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.

cated with anhydrous sodium sulphate. The estrogenic factor ma first be removed with ethyl ether.[2]

By modifying the method it has been possible (a) to obtain a stronger follicle stimulating effect (Reaction I) and (b) to demonstrate a luteinizing effect (Reaction III) at certain periods in the cycle of non-pregnant, normal, menstruating women.

The cycle of excretion in the urine has also been studied, employing the Katzman-Doisy benzoic acid method.[3]

By means of these methods a normal woman has been studied throughout 3 menstrual cycles, and 3 other less regularly menstruating females have each been carried through one cycle of both blood and urine.

The maximum concentration of gonadotropic hormone in the blood occurred on the 9th to the 12th day. In this case Reaction I was obtained on the 9th day and Reaction III on the 12th day with 40 cc. of blood, giving a concentration of 25 R.U. of gonadotropic hormone per liter of blood. At other periods in the cycle, negative results were obtained with equal quantities of blood.

In the urine negative results with quantities up to 500 cc. were obtained except on the 10th to the 14th day of the cycle. During this period both follicle stimulating and luteinizing reactions were found with amounts varying from 40 to 500 cc. The concentration of the hormone in the urine was between 2 and 25 R.U.L. The maximum excretion in one day was 44 R.U. The total excretion in the first cycle amounted to 80 R.U. and in the second cycle to 54 R.U.

The method of assaying consists in injecting the extract in 5 divided doses over a 3-day period, into 20-24 gm. white female rats, the animals being killed 96 to 100 hours after the first injection. The ovaries are studied in serial section.

Ovaries containing from 2 to 3 large cystic follicles were recorded as faintly positive.

Ovaries honeycombed with large cystic follicles were recorded as a strong Reaction I.

Ovaries containing corpora lutea were tabulated as Reaction III.

Summary. By an improved method increased concentration of the follicle stimulating factor and the presence of luteinizing factor can be demonstrated in the blood of cyclical, menstruating women during the 9th to 12th day of the cycle. 2. In the urine a similar accumulation of both factors was noted between the 10th to the

[2] Frank, R. T., and Goldberger, M. A., *J. A. M. A.*, 1928, **90**, 376.

[3] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **106**, 125.

14th day. 3. The presence of the luteinizing reaction has hitherto been associated with pregnancy and chorionepithelioma.

8043 P

Antibody Production by the Rabbit Against an Ectoparasite.

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The itch mite, *Psoroptes communis* var. *cuniculi,* is a common ectoparasite upon the rabbit, large numbers being found upon the skin surface deep within the fold of the outer ear. The skin where the mites occur becomes necrotic and is sloughed off. If a small piece of the slough be examined with a low-power microscope, many mites can be seen crawling over it.

It has occurred to the writer that repeated skin puncture by such numbers of ectoparasites, each injecting in the form of its saliva a small quantity of protein peculiar to the parasite, should lead to the production of specific antibody against the attacking species. We have endeavored to determine if this be true by testing for the presence of precipitin in 10 adult rabbits, 5 known to be infested with the parasites, these being observed in each case under the microscope, and 5 others apparently free of such infestation, neither the parasites nor their characteristic effects being detected upon careful search.

The antigen—an extract of the mite—was prepared in the following manner. Pieces of the sloughed skin were removed with forceps from the infested ears and placed in a Petri dish. As we observed them with a low-power dissecting binocular, the mites could be seen to leave the skin and crawl about over the glass plate. They were picked up, best in groups of several, on the point of a dissecting needle and transferred to a small mortar. After about 300 mites were thus transferred, they were mashed with a pestle and triturated for 10 minutes with 1½ cc. of Coca's solution. The mixture was then centrifugated at high speed and the *very slightly* opalescent supernatant fluid decanted. This fluid was used as the antigen.

In the precipitin tests, which were carried out in small-bore tubes, 0.1 cc. of the antigen was layered over an equal volume of the clear