Section of the moderator nerves at no time produced any pressor or depressor response in this animal.

Summary. From the foregoing results it seems clear that in the dog, removal of both kidneys does not prevent the immediate appearance of a pressor response to moderator nerve section. The reflex response to such a procedure is at least equal to that seen in normal dogs and if anything, seems to be heightened. Chloralose and ether were each used as anesthetic agents in both groups of experiments. It so happened that one of the most marked pressor responses occurred after the use of each kind of anesthetic agent in both the normal and nephrectomized group of dogs. In view of the slightly different initial blood pressure levels in the two groups, as well as the difficulty in ascertaining the exact depth of anesthesia in each experiment, close quantitative comparison of the pressor responses in the two groups is not justified. It would seem, however, that circulating pressor substances arising from the kidney (*e. g.*, angiotonin) are not demonstrably important in the production of acute neurogenic hypertension.

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Comparative Study of Effect of Sulfadiazine and Sulfathiazole on *Staphylococcus aureus.*

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(Introduced by Chester S. Keefer.)

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Sulfadiazine has been found to be as effective as sulfapyridine and sulfanilamide in the treatment of experimental pneumococcal and streptococcal infections,[1, 2] and its administration to man causes relatively few toxic symptoms.[3, 4, 5] In staphylococcal infections

[1] Feinstone, W. H., Williams, R. D., Wolff, R. T., Huntington, E., and Crossley, M. L., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 427.

[2] Robbin, R. O., Jr., and Winnek, P. S., *J. Am. Chem. Soc.*, 1940, **62**, 1999.

[3] Peterson, O. L., Strauss, E., Taylor, F. H. L., and Finland, M., *Am. J. Med. Sci.*, 1941, **201**, 351.

[4] Plummer, N., and Ensworth, H. K., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 734.

[5] Reinhold, J. G., Flippin, H. F., Schwartz, L., and Domm, A. H., *Am. J. Med. Sci.*, 1941, **201**, 106.

in mice, the percentage of survivals following treatment with sulfadiazine compared favorably with the number of survivals following the administration of sulfathiazole.[1] The results of comparing the relative effectiveness of sulfanilamide and sulfadiazine against hemolytic streptococci were reported in a previous paper.[6] At this time, we report the results of a comparative study of the effect of sulfadiazine and sulfathiazole on various pathogenic strains of *Staphylococcus aureus.*

Methods. The methods employed in this study have been described previously.[7, 8] In brief, the culture medium was defibrinated whole blood obtained from individuals without evidence of infection. The two drugs, sulfadiazine or sulfathiazole, were either added directly *in vitro* or were administered by mouth before withdrawal of the blood. Chemical analysis of all blood containing the drug was made.* Eight saline dilutions of an 18-hour broth culture of *Staphylococcus aureus* were made and 0.1 cc of each dilution was added to 0.5 cc of the defibrinated blood. The culture tubes were sealed and rotated in the incubator for 48 hours. The tubes were then examined for hemolysis; those cultures showing no hemolysis were opened and the contents plated out after proper dilutions had been made. The agar used in these plates contained para-amino benzoic acid.[9] Following incubation of the plates the colonies were counted.

Action of Sulfadiazine and Sulfathiazole When Added in vitro to Whole Blood. It was first necessary to determine what effect the addition of sulfadiazine *in vitro* had on the bactericidal power of blood obtained from normal individuals. Five milligrams of the powdered drug were added to 20 cc of defibrinated blood. After thorough mixing for one hour, various dilutions were made with drug-free blood as the diluent. Each dilution was then used as culture medium and the bactericidal action against the staphylococcus observed.

Fig. 1 shows the results obtained in 2 such experiments.

The addition of sulfadiazine to defibrinated blood caused an

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6 Keefer, C. S., and Rammelkamp, C. H., to be published.

7 Rammelkamp, C. H., and Keefer, C. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 664.

8 Rammelkamp, C. H., and Keefer, C. S., *New Eng. J. Med.*, 1940, **223**, 877.

* Miss Lucy Perry made all chemical determinations. Only the non-acetylated amounts of the drugs are recorded in this paper. The sulfadiazine used in this study was supplied through the courtesy of Lederle Laboratories, Inc.

9 Strauss, E., Dingle, J. H., and Finland, M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 131.

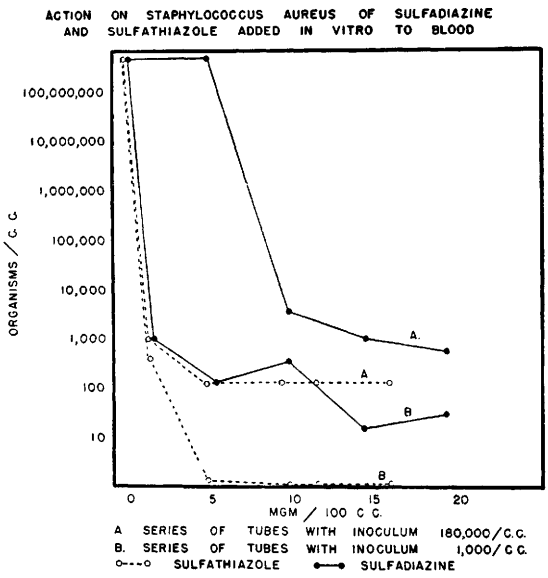

FIG. 1.
Points represent number of organisms present after 48 hours' incubation.

increase in the bactericidal power of the blood. When the original inoculum was greater than 1,000 organisms per cubic centimeter of blood, complete killing was not observed. This was found to be true even with concentrations of sulfadiazine as high as 20 mg per 100 cc. Blood containing less than 1,000 organisms per cubic centimeter usually exhibited some bacteriostatic action with concentrations as low as 2 mg per 100 cc. This action became bactericidal when levels of 5 to 10 mg per 100 cc were obtained. Concentrations above 10 mg per 100 cc produced only a slight increase in the bactericidal power.

We have shown previously[5] that the addition of sulfathiazole to whole blood caused bacteriostasis and an increased killing power against *Staphylococcus aureus*. In the present experiments sulfathiazole was somewhat more effective than sulfadiazine against pathogenic staphylococci when whole blood was used as the culture medium. In general, sulfathiazole completely killed inoculations of 10,000 organisms or less, which is about 10 times the bactericidal power observed following the addition of sulfadiazine to the media. A further point observed in these *in vitro* studies was that the action of sulfathiazole became manifest at low concentrations. When large inocula were used, bacteriostasis was more marked in the sulfathiazole-containing tubes.

Effect of Ingestion of Sulfadiazine on Bactericidal Power of

Blood. In this group of experiments, the blood was studied for bactericidal activity after the drug had been exhibited by mouth. A sample of blood was withdrawn before the administration of 4 or 5 g of sulfadiazine, and additional specimens were taken at intervals varying from 40 minutes to 4 hours after the ingestion of the drug. These samples were defibrinated and then used in the bactericidal tests.

Fig. 2 shows 2 typical responses observed in 30 experiments. It was clearly demonstrated that the administration of sulfadiazine by mouth to normal individuals increased the bactericidal effect of the blood against pathogenic staphylococci. We then studied more thoroughly the relation of the concentration of sulfadiazine in the blood to the bactericidal power observed. Fig. 3 illustrates the results obtained in one of these experiments. With a concentration of 1.3 to 1.6 mg per 100 cc no effect against pathogenic staphylococci was noted, even with a small inoculum of 20 organisms per cubic centimeter of blood. When the concentration reached 2.5 mg per 100 cc and the inoculum was less than 20,000 organisms per cubic centimeter, a definite bactericidal effect occurred. With concentrations up to 4.2 mg per 100 cc this action was even more marked. Levels of 4.8 and 5.2 mg per 100 cc caused some bacteriostatic action in blood containing 200,000 organisms per cc.

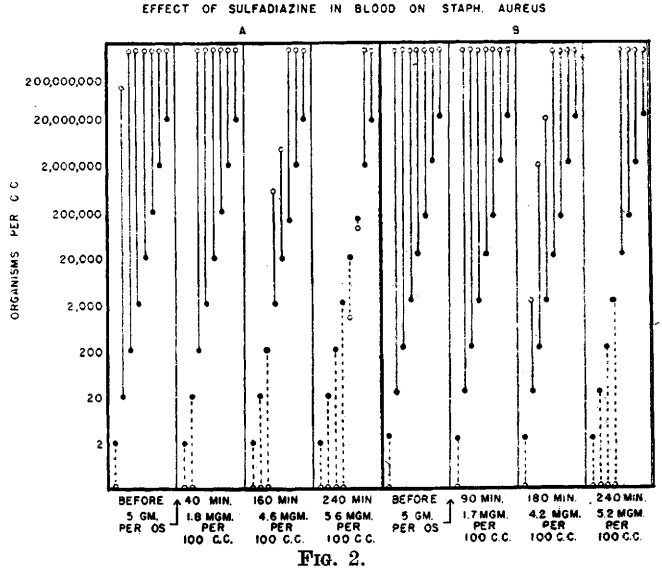

FIG. 2.

Solid dots represent original inoculum of *Staphylococcus aureus*. Circles represent number of organisms present after 48 hours' incubation. Solid lines indicate growth; interrupted lines indicate killing.

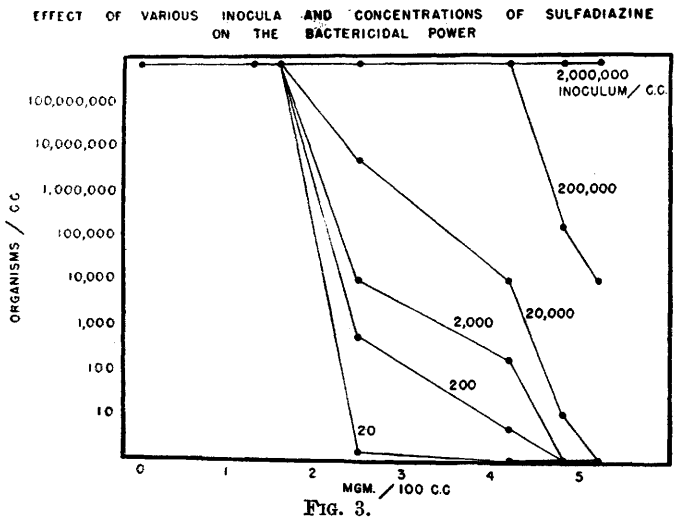

FIG. 3.

Solid dots represent number of organisms present after 48 hours' incubation. Lines connect cultures with the same original inoculum.

These studies showed, then, that concentrations of sulfadiazine of 4 to 5 mg per 100 cc are desirable in order to obtain effective bactericidal action.

Comparative Bactericidal Effect of Sulfadiazine and Sulfathiazole When Administered by Mouth. From the studies reported above, it was obvious that the addition of sulfathiazole to blood caused a greater increase in the bactericidal effect than sulfadiazine. Further, it was found that the maximal bactericidal action occurred at a lower concentration in the sulfathiazole-containing cultures. As a result of previous studies with sulfathiazole[8] it was our impression that sulfathiazole exerted a more marked action than sulfadiazine when these drugs were given by mouth.

The bactericidal power of these two drugs after oral administration was then examined and compared. Normal individuals were first given 4 g of sulfathiazole by mouth. A week later the same individual was given 4 g of sulfadiazine. In each instance blood was withdrawn before administration of the drug and several times thereafter. Bactericidal tests were carried out using an inoculum that was comparable in the two tests.

In Fig. 4 the results of 5 such comparative tests are recorded; these represent the typical results obtained in 9 such observations. It is at once evident that sulfathiazole given orally causes a greater increase than sulfadiazine in both the bactericidal and the bacteriostatic power of the blood. Occasionally the action of the two drugs is about equal, as is seen in test C. In test E the level of

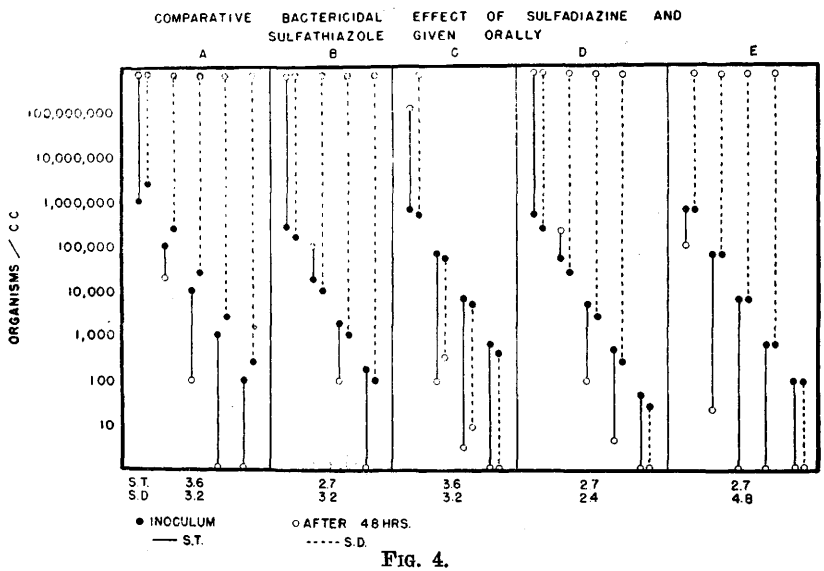

FIG. 4.
S.T.—sulfathiazole; S.D.—sulfadiazine.

sulfadiazine was 4.8 mg per 100 cc, and even in this individual a concentration of sulfathiazole of 2.7 mg per 100 cc was more effective against the staphylococcus.

Discussion. The present studies indicate that sulfadiazine increases the bactericidal power of normal blood against *Staphylococcus aureus,* both when added directly *in vitro* and when administered by mouth. The addition of the two drugs to similar samples of blood shows that sulfathiazole is somewhat superior to sulfadiazine against the staphylococcus. Further, the action of sulfathiazole becomes manifest at lower concentrations. Attempts to determine the level of sulfadiazine which produces maximal killing show that a concentration of 4 mg or above is usually necessary. Levels above 10 mg per 100 cc do not increase this action appreciably. This is in contrast to the action of sulfathiazole which produces maximal bactericidal action at a somewhat lower level.[7]

When the two drugs are administered orally, sulfathiazole invariably produces as great or greater an increase in the bactericidal action of the blood. This was true even though the concentration of sulfadiazine was twice that of sulfathiazole.

These results, then, indicate that sulfadiazine is not as effective in whole blood as sulfathiazole against the staphylococcus, even though high concentrations are obtained. It is felt, however, that sulfadiazine should prove useful in the treatment of patients with staphylococcal infections who do not tolerate sulfa-

thiazole. It would also appear useful in the treatment of staphylococcal infections of the meninges inasmuch as sulfadiazine diffuses readily into the spinal fluid.

Conclusions. Sulfadiazine increases the bactericidal action of normal blood when added *in vitro* or when exhibited orally. Comparative tests show that sulfathiazole is somewhat superior to sulfadiazine in its effect on *Staphylococcus aureus.*

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Absorption and Metabolism of Estrogens. I. Estrogen Assay by Intrasplenic Injection.

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It has been stated that estrogens are metabolized by the liver of the rat and completely inactivated. The best evidence for this is the excellent work of Heller[1] on tissue slices incubated with various estrogens. Methods of indirect attack *in vivo* have consisted in attempting to study effects of endogenous or exogenous estrogens in animals in whom the liver had been poisoned.[2, 3] There have also been attempts to recover estrogens by hashing the whole animal after injection.[4] These have also led to the belief that estrogens are completely inactivated by the liver.

The present study of which this is a preliminary report was undertaken with the view of direct attack on this problem.

The estrogen in question was injected directly into the spleen in 0.05 cc of oil solution. The spleen was exposed through a midventral incision and delivered to the outside; the site of entrance of the needle into the spleen was tied with an encircling silk ligature to prevent leakage of the oil. The injections were made into castrate females primed 14 days previously with 50 γ of estrone. Vaginal smears were taken with a moist, cotton tipped toothpick at 48, 60, and 72 hours after injection, and a fully cornified smear in any one or more of the three periods was considered as positive. These pro-

1 Heller, C. G., *Endocrinology,* 1940, **26**, 619.

2 Pincus, G., and Martin, D. W., *Endocrinology,* 1940, **27**, 838.

3 Talbot, N. A., *Endocrinology,* 1939, **25**, 601.

4 Zondek, B., and Sklow, J., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 276.