A and to 186 mg (range: 154-243) in dog B. The fasting plasma levels of ascorbic acid decreased to 0.32 mg% in dog A and to 0.34 mg% in dog B. This was accompanied by an average decrease in reabsorptive Tm of 17.5% in dog A, 58% in dog B, and 45.3% in dog C. The clearance ratio rose 11, 23, and 20% respectively.

Conclusions. Estradiol increases the clearance of ascorbic acid in the dog by reducing tubular reabsorption, the clearance approaching that of creatinine in some cases. As a result, the fasting plasma levels of ascorbic acid fall during the treatment.

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A Test Paper for the Rapid Estimation of the Level of Sulfonamide in Serum.

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The reaction of Ehrlich's reagent, *p*-dimethylaminobenzaldehyde, with aromatic amines in acid solution has been made the basis of a colorimetric determination of sulfonamides by Werner[1] and by Morris.[2] Fuller[3] has employed this substance in test papers for estimating the level of sulfonamide in laked blood after precipitation of proteins with an acid reagent, comparing the yellow color produced with standard color strips. The present paper describes the preparation of acid-containing test papers with which the level of sulfonamide can be estimated directly in serum without the addition of precipitating or acidifying reagents.

One gram of *p*-dimethylaminobenzaldehyde (straw-colored material is usable) is dissolved in 2 cc of concentrated hydrochloric acid and to the solution is added 0.8 cc of syrupy phosphoric acid (sp. gr. about 1.7) and water to make 100 cc. Large pieces of a good grade of absorbent paper (such as is used in making litmus paper) are soaked in the solution and immediately hung up to drain. When superficially dry, the papers are stored in the dark in covered glass jars for from 5 to 10 days, during which time most of the hydrochloric acid evaporates. The edges of the paper, which are usually yellow, are then trimmed off and the paper is cut into small strips and preserved in suitable vials. The strips are almost colorless, but become discolored if they come in contact with the skin at any time during preparation or use. The phosphoric acid remaining in the papers is sufficient to give a pH of approximately 3 when they are moistened with serum.

Estimation of the sulfonamide level is made by spreading evenly the serum to be tested over one end of an impregnated strip by means of a fine dropper, care being taken that unabsorbed fluid does not remain. In this way, the serum is spread over the paper by the movement of the dropper rather than by the capillarity of the fibers, and the reagents are consequently not leached out of areas which are oversaturated. Within 10 seconds a maximum development of color occurs. To avoid translucence of the paper interfering with the reading, the wet strip is held close to a white background or placed on white paper or white porcelain. The color is then judged in comparison with a graded series of dry standard color strips. The color ranges from a pale violet with normal human serum to a bright yellow with serum containing 15-20 mg sulfadiazine per 100 cc. A comparison of values obtained by the test-paper method with those secured by the method of Bratton and Marshall[4] are set forth in Table I.

1 Werner, A. E. A., *Lancet*, 1939, **1**, 18.

2 Morris, C. J., *Biochem. J.*, 1941, **35**, 952.

3 Fuller, A. T., *Lancet*, 1942, **1**, 760.

4 Bratton, A. C., Marshall, E. K., Babbitt, D., and Hendrickson, A. R., *J. Biol. Chem.*, 1939, **128**, 537.

TABLE I.
Comparative Sulfadiazine Estimations.

Serum sample No.	Method of Bratton and Marshall mg%	Estimation by test papers — Observer* A mg%	B mg%	C mg%	D mg%
1	0.0	0	0	0	0
2	0.0	0	0	0	0
3	0.0	0	0	0	0
4	5.7	6	7	4	4
5	4.3	4	5	4	3
6	5.1	5	6	4	4
7	14.2	14	11	12	11
8	9.3	10	9	8	11
9	9.5	10	—	—	—
10	6.2	6	7	10	11
11	6.4	6	8	8	8
12	3.6	4	6	6	5
13	7.3	8	9	6	8
14	0.0	0	0	0	0
15	7.2	6	4	4	4
16	7.5	8	—	—	—
17	7.1	8	6	6	6
18	8.0	8	8	8	8
19	6.2	6	9	4	8
20	14.2	14	13	12	12
21	7.8	8	5	8	11
22	6.8	8	8	6	10
23	8.3	8	10	8	11
24	5.5	4	7	4	4
25	7.3	6	7	6	8
26	0.0	0	0	0	0
27	4.8	4	6	8	4
28	10.3	10	11	12	10
29	10.0	10	10	8	10
30	11.2	10	10	8	12
31	9.7	—	8	12	12

* A—Experienced observer. B, C, D—Inexperienced observers.

Satisfactory color standards may be made by coloring paper strips with either water colors or soluble dyes. Suitable graded concentrations of picric acid in combination with Orange G to furnish the weak reddish component have been satisfactory. Standards made by selection of appropriate tints from samples of colored construction papers should prove satisfactory. The colors are calibrated by comparison with test strips moistened under the usual conditions with human sera containing known amounts of sulfonamide as determined in serum by standard methods. A single color standard is suitable for estimation of sulfapyridine, sulfathiazole or sulfadiazine; for sulfanilamide the values read from this standard should be divided by 1.5.

The standard does not apply to urine and body fluids containing variable amounts of protein. The colors can be grouped in intensities corresponding to 0-3, 3-6, 6-9, 9-12 and over 12 mg of sulfadiazine per 100 cc or in other convenient groupings. After some practice readings may be obtained which agree within 1 mg (plus or minus) with determinations made by the method of Bratton and Marshall. In any event, in the hands of a worker experienced in the clinical interpretation of sulfonamide levels, serum concentrations should be readily classifiable into groupings such as "zero," "subtherapeutic," "therapeutic" and "toxic." The limits of concentration for any group will depend, as in the method of Bratton and Marshall, on the type of sulfonamide administered. Sulfonamide values for whole blood are not directly transferable to serum values, since the latter are usually higher for the same blood sample.

The reaction on the test papers is given by free sulfonamides and other primary aromatic

amines including *p*-aminobenzoic acid, *i.e.*, by the same compounds that give color in the method of Bratton and Marshall.[4] Urea in pathological concentrations yields significant amounts of color with Ehrlich's reagent. Pyrrols and indoles, however, do not react with the reagent at the acidity attained in the test. Moderate hemolysis in the serum seems not to interfere seriously with color matching. The chief bars to accuracy appear at the present time to be (a) the possibility that the test papers or the standards may prove to be unstable under certain conditions of storage or exposure and (b) the fact that since the intensity of the color is influenced by protein and the test is empirically standardized for normal protein content of serum, alterations of the proteins in pathological sera might lead to inaccuracies. The first difficulty can be removed in part by keeping test papers in a cool place away from direct light and moisture. The other limitations as yet have not been adequately explored. Results of attempts to replace the phosphoric acid by oxalic or citric acids in the preparation of test papers indicate that modification in this direction is possible.

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The Chemotherapeutic Effect of Esters of Penicillin.*

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In a recent communication[1] the preparation of esters of penicillin was reported. Although aliphatic esters show no bacteriostatic action *in vitro*, it was shown that they are highly effective chemotherapeutic agents in experimental infections due to hemolytic streptococci. Evidence was presented to show that they are effective *in vivo* both by the subcutaneous and by the oral route of administration. This activity is undoubtedly due to the hydrolysis of the esters with a slow liberation of active penicillin.

In this paper experiments will be described which confirm and extend the previous observations.

Materials and Methods. The penicillin employed throughout the study was prepared by the chloroform method.[2] The free acid was esterified with diazoethane or diazo-n-butane prepared from the corresponding derivatives of nitroso-urea. Remaining acidic fractions were separated from the ester by alkaline phosphate extraction.† Unless otherwise specified, the esters were dissolved in absolute alcohol before use and the solutions diluted with four volumes of propylene glycol.

In all experiments 15-hour blood broth cultures of a highly virulent strain of Group A hemolytic streptococci (strain C203Mv) were used. Mice were infected by the intraperitoneal route with 1 cc of varying dilutions of culture. Treatment was carried out by either the subcutaneous or the oral route of administration. All experiments were controlled with a series of untreated animals.

Subcutaneous Administration. In the first experiments mice infected with 1 cc of 10^{-3}, 10^{-4}, and 10^{-5} dilutions of hemolytic streptococci were treated with ethyl esters of peni-

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1 Meyer, K., Hobby, G. L., and Chaffee, E., *Science*, 1943, **97**, 205.

2 Meyer, K., *et al.*, *Science*, 1942, **96**, 20.

† Oily fractions removed from both the ethyl and butyl esters contained ethers and addition products of the diazo compounds, probably pyrazoline derivatives. These oily fractions were only weakly active *in vivo* and were locally irritating.