tests, a separate serum pool being made from monkeys vaccinated with each strain. In the neutralization test, the final virus concentration was 100 $P.D._{50}$ per cc and serum dilution 1:2. This mixture was incubated for 4 hours in the refrigerator just prior to intracerebral inoculation of 5 monkeys with 1.0 ml each.

Results. Tables I and II summarize the results.

Conclusions. These results in conjunction with those of the previous study indicate that 3 groups of poliomyelitis virus have been demonstrated. I. One group encompasses at least 9 viruses compared in this study: Bk, McK, Ca., Fr, Mi, Gu, Ko, Cp. and Br. This group, which includes the greatest number tested to date has been designated tentatively as Group I. II. Another group so far comprises the La and MV viruses. III. A third virus, Le, differs sufficiently from the above two groups to represent a third group.

16912 P

Relationship of Body Specific Gravity to Body Fat and Water Content.*†

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It has been demonstrated by Behnke and his coworkers[1-3] that the specific gravity of the human body can be used as an index of the proportion of fat present. Rathbun and Pace[4,5] measured the specific gravity of eviscerated guinea pigs and also analyzed the bodies chemically for the fat content. They found that the body specific gravity of the animals decreased as the fat content increased, and that the range of specific gravity for the guinea pigs closely approximated that found by Behnke in his series of normal human males. The relationship between body specific gravity of the eviscerated guinea pig and the proportion of body fat was found to be a very exact one and agreed closely with the theoretical equation:

$$\% \text{ fat} = 100 \left(\frac{5.548}{\text{specific gravity}} - 5.044 \right)$$

The use of body specific gravity as an index of the amount of body fat in humans has been examined further in this laboratory. In an effort to describe more accurately the relationship which exists between metabolic rate and age, studies are being carried out to ascertain whether the "lean body mass," *i.e.*, body weight minus the fat content, may not be a better standard of reference for the metabolic functions of the body than surface area or weight of the body.

The difficulties encountered in weighing elderly people under water, a part of the procedure for measuring specific gravity of the body, were so great that it was decided to try to measure body water instead and calculate the lean body mass from this determination. A chemical method for the measurement of total body water, by the use of antipyrine (1-phenyl-2:3-dimethyl-5-pyrazolone) was then developed in this laboratory and the values obtained agreed closely with simultaneous measurements of body water by deuterium oxide.[6]

* This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

† Part of the expenses of this study were defrayed by the Josiah R. Macy, Jr. Foundation.

1 Behnke, A. R., *Harvey Lecture Series*, 1941-1942, **37**, 198.

2 Behnke, A. R., Feen, B. G., and Welham, W. C., *J.A.M.A.*, 1942, **118**, 495.

3 Welham, W. C., and Behnke, A. R., *J.A.M.A.*, 1942, **118**, 498.

4 Rathbun, E. N., and Pace, Nello, *J. Biol. Chem.*, 1945, **158**, 667.

5 Morales, M. F., Rathbun, E. N., Smith, R. E., and Pace, Nello, *J. Biol. Chem.*, 1945, **158**, 677.

TABLE I.
Relationship of Body Specific Gravity, Body Fat, and Body Water.

Subject	Specific gravity	% body fat		% body water		% water from lean body mass
		from spec. grav.	from antipyrine	from spec. grav.	from antipyrine	
1	1.021	39.0	41.5	44.4	43.4	71
2	1.032	33.2	29.0	49.3	51.8	77
3	1.032	27.5	30.4	53.0	50.7	70
4	1.044	27.0	25.2	53.4	53.2	73
5	1.045	26.5	31.0	54.0	50.0	68
6	1.057	20.5	17.2	58.2	60.5	76
7	1.061	18.5	16.3	58.6	58.8	72
8	1.061	18.5	20.4	58.6	58.0	72
9	1.064	17.0	19.9	59.6	58.5	72

Thus in the same individuals it became possible to measure body water directly by a chemical method and by specific gravity, using Pace and Rathbun's formula.[7] This circumstance permitted a verification of both methods in a number of individuals. Pace *et al.* have previously shown a good check in one individual between the calculation of body water made from specific gravity and direct measurement by tritium oxide.[8]

Methods. The specific gravity of nine normal individuals was determined by weighing them in air and under water according to Behnke's procedure. On the same day, total body water of the subjects was measured by the use of antipyrine. In one individual, total body water was simultaneously determined by the use of both antipyrine and deuterium oxide, with good agreement by the two methods. Body fat and body water were then calculated or measured by two independent procedures.

Body fat was calculated from specific gravity[4,5] and from the antipyrine figure for total body water

$$\% \text{ fat} = 100 \left(\text{body wt} - \frac{\text{wt body water}}{0.73} \right)$$

on the assumption that the defatted tissues of the body contain on the average, 73% water.

Body water values were ascertained by direct measurement with antipyrine and calculated from specific gravity

$$\% \text{ body water} = 100 \left(4.424 - \frac{4.061}{\text{sp.gr.}} \right)$$ [7]

Results. The figures for per cent body fat calculated from specific gravity and from total body water determined by antipyrine agree fairly well (Table I) for the 9 individuals studied.

Likewise, there is good agreement of the values for per cent body water calculated from specific gravity and estimated chemically by the use of antipyrine (Table I).

In these tables the subjects are listed in order of increasing specific gravity. The inverse relationship of body water to body fat is readily noted; as the body specific gravity increases, total body water increases, while body fat decreases. Furthermore, the water content of the lean body mass is fairly constant.

Discussion. The fact that body specific gravity can be used to predict fat and water content in normal humans has received scant notice in the recent literature. The concept presented by Behnke and his co-workers that fat tissue is the chief variable of the body and that the fat-free tissue of the body or "lean body mass" is relatively constant in composition, is an important one. Variability in total body water depends, from this point of view, largely on the amount of body fat. This relationship seems not to have been widely appreciated.

In the literature, figures for the proportion of body water have run from 59.3% reported

[6] Soberman, R., Brodie, B. B., Levy, B., Axelrod, J., Hollander, V., and Steele, J. M., *J. Biol. Chem.*, in press.

[7] Pace, Nello, and Rathbun, E. N., *J. Biol. Chem.*, 1945, **158**, 685.

[8] Pace, Nello, Kline, L., Schachman, H. D., and Harfenist, M., *J. Biol. Chem.*, 1947, **168**, 459.

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.

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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]

* Aided by grants from C. S. Howard Donation and the Fleischner Endowment Fund.

† This laboratory is supported by the Divisions of Medicine and Pediatrics of the University of California Medical School, and by the Department of Public Health of the City and County of San Francisco.

‡ 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.