were considered: The total of cysts plus spots in each group; the total of cysts alone and of spots alone in each group; the average of cysts plus spots in each group. In addition, the method of pairing and the t-test was applied and also the z-test to determine the importance of sex.

Results. The total number of cysts plus spots is higher in splenectomized mice (148) than in normal ones (116). In splenectomized mice, 17 cysts developed to proper size (131 spots), as compared with only 6 cysts in the non-operated mice (110 spots). The average number of cysts plus spots in splenectomized mice is 5.28 ± 0.70, in normal mice 4.14 ± 0.76. This average number of cysts plus spots in the 2 groups does not differ significantly because the error attached to it is too great. When the t-test is applied, however, it becomes evident that the number of cysts found in splenectomized mice differs significantly from the number found in the normal group. This is especially true for individuals with medium resistance. When either no cysts plus spots or a large number are found, the difference between the 2 groups is not obvious. The z-test showed that sex differences are insignificant. It is evident that besides splenectomy, there are other unknown factors contributing to the variability of the number of cysts which are of equal or greater importance.

Conclusion. In inbred mice of a strain which is somewhat resistant to infection with eggs of *Taenia taeniaeformis*, splenectomy increases the susceptibility to infection except in those comparatively few mice with an individual resistance that is either abnormally high or abnormally low.

11878

Distribution of Sulfanilamide in the Ocular Fluids and Tissues After Local Application.

S. Y. P'AN. (Introduced by Hamilton H. Anderson.)

From the Department of Pharmacology, Peiping Union Medical College, Peiping, China.

Marshall[1, 2] first demonstrated that after oral administration,

[1] Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.

[2] Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. Pharm. and Exp. Therap.*, 1937, **61**, 196.

sulfanilamide is rapidly absorbed and is present in approximately equal concentrations in body tissues except bone and fat. Rambo,[3] Bellows and Chinn[4] and Mengel[5] have shown that the drug is present in the aqueous or vitreous humors or ocular tissues, after oral administration in animals or man. There are few reports on the absorption of sulfanilamide after local application into the conjunctival sac and the results reported are not uniform. Mengel[5] and Guyton[6] found that when a solution of sulfanilamide was applied into the conjunctival sac, concentrations of from less than 0.1 to 0.8 mg % were found in the aqueous humor. Bellows and Chinn[4] dusted sulfanilamide powder into the conjunctival sacs of dogs and allowed it to remain for 2 hours. They were unable to detect any trace of the drug in either the aqueous or vitreous humors. Lou and P'an,[7] on the other hand, found that sulfanilamide was present in significant amounts in the aqueous humor of human eyes after local instillation of a warmed 1% solution (concentration in aqueous humor varied from 1.0 to 4.2 mg %) or powdered sulfanilamide (concentration in aqueous humor varied from 4.5 to 44.4 mg %). The distribution of sulfanilamide in the various ocular tissues and fluids after local application has not hitherto been reported and is, therefore, the subject of this study.

Except in 2 rabbits in which sulfanilamide was applied to both eyes, 100 mg of finely divided sulfanilamide was applied to the conjunctival sac of one eye using the other eye as a control in each of the remaining 17 albino rabbits. Animals weighed from 1.5 to 2.2 kilos. The treated eye was kept closed by the application of a clamp. After an interval of one hour the animal was anesthetized with ether and samples of aqueous humor and blood were drawn. Both eyes, including the conjunctivae, were then removed and immediately dissected into the following components: conjunctiva, cornea, sclera, lens, vitreous humor and the chorioretinal layers. Tissues were washed three times in 0.9% NaCl solution, weighed and ground with sand and an appropriate amount of 5% para-toluene-sulfonic acid was added so that each cc contained from 10 to 50 mg of the tissue. These samples were centrifuged and filtered. To 2 cc of the filtrate was added 2 cc of water, and this was treated with sodium nitrite and dimethyl-alpha-napthalamine and compared with an appropriate standard according to the method of Marshall[8] in an Evelyn photo-

[3] Rambo, V. C., *Am. J. Ophth.*, 1938, **21**, 739.

[4] Bellows, J. G., and Chinn, H., *J. A. M. A.*, 1939, **112**, 2023.

[5] Mengel, W. G., *Arch. Ophth.*, 1939, **22**, 406.

[6] Guyton, J. S., *Am. J. Ophth.*, 1930, **22**, 833.

[7] Lou, T. H., and P'an, S. Y., *Chin. M. J.*, 1940, **58**, 167.

[8] Marshall, E. K., Jr., *J. Biol. Chem.*, 1937, **122**, 263.

electric colorimeter using filter No. 520. Samples of blood or aqueous humors were tested by adding 0.1 cc of the sample to 5 cc of water and precipitating with 2 cc of 15% para-toluene-sulfonic acid. This was filtered and 4 cc of the filtrate was used for the determination. Duplicate analyses were made whenever adequate samples were available.

Half of the tests were made according to the micromethod of MacLachlan *et al.*[9] using 0.1 cc of fluid or blood. The ocular tissues were ground with sand and 5% para-toluene-sulfonic acid was added as described above, 2 cc of the filtrate being used in these tests, comparisons being made with an appropriate standard in a Hasting's bicolorimeter.* The third washings of the various tissues were examined for the presence of sulfanilamide and found to be negative.

The mean concentrations of free sulfanilamide found in the ocular fluids and tissues of the 19 rabbits tested after local application of the drug are, in their decreasing order as follows: Cornea—185.4±13.2; conjunctiva—89.8±14.0; aqueous humor—54.8±5.3; sclera—23.3±4.6; chorioretinal tissue—12.3±1.2; lens—8.7±1.8; and vitreous humor—1.1±0.2 mg %.

No trace of sulfanilamide was found in the ocular fluids and tissues of untreated eyes, or in the blood, except in 2 instances where 1.8 and 1.9 mg % was detected. Except in the vitreous humor, lens and chorioretinal tissues the concentrations of sulfanilamide in the ocular fluids and tissues are higher than those obtained after the oral administration of moderate amounts of the drug in man and rabbits.[3, 5, 6, 7, 10]

Local application of powdered sulfanilamide into the conjunctival sac would appear to be the method of choice for obtaining adequate concentrations of the drug in ocular tissues and fluids, since tremendous doses would be required orally or parenterally to maintain similar levels with the consequent danger of untoward effects. Sulfanilamide has had some effect in controlling certain infections limited to the ocular region, especially trachoma and gonoblennorrhea.

[9] MacLachlan, E. A., Carey, B. W., Jr., and Butler, A. M., *J. Lab. Clin. Med.*, 1938, **23**, 1273.

* Ten duplicate tests were made with each colorimeter on samples of rabbit's blood to which sulfanilamide was added to make concentrations of 50 and 100 mg %. Similar results were obtained with each colorimeter, within 4 % of the known amount at 50 mg % and within approximately 5.5 % of the known amount at 100 mg %.

[10] Long, P. E., and Bliss, E. A., *The Clinical and Experimental Use of Sulfanilamide, Sulfapyridine and Allied Compounds*, New York, The Macmillan Co., 1939, pp. 151-153.

When such diseases are localized local application of the drug in powdered form would appear to be preferable to other methods of administration where general systemic absorption is not essential, especially in hypersensitive patients.

In rabbits a slight to moderate congestion of the conjunctiva was observed after sulfanilamide had been applied locally. Microscopic examination of the cornea removed from four of the treated eyes showed no significant changes while the conjunctiva exhibited a moderate degree of desquamation and a very slight leucocytic infiltration just beneath the epithelium. These changes may be due to the mechanical rather than the chemical effects of the powdered drug.†

Summary. Sulfanilamide was found to be present in significant concentrations in ocular tissues and fluids after its local application in powdered form into the conjunctival sacs of rabbits. No appreciable untoward reactions were encountered in the 19 rabbits studied. Local application of the drug for 1 hour did not result in an appreciable systemic absorption, since no sulfanilamide could be detected in blood samples, with two exceptions, or in ocular tissues and fluids of the untreated eyes of test animals.

11879 P

Specific Polysaccharide as Cutaneous Test for Evaluation of Serum Therapy in Influenza Bacillus Meningitis.

JOHN H. DINGLE AND LEAH R. SEIDMAN.
(Introduced by J. F. Enders.)

From the Infants' and Children's Hospitals and the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and from the Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

The use of specific polysaccharides injected intracutaneously as a means of evaluating the adequacy of serotherapy was first advocated in pneumonia by Francis,[1] and has since been employed by others[2, 3, 4]

† The author is indebted to Dr. C. H. Hu for examining the histological sections and to Dr. S. H. Liu for the use of the Evelyn photoelectric colorimeter.

1 Francis, T., Jr., *J. Exp. Med.*, 1933, **57**, 617.

2 Finland, M., and Brown, J. W., *J. Clin. Invest.*, 1938, **17**, 479.

3 Felton, L. D., and Prather, P. F., *Pub. Health Rep., U.S.P.H.S.*, 1939, **54**, 1053.

4 Wood, W. B., Jr., *J. Clin. Invest.*, 1940, **19**, 95, 105.