7. Högman, C. F., Killander, J., Acta Pathol. Microbiol. Scand., 1963, v55, 357.

8. Daniel, T. M., Weyand, J. G. M., Stavitsky, A. B., J. Immunol., 1963, v90, 741.

9. Talmage, D. W., Dixon, F. J., Bukantz, S. C., Dammin, G. J., ibid., 1951, v67, 243.

10. Benedict, A. A., Brown, R. J., Ayengar, R., J. Exp. Med., 1962, v115, 195.

11. Greenbury, C. L., Moore, D. H., Nunn, L. A. C., Immunology, 1963, v6, 421.

12. Schultze, H. E., Clin. Chim. Acta, 1959, v4, 610.

13. Uhr, J. W., Finkelstein, M. S., Franklin, E. C., Proc. Soc. Exp. Biol. and Med., 1962, v111, 13.

14. Franklin, E. C., ibid., 1962, v109, 338.

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A Micromethod for Determination of Serum Triglycerides.* (29824)

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Any study of the lipid metabolism of individual infants or small animals necessitates the employment of suitable micromethods in order to keep to a minimum the volume of blood serum that is required. Micromethods for determination of cholesterol and phospholipids are available but the original triglyceride method of Van Handel and Zilversmit(1) called for the use of 1 ml of serum although a subsequent report by Van Handel (2) decreased the volume of serum to 0.5 ml. Brody and Carlson(3) independently devised a serum triglyceride method similar in principle to the Van Handel methods(1,2) but which employed 1 ml of serum. In the present study, the method as detailed by Van Handel(2) was scaled down without loss of accuracy so that duplicate determinations for measurement of serum triglyceride concentration can be performed on 0.1 ml serum.

The concentration of reagents and their preparation are essentially those described by Van Handel(2). A 20-80 mesh fraction of Zeolite† was prepared from the 20 mesh grade by partially pulverizing and sifting it. The material was activated by heating at 125°C for 4 hours and placed in a tightly stoppered bottle. It was found that the 20-80 mesh fraction was as reliable as the 80-100 mesh fraction for human serum analyses and easier to handle.

For each serum sample, 0.2 g portions of Zeolite are placed in a series of 15 ml glass-stoppered centrifuge tubes. 1.0 ml of chloroform is added to each tube and the contents agitated on a mechanical mixer.‡ 0.1 ml of serum is placed in each tube, mixed, and an additional 1.0 ml of chloroform is pipetted into each tube. The tubes are stoppered, mixed mechanically for 2 minutes and thereafter occasionally before being allowed to stand overnight. Phospholipids are quantitatively adsorbed on the Zeolite. A standard of purified corn oil(2) or glyceryl tripalmitin in chloroform is treated in a similar fashion.

After standing at least 4 hours, or overnight, the extracts are filtered through Whatman #540 filter paper into stoppered tubes. Duplicate 0.5 ml portions of chloroform extract are placed in 15 × 125 mm test tubes and evaporated to dryness in a water bath. A stream of air is introduced to accelerate the removal of the last portions of solvent.

The glycerides are saponified by heating in the test tubes with 0.5 ml of 0.4% KOH (w/v) in ethanol for 20 minutes at 60-65°. One extra tube with alcoholic KOH is added to the series to serve as a reagent blank. The tubes are stoppered with glass marbles to minimize evaporation and only the bottoms of the test tubes are immersed in the water bath in order to avoid loss of glycerol by volatilization.

After saponification, the tube contents are

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† W. A. Taylor Co., Baltimore, Md.

‡ Turbo-Mixer, TechniLab Instruments, Los Angeles, Calif. or Vortex Mixer, Scientific Industries, Springfield, Mass.

acidified by addition of 0.5 ml of 0.2 N H_2SO_4, and a carborundum chip to minimize bumping is added. The tubes are immersed to half their length in a boiling water bath for 10 to 12 minutes until the odor of ethanol has disappeared. The tubes are cooled and the glycerol is converted to formaldehyde by oxidation with 0.05 ml of 0.025 M $NaIO_4$ at room temperature for 10 minutes. Excess $NaIO_4$ is reduced by treatment with 0.05 ml of 0.5 M Na AsO_2 for 10 minutes.

Five ml of 0.2% chromotropic acid in sulfuric acid are added to each tube and stirred mechanically.‡ The tubes are placed in an aluminum block heater§ adjusted to 100-105°C and heated for 30 minutes. The tubes are cooled and the intensity of color is measured spectrophotometrically‖ at 570 mμ. The reading of the blank against water, using 19 mm cuvets, ordinarily does not exceed 0.060.

The precision of the micromethod for determination of serum triglycerides was compared with that of the macromethod by analyzing the same serum sample on 24 separate occasions in duplicate by each method. The results are shown in Table I. The mean value obtained by the macromethod was 121.5 mg per 100 ml, with a standard deviation of 3.5 mg/100 ml while corresponding values for the microprocedure were 122.4 ± 2.6 mg/100 ml. The coefficients of variation for the macro- and micromethods were 2.8 and 2.1 respectively. Thus, the microprocedure yields essentially the same values for serum triglyceride determination as does the macromethod and has a slightly lower standard deviation and coefficient of variation.

TABLE I. Summary of 24 Duplicate Determinations of Serum Triglycerides.

Method	Triglycerides Mean, mg/100 ml	S.D., mg/100 ml	C.V.
Macro	121.5	3.5	2.88
Micro	122.4	2.6	2.12

S.D. = Standard deviation.

C.V. = Coefficient of variation = $\frac{\text{S.D.}}{\text{mean}} \times 100$.

Summary. A micromethod for direct determination of serum triglyceride concentration is described by means of which duplicate measurements may be carried out on 0.1 ml of serum.

§ Research Specialties Co., Richmond, Calif.

‖ Coleman Jr. Model 6 Spectrophotometer, Coleman Instruments, Maywood, Ill.

1. Van Handel, E., Zilversmit, D. B., J. Lab. Clin. Med., 1957, v50, 152.

2. Van Handel, E., Clin. Chem., 1961, v7, 249.

3. Brody, S., Carlson, L. A., Clin. Chim. Acta, 1962, v7, 694.

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Potassium Loss from Human Erythrocytes Exposed to Amphotericin B. (29825)

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Amphotericin B, a heptaene antibiotic derived from *Streptomyces nodosus,* is effective in the treatment of a number of systemic fungal infections. Because of recent reports that the drug causes hemolysis of canine erythrocytes *in vivo*(1), and hemolysis of human erythrocytes *in vitro*(2,3), the effect of the drug on human erythrocytes was studied further using an *in vitro* system.

Materials and methods. Freshly drawn human erythrocytes were freed of plasma and buffy coat by centrifugation, washed 3 times in buffered saline solution (pH 7.4, containing 135 mM NaCl, 10 mM Na_2HPO_4, and 5 mM KCl) and then suspended in 1½ volumes of buffer. Amphotericin B (Fungizone for Infusion, Lot #3A80431, E. R. Squibb & Sons) was dissolved in distilled water and