaerobic conditions, such as may exist in renal ischemia, decarboxylation without subsequent deamination can occur, thus leading to the accumulation of pressure amines, and Bing and co-workers[2,3] demonstrated that experimental hypertension can be produced by injection of amino acids into the ischemic kidney of cats. Under normal aerobic conditions, on the other hand, such amines do not accumulate since they are readily destroyed by the catalytic action of amino oxidase. Friedman, Soloway, Marrus and Oppenheimer[4] demonstrated that certain quinones, which may inactivate pressure amines, may have well-defined blood pressure reducing qualities in hypertensive rats. Grollman and Harrison,[5] found some blood pressure lowering substance in body and liver oils of fish and assumed that this substance might also be a quinone. It was, therefore, suggestive to investigate the

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4 Friedman, B., Soloway, S., Marrus, J., and Oppenheimer, B. S., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 195.

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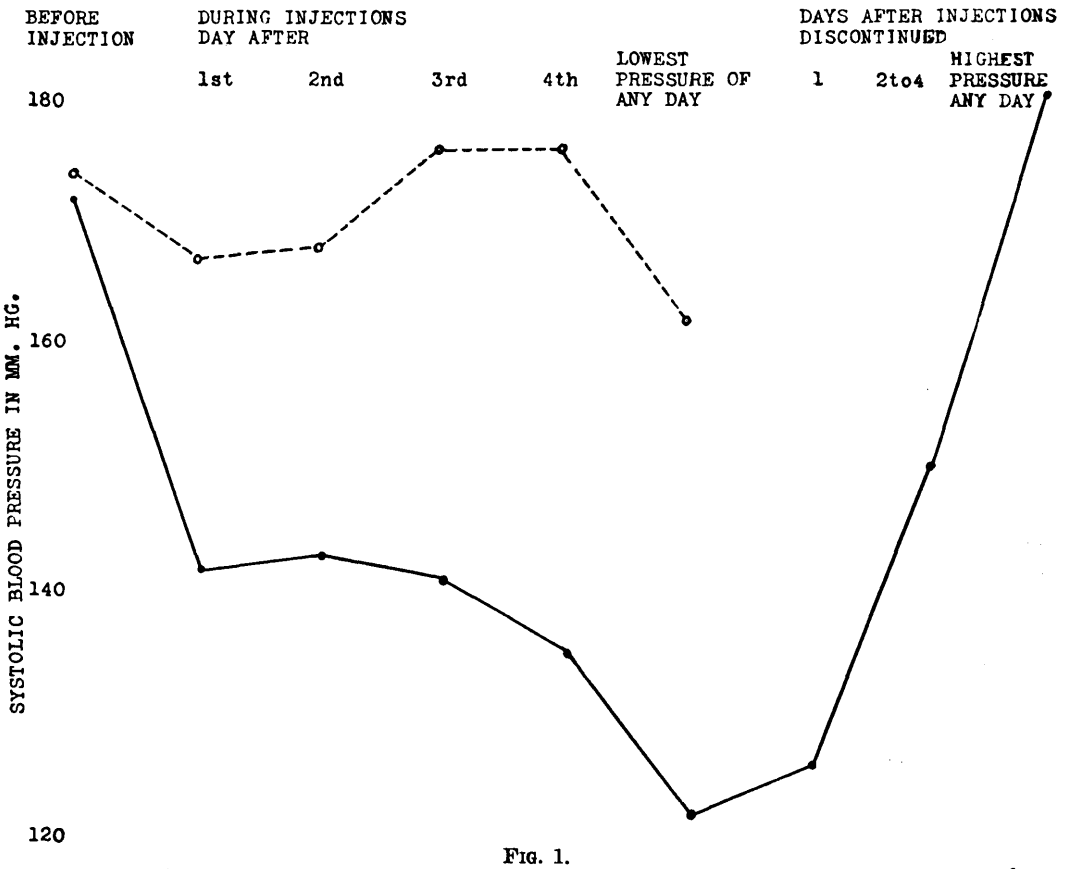

FIG. 1.

The solid line shows the average of 9 experiments before, during, and after 5 to 10 mg of Menadione daily. Another hypertensive animal did not respond to Menadione injections. The dotted line shows the average blood pressure figures of 5 rats before and during administration of 10 mg of Synkayvite daily.

blood pressure of experimental hypertensive rats after quinones of the vitamin K group had been administered.

Methods. The rats were rendered hypertensive by wrapping of both kidneys with silk. The blood pressure was determined by the platysmographic method of Grollman, Harrison and Williams.[6]

The substances used were 2-methyl-1,4-naphthoquinone, Menadione (Merck) which was dissolved in oil and given intramuscularly, and the water soluble hydroquinone compound 2-methyl-1,4-naphthohydroquinone, diphosphoric acid ester tetrasodium salt, Synkayvite (Roche), which was also applied by intramuscular injection.

[6] Grollman, A., Harrison, T. R., and Williams, J. R., *J. Clin. Invest.*, 1939, **18**, 373.

Results. The results of the experiments are shown in the accompanying figure, from which it is evident that vitamin K (Menadione, Merck) is capable of lowering the blood pressure of hypertensive rats in a way similar to other quinones and to kidney extract. The hydroquinone compound (Synkayvite, Roche) showed no pressure-reducing activity. For any trial of treatment of human hypertension with vitamin K it must be borne in mind that much higher doses might have to be administered than those used for treatment of vitamin K deficiencies, since the doses found to be effective in hypertensive rats are relatively high.

That the result obtained with the Menadione is not due to the oil is shown by the fact that several other quinones, not reported

in this paper, in the same solvent, had no effect.

Summary. Vitamin K produces lowering of blood pressure in rats rendered hypertensive by silk perinephritis. The hydroquinone compound Synkayvite shows no such effect. The trial of vitamin K in human hypertension is being considered.

14502

Filter Paper Disc Modification of the Oxford Cup Penicillin Determination.*

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At the present time biological assay methods are the sole means of determining penicillin potency. A number of such methods are in use in various laboratories;[1] however, the Oxford cup method,[2] with several modifications,[3] has continued to be regarded as a procedure in which a commensurate accuracy and speed may be obtained with a minimum of labor. The following filter paper disc modification should reduce the labor and time involved in setting up this test, while the ease with which extra replicates may be run should increase the test's accuracy.

The principal modification lies in the use of a thick filter paper disc saturated with the penicillin sample, substituted for the sample-containing small cylinder used in the Oxford cup method. These discs may be conveniently set up on the seeded plates at a rate of about 6 per minute, a rate considerably more rapid than that in the original method. Another advantage of the discs is that the test plates may be manipulated freely to facilitate reading. Experience with a large number of tests has indicated that the zones of inhibition obtained with the discs are more consistent and more sensitive to variations in the penicillin content of the sample than the zones obtained with the cup method. This improvement may be due to a more consistent contact of the penicillin solution with the agar and to a more even diffusion from the disc.

The test organism, *Staphylococcus aureus H*† (Oxford strain), tends to produce a somewhat granular growth in nutrient broth. With the use of peptone broth as the seeding medium, a more diffuse growth is obtained and this in turn effects a more even seeding of the test plates.

Hobby[4] has criticized the plate method as having disadvantages arising from discrepancies in the depth and dryness of the agar and in the lag phase of the test organism. An attempt was made to control these factors by the use of a stabilized culture adapted to the seeding medium, and through a more complete standardization of the preparation and the treatment of the seeded plates.

Aside from the above modifications, the following procedure conforms closely to that of the Oxford method.

Procedure. The test organism, *Staphylococcus aureus H*, was transferred from an agar slant twice through peptone broth‡ for 24-hour

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† Culture furnished through the courtesy of Dr. J. W. Foster, Merck & Co., Rahway, N.J.

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‡ 1% peptone, 0.5% NaCl.