given and at certain intervals after anesthesia was induced. Determinations of the total cholesterol were made on the serum according to the method of Bloor, Pelkan and Allen.[1] The dogs were in the fasting state in all instances except dog No. 304, which had received a high-cholesterol meal 2 hours before anesthesia was produced.

The results are shown in Table I.

Conclusions. Nembutal in amounts sufficient to produce deep narcosis lasting several hours in dogs is without significant effect upon the level of the total serum cholesterol.

8041 P

An Improved Method for Determination of the Gonadotropic Hormone.

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The following method for the extraction of gonadotropic hormone from human blood is a modification and improvement of the acid alcohol method previously reported from this laboratory.[1]

With the old method only a faint Reaction I was demonstrable in 40 cc. of blood taken on the 8th to the 12th day of the cycle. With the new method it has been possible to obtain both strong Reaction I and III with the same amount of blood at a comparable point in the cycle.

Method. (1) 40 cc. of vein blood is mixed with 30 gm. of anhydrous sodium sulphate, stirred to dryness and pulverized. (2) 200 cc. of 60% alcohol, acidified with 50% acetic acid to pH 5, is then added to the powder and shaken for one hour in a mechanical shaker. (3) The mixture is centrifuged and the supernatant alcohol decanted and kept. (4) A similar second extraction of the residual sludge is made. (5) The 2 alcohol extracts are each concentrated by evaporation under a fan at room temperature to a volume of 50 cc. and then centrifuged. (The concentration can be

1 Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, **52**, 191.

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1 Frank, R. T., Goldberger, M. A., and Spielman, F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 999.

hastened by vacuum distillation, temperature not to exceed 23°C.). (6) The precipitate and scum which separate during the concentration are removed and shaken for 10 minutes with 10-20 cc. of dilute NaOH, the final pH of the mixture not to exceed 8.5 (thymol blue) (A). (7) The two 50 cc. alcohol extracts are united and allowed to evaporate at room temperature (fan) almost to dryness (B). (8) The residue (B) is added to the aqueous alkaline extract of the first precipitate (A), then shaken and the pH of the whole adjusted to 8-8.5. The extract is then shaken vigorously for 5 minutes and allowed to stand for 10 minutes at room temperature (C). (9) The extract (C) now is centrifuged and the pH of the supernatant fluid is then adjusted with dilute acetic acid to 7-7.5 (using phenol red) (D). (10) The extract (D) (the total volume of which should not exceed 5 cc.) is then allowed to stand overnight in the refrigerator at a temperature of 35-40°C. Crystals of sodium sulphate separate out. The supernatant fluid is then poured off (E). (11) The final extract (E) is injected into an immature rat weighing 20 to 24 gm. in 5 divided doses, over 3 days. The animal is killed in 96-100 hours after the first injection. The intensity of the reaction is determined by examination of serial sections of the ovaries.†

8042 P

Gonadotropic Blood and Urine Cycles in Normal Menstruating Woman.

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A method for the determination of gonadotropic factor in the blood of menstruating, non-pregnant women was published from this laboratory.[1] This method consisted in the extraction by means of 60% acid alcohol of the residual sludge of 40 cc. of blood desic-

† The method of assay and the clinical application of the test are discussed in the succeeding report.

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1 Frank, R. T., Goldberger, M. A., and Spielman, F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 999.