as the result of a desiccation experiment[9] to 72.3 as measured by use of deuterium oxide.[10] The variations may, of course, have been due to the employment of different methods, but in the light of the present study, seem more likely to be the result of varying degrees of obesity.

These data serve to emphasize the necessity for taking into account the amount of adipose tissue of the body before comparison of the proportion of body water between individuals has much significance. They serve also to corroborate the usefulness of specific gravity as a measurement of the proportion of fat and water in the body.

Summary. 1. Body fat and water can be calculated from body specific gravity with considerable accuracy.

2. Use of an independent method of measuring body water clearly shows the close inverse relationship of per cent body fat and per cent body water.

3. The proportion of water in the body is highly variable unless it is expressed in terms of fat-free tissue (lean body mass.)

[9] Gregersen, M. L., MacLeod's Physiology in Modern Medicine, 8th ed., 1938, p. 903.

[10] Moore, F. D., *Science*, 1946, **104**, 157.

16913

Assay of Aureomycin in Body Fluids: Observations on Individuals Receiving Aureomycin.*

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In order to place therapy with the promising new antibiotic, aureomycin, on a rational basis, the development of a satisfactory method of assay in body fluids is necessary. It is the purpose of the present communication to describe such a method of assay and to report some of the initial results from the application of this method in the study of the use of aureomycin.‡

At the time of the preparation of this report, only two groups of investigators have reported the results of attempts to assay the drug. The first group, using a *B. subtilis*-like organism, reported levels in humans following ingestion of various amounts of the drug.[1] The results were expressed as "units" which represented one-seventh of a microgram of the antibiotic. Paine, Collins, Finland, and Wells,[2-4] in studying aureomycin, reported on plasma levels of the drug as well as a quantitative method applicable to the urine. They stated that their method for determining the plasma levels was not satisfactory.[3,4] The urine levels were expressed as dilutions of urine which inhibited the test organism, streptococcus No. 98, rather than in terms of concentration of the drug.

Method. In this laboratory a modification of the Rammelkamp tube dilution technic[5]

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‡ The aureomycin used in this investigation was provided by Lederle Laboratories, Division of the American Cyanamid Company.

[1] Cox, H. R., personal communication.

[2] Paine, T. F., Collins, H. S., Finland, M., *J. Bact.*, 1948, **56**, 489.

[3] Collins, H. S., Wells, E. B., Paine, T. F., Finland, M., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 174.

[4] Finland, M., Collins, H. S., and Paine, T. F., *J.A.M.A.*, 1948, **138**, 946.

[5] Rammelkamp, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 95.

TABLE I.
Composition of Study Group Presented According to Route, Dose, and Period When Assay Was Done.

Route	Dose	Assays following initial dose		Assays following subsequent doses	
		No. persons	No. determinations	No. persons	No. determinations
Oral	0.16-0.6 g	1	1	5	10
	1.0 "	11	36	13	35
	1.5-2.5 "	2	8	2	7
I.V.	50 mg	9	47	2	4
	100 "	1	3	1	5
I.M.	50 "	5	12	—	—
	100 "	1	4	2	4
	200 "	1	1	—	—

which has been used for several years for the assay of penicillin and streptomycin in body fluids, has been applied to the assay of aureomycin. The method has proved practical and forms the basis for the present report.

Technic of the Test; Test Organism. The bacterium used in the test was a strain of beta hemolytic streptococcus (JB) which has been used in this laboratory for antibiotic assay for the past 5 years. This strain is sensitive to 0.0039 unit of penicillin per ml and to 0.015 μg of streptomycin per ml. A 24-hour culture of this organism in brain-heart infusion broth was centrifuged in a Hopkins tube[6] and then diluted with a buffer broth to be described below to make a final concentration of 4000 organisms per 0.5 ml of the broth.

Media. The broth used for the assay was a beef heart infusion (Difco) broth buffered with sodium phosphate to a pH of 7.6 and with 10% neopeptone. Ascitic fluid was added at the time of the test to a final concentration of 20%.

Serial Dilution of the Unknown Fluid. The serum or other body fluid was stored at about –40°C unless tested immediately. Fluids, such as urine, which were not sterile were filtered through Seitz pads. A series of 8 to 10 tubes were set up, each containing 0.2 ml of the buffer broth. To the first of these was added 0.2 ml of the material to be tested and serial 2-fold dilutions were made. The broth suspension of the test organism, in a volume of 0.5 ml, was then added to each tube, making a final volume of 0.7 ml. The tubes were well shaken and placed in an incubator at 37°C for 18 hours. The end-point of the test was taken as the highest dilution of the unknown material showing no growth visible to the unaided eye.

Control. With each assay, the test organism was set up with a known concentration of aureomycin which was prepared by diluting a standard solution of the drug. This standard solution contained a concentration of 10 μg by weight per ml and was stored between tests at –40°C. In the control set-up, 0.2 ml of this solution, containing 2 μg, was diluted in 2-fold steps with the buffer broth through 8 tubes. To each of these was added 0.5 ml of the test organism suspension. The final concentration of aureomycin in these tubes was therefore from 1.0 μg per ml down to 0.007 μg per ml. In this laboratory the standard organism used usually was inhibited in the tube containing 0.031 μg per ml and occasionally varied one tube in either direction, or from 0.062 μg per ml to 0.015 μg per ml.

As a further control over the technic, a known concentration of aureomycin, 5 μg per ml, was included in each run and was diluted as an unknown. In any run where this gravimetric value did not check with the bioassayed value, the results of the determinations on the unknown were considered inaccurate and the materials retested.

Example of Calculation. If, in determining the level of aureomycin in serum, the growth was inhibited up to a dilution of 1:8 and the control series showed the test organism to be inhibited at a concentration of 0.031

[6] Todd, J. C., Sanford, A. H., Clinical Diagnosis by Laboratory Methods, W. B. Saunders, 1935, p. 703.

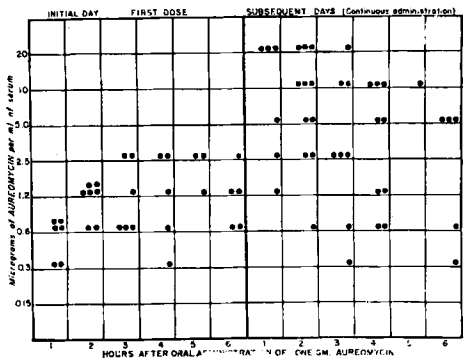

FIG. 1.

Graph illustrating blood aureomycin concentrations following an initial oral dose of one gram, as compared with blood levels following subsequent oral doses of one gram every 4 to 6 hours in patients receiving continuous therapy.

μg per ml, then the serum level was $8 \times 0.031 \times 5$ or 1.24 μg per ml of serum.

Study Group. Thirty individuals receiving aureomycin in a variety of doses and by several routes were studied in the present investigation, as presented in Table I. These individuals included patients under treatment for various infectious diseases and a group of normal individuals.

Results: Serum Levels Following Administration by Various Routes. Over the period of 6 months during which this method of assay has been used in this laboratory, certain general observations have been made. First, the test organism has not shown significant variation in its aureomycin sensitivity as determined by the control assay with known concentrations of the antibiotic. Retesting of serum specimens which had been stored over a period of 4 months at −40°C, showed no deterioration of the antibiotic content. No deterioration of the antibiotic in serum, plasma, or whole blood stored at 4°C for as long as 50 hours has been observed, although this possibility remains.[2] Therefore, all serum specimens were frozen at −40°C as soon as possible after being obtained.

Oral Route. Following the oral administration of an initial dose of 1.0 g of aureomycin in adults, significant concentrations of the drug were present in the blood within one hour, and the maximum concentrations were reached within 2 to 4 hours. The results of serial determinations on a group of 11 such individuals is presented in Fig. 1. The peak concentrations found in different individuals fell between 0.6 and 2.5 μg per ml of serum. Measurable amounts of the drug persisted in the serum for at least 6 hours, and in many individuals no decrease in concentration was observed at that time.

In most individuals to whom aureomycin was administered on a continuous schedule every 4 or 6 hours, the levels of the drug tended to increase gradually (Fig. 1). This was noted as early as after the second dose of the drug, but usually was more marked after several days. In some cases the later levels exceeded the earlier ones by many fold.

When the drug was given in doses of 0.5 or 1.5 g, the blood concentration did not appear to be affected to a degree proportional to the differences between these doses and the 1.0 g dose, although some difference was usually observed. Doses of aureomycin in children reduced roughly according to body weight appeared to produce concentrations comparable to those attained in adults following administration of 1.0 g.

Intravenous Route. Within 5 minutes following the intravenous administration of 50 mg of aureomycin dissolved in 5.0 ml of 0.784% Na_2CO_3 solution, drug concentrations were reached which were equal to or exceeded the maximum values obtained following oral administration of 1.0 g. Results of serial determinations of drug concentrations in 11 individuals following this dose are presented in Fig. 2. Serum levels rapidly declined dur-

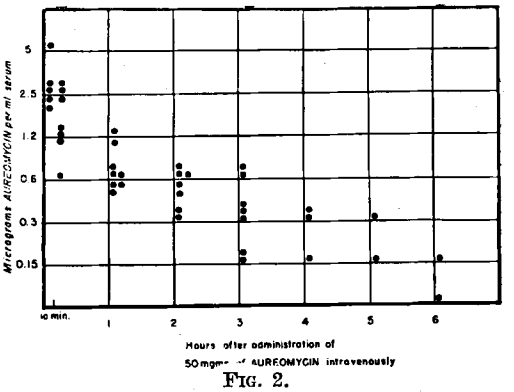

FIG. 2.

Graph illustrating blood aureomycin concentrations following the initial intravenous administration of 50 mg.

ing the first hour and decreased slowly thereafter. Measurable concentrations were present as long as 6 hours after administration.

Intramuscular Route. When the drug was administered by the intramuscular route in doses of 50 to 200 mg dissolved in 5.0 ml of a Sorensen phosphate buffer (pH 7.2) and mixed with an equal volume of 2% procaine, measurable levels were rarely noted during the following 4 hours. In only one of 21 determinations was a concentration greater than 0.15 μg per ml observed.

Urine Levels. Aureomycin concentrations were readily measured in the urine using this method of assay. Following doses of the order described above, concentrations of from 5 to 50 μg per ml were observed. Much smaller amounts were observed during the 4-hour period following the ingestion of 1.0 g than following the intravenous administration of 50 mg.

Cerebrospinal Fluid Levels. From 1 to 3 hours following the ingestion of 1 g, no measurable amount of aureomycin was observed in the cerebrospinal fluid in 3 assays on 2 individuals with meningeal irritation. In one normal child aureomycin was not demonstrated in the cerebrospinal fluid after the ingestion of 2 grams over a period of 24 hours.

Sensitivity of Organisms. The sensitivity of a variety of pathogens isolated in this laboratory varied from 0.017 μg per ml to 285 μg per ml. Most organisms tested were sensitive to less than 1.0 μg per ml.

Discussion. The tube dilution method of antibiotic assay in body fluids, although having certain disadvantages, represents a practical and convenient procedure. Variations in the end-point may occur with variation in the size of the inoculum of test organism.[2] These, however, are minimized by the use of a constant, small inoculum. Although the use of the inhibition of visible growth at 18 hours of incubation as the end-point may not coincide with complete inhibition as evidenced by subculture, neverthless, this discrepancy is relatively constant.

One of the problems inherent in any method of assay of an antibiotic is the rate of deterioration during handling and under conditions of incubation. This factor may be more important in the case of aureomycin than in the assay of penicillin and streptomycin.

Since high blood concentrations were often not reached for several days when the drug was given by mouth, a combination of the more efficient intravenous route and the more convenient oral route may be advisable in the treatment of many severe acute infections. The intramuscular route in our hands has been of very limited value because measurable serum levels were so rarely obtained. Frequent painful local reactions have occurred.

Summary. 1. A serial tube-dilution method of determinations of aureomycin concentrations in body fluids using an hemolytic streptococcus as the test organism is described.

2. Serum levels of aureomycin ranged from 0.3 μg to 2.5 μg during a 6-hour period following the initial oral dose of 1 g.

3. Serum levels or aureomycin following doses of 1 g on subsequent days in patients receiving continuous therapy ranged from 0.3 μg to 20 μg during a 6-hour period.

4. Serum concentrations of aureomycin following the intravenous administration of 50 mg ranged from 0.6 to 5 μg 5 minutes after injection and declined gradually over a 6-hour period.