TABLE I.
Summary of Data.

Manganese content of diet	Females		Males	
	Low	High	Low	High
No. of rats	13	13	5	5
Final weight, g	190.8	191.1	237.0	237.6
Length of fresh tibia, cm*	3.38	3.43	3.44	3.49
Density of fresh bone, g/cc†	1.536	1.570	1.461	1.479
Breaking strength of femur, kg	7.90	9.35	7.89	9.91
Phosphatase, units/g fresh bone	12.69	14.46	17.02	17.43

* Avg of 2 bones.
† Avg of all bones.

lower density of the bones of the manganese-deficient rats observed in this study cannot be explained by a reduction in ash content of the bones since the percentage ash was the same for both groups. Caskey, Norris, and Heuser (unpublished results) found that the bones of manganese-deficient chicks contained slightly more fat and less water than those of normal chicks. If the same is true of the rat, it could explain the lower density without corresponding decrease in ash content.

The percentage of calcium and phosphorus in the fat-free dry bone was not lowered in the manganese-deficient animals. These results agree with those of Wachtel *et al.*[2] The percentage of magnesium was also the same for both groups.

The breaking strength of the bones of manganese-deficient rats was much less than those of the controls. The odds that the difference was significant were greater than 999:1. These results are in agreement with those reported by Smith, Medlicott, and Ellis[7] for the rabbit. The difference may be due in part to the lower density of the bones of deficient rats or to structural changes in the matrix of the bone, not affecting density and mineral content. The possibility also exists that there may be changes in the crystal structure of the bone salts.

The amount of phosphatase activity of the fresh bone was less in the manganese-deficient rats. The difference was found to be highly significant with odds of 830:1. These results are not in agreement with those of Wachtel *et al.*[2]

Summary. The length, density, breaking strength, and phosphatase content of the bones of manganese-deficient rats were lower than in pairmates of the same weight receiving adequate manganese. There was no difference between the two groups in percentage of ash, calcium, phosphorus, and magnesium in the fat-free dry bone.

7 Smith, S. E., Medlicott, M., and Ellis, G. F., *Arch. Biochem.*, 1944, **4**, 281.

15049

A Method for Determination of Streptomycin in Body Fluids.

ROBERT B. STEBBINS AND HARRY J. ROBINSON. (Introduced by H. Molitor.)
From the Merck Institute for Therapeutic Research, Rahway, N.J.

The introduction of streptomycin as a chemotherapeutic agent for the treatment of bacterial infections[1-4] has emphasized the need for a quantitative method for the determination of the drug in body fluids.

1 Jones, D., Metzger, H. J., Schatz, A., and Waksman, S. A., *Science*, 1944, **100**, 103.

2 Robinson, H. J., Smith, D. G., and Graessle, O. E., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 266.

In a recent communication, Foster and Woodruff described a method for the determination of streptothricin in aqueous solution.[5] Attempts to apply this procedure directly to the quantitative determination of streptomycin in blood, urine and body tissues were not satisfactory for, among other things, it was found that the blood of certain animal species, as well as man, contains a substance which produced a marked inhibitory effect upon the test organism *(Bacillus subtilis)*. Frequently this substance was present in amounts sufficient to produce a zone of inhibition larger than that obtained with low concentrations of streptomycin. Furthermore, the method of Foster and Woodruff, when applied to the assay of streptomycin, was not sufficiently sensitive to measure accurately concentrations of the drug in serum below 20 units per cc.

The foregoing difficulties were largely eliminated by the use of a streptomycin sensitive organism *(Staphylococcus aureus SM)* which was not inhibited by normal blood, by increasing the pH of the medium and by decreasing the salt concentration. These two latter factors were shown by Foster and Woodruff to influence the activity of streptothricin and later[6] were shown to enhance the activity of streptomycin in culture fluids, etc., when *Bacillus subtilis* was employed as the test organism.

The assay described in this communication is suitable for the determination of streptomycin in blood, urine and tissue fluids of mice, rats, rabbits, dogs, monkeys and man.

Materials. A strain of *Staphylococcus aureus SM* is used as the test organism. The culture is maintained in a uniform condition by daily transfer in F.D.A. nutrient broth at 37°C. The assay medium is a modified F.D.A. nutrient agar.* Including the beveled glass cylinders known as penicylinders,† the glassware required is ordinary standard bacteriological equipment.

Procedure. Using a 6-hour culture of the test organism grown in F.D.A. broth, a serial 10-fold dilution is made through 10^{-4} in broth. A final dilution to 10^{-5} is then made directly into the melted nutrient agar which has previously been cooled to 45°C. With the aid of a calibrated wide mouth pipette, 10 cc of this melted seeded agar is added to each of a series of petri dishes and these are set aside until the agar solidifies. Next, the beveled glass cylinders are warmed slightly by passage through a Bunsen flame and immediately placed upon the surface of the agar plate. In this manner a seal between the glass cylinder and the agar is effected. Four such cylinders are placed in each agar plate. The glass cylinders are then filled with the properly diluted samples for assay and the plates incubated at 30°C for 16-18 hours. At the end of this period the diameters of the zones of inhibition are measured in millimeters.

Assay of Blood, Urine and Tissue Extracts. In determining the concentration of streptomycin in blood, it is necessary to construct a standard curve of reference for each assay using an aliquot of the drug solution which is being administered to the patient. This is done by making dilutions of streptomycin containing 1, 2, 4, 8, and 16 units per cc in serum obtained from each individual prior to treatment or from any normal donor. The diameters of the resulting zones of inhibition are plotted as ordinates (arithmetic scale) against the concentrations of streptomycin as abscissa (logarithmic scale). The values obtained in a typical assay are shown in Table I, and the standard curve obtained therefrom in Fig. 1.

Test serums containing unknown amounts

* Peptone (siccum-Armour)	10.0 g
Bacto Beef Extract (Difco)	5.0 "
Salt (NaCl)	2.5 "
Agar-agar	10.0 "
Distilled water	1,000 cc

Adjusted to pH 7.5-8.0 with N/1 NaOH

† This is a trade name for a special type of glass cylinder developed for the assay of penicillin.

[3] Feldman, W. H., and Hinshaw, H. C., *Proc. Staff Meet., Mayo Clinic*, 1944, **19**, 596.

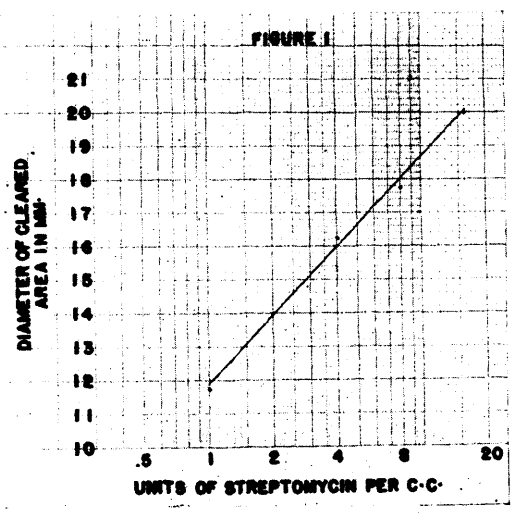
[4] Heilman, F. R., *Proc. Staff Meet., Mayo Clinic*, 1944, **19**, 553.

[5] Foster, J. W., and Woodruff, H. B., *J. Bact.*, 1943, **45**, 408.

[6] Foster, J. W., and Hendlin, D., to be published.

TABLE I.
Diameters of Cleared Areas Produced by a Streptomycin Standard with Human Serum as the Diluent.

Units per cc	1 mm	2 mm	4 mm	8 mm	16 mm
Plate 1	12.0	14.0	16.5	18.0	20.0
Plate 2	11.5	14.0	16.0	17.5	20.0
Avg	11.75	14.0	16.25	17.75	20.0



A standard curve for streptomycin in human serum.

f the drug are diluted with any normal human serum to contain approximately 5 to 15 units per cc. This will insure a reading which will fall on the standard curve. Dilutions of the test sample as well as each unitage level of the standard should be run in duplicate. The drug concentration in the serum sample is obtained by determining from the standard curve the streptomycin concentration corresponding to the diameter of the zone of inhibition and correcting for the dilution.

In the assay procedure for urine, feces or tissue extracts distilled water replaces serum as the diluent.

The results of a study of the distribution of streptomycin in blood, urine and tissue fluids in a variety of animal species will be reported elsewhere.

Statistical Analysis of the Method.‡ The curve relating unitage of streptomycin per cc in the cylinder to the diameter of the cleared area on the plate was investigated in three assays with several individual human sera, water, and certain combinations of serum and water as diluents for the drug. The data in these assays were analyzed in accord with methods described by Bliss for the biological assay of drug potency.[7,8]

These assays showed that the diameter of the cleared area in millimeters could be plotted as a straight line against the logarithm of the unitage in the range from 1 or 2 units to 20 units per cc. There was no significant change in sensitivity of the assay with change in unitage level. Individual or pooled sera could be used interchangeably as diluent without affecting relative unitage of streptomycin within a given assay.

The apparent unitage of streptomycin when assayed in a 2-fold dilution of serum with water was 89 ± 6%§ of the unitage in serum alone, and the apparent unitage in the 4-fold dilution of serum with water was 82 ± 6% of the unitage in serum. The apparent unitage in water, however, was 79 ± 8% and 57 ± 7% of the unitage with serum as the diluent in 2 different assays.

The slopes of the standard curves in the above 3 assays were 8.6, 6.7 and 5.9 (mm change in zonation per 10-fold change in unitage level). All the curves within each assay were parallel regardless of diluent, although slight deviation from linearity was observed at the 1 unit level in the first assay and some curvature was present in the third assay. The inherent precision of the method, as indicated by s/b = 0.068, 0.052 and 0.055 for the 3 assays, is quite satisfactory for a biological assay.[9] The variation between plates was of borderline significance.

‡ The authors wish to thank Dr. Walther H. Ott for permission to include in this paper his report on the statistical analysis of the data.

[7] Bliss, C. I., and Marks, H. P., *Quart. J. Pharm. and Pharmacol.*, 1939, **12**, 182.

[8] Bliss, C. I., *J. Am. Stat. Assn.*, 1944, **39**, 479.

§ Standard error.

[9] Bliss, C. I., and Cattell, M., *Ann. Rev. Physiol.*, 1943, **5**, 479.