20 rabbits (previously isolated for at least 4 weeks) were submitted to electrical stimulation. For this purpose an interrupted tetanic current was employed, using bipolar electrodes of fine Nichrome wire, insulated to the finely pointed tips and the latter spaced one millimeter apart. In most instances the strength of stimulus was adjusted to give a maximal constriction of the ear vessels. Variations in procedure are shown in Table I. In all instances exploratory operations were made 18 to 24 hours after stimulation. The animals, in addition, were re-examined 2 to 4 weeks later to determine the condition of the ovaries. One animal in each series was then sacrificed and the pituitaries were examined histologically. They did not differ in appearance from those of control animals.

In the first series 'Nembutal' (pentobarbital sodium) was used for anesthesia. Since some barbiturates apparently exercise an inhibiting effect both on the parasympathetic and the sympathetic systems,[4, 5] light ether anesthesia was used in subsequent series with, however, no difference in results.

While these experiments were in progress, Hinsey and Markee[6] reported that section of the sympathetic trunks or ablation of the superior cervical ganglia failed to prevent subsequent ovulation and pregnancy—an observation which the writer has had opportunity to confirm. Their results, as well as those reported here, seem definitely to show that the nervous pathway, if, indeed, there is a nervous pathway, is other than through the ganglia in question.

7460 C

Further Studies on Loss of Sensitivity to Anterior Pituitary-Like Hormone of Pregnancy Urine.

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It is well known that both the anterior pituitary sex hormone and the anterior pituitary-like hormone of pregnancy urine (A.P.L.) lose their ovary-stimulating effect after a certain time, if given daily over a long period. Hisaw[1] has recently been able to show that this

4 Stavisky, G. W., *J. Pharm. and Exp. Therap.*, 1931, **43**, 499.

5 Clark, G. A., *J. Physiol.*, 1931, **73**, 297.

6 Hinsey, J. C., and Markee, J. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 270.

loss of sensitivity may be obtained in rabbits even by the administration of subthreshold doses of anterior pituitary sex hormone, and we[2, 3] found that the loss of sensitivity is limited to the gonadotropic preparation with which the animals have been treated previously. Animals which became insensitive to A.P.L. remained responsive to pituitary implants and vice versa.

In an attempt to determine whether this loss of sensitivity is due to the formation of specific hormone inhibiting substances as postulated by Collip[4] or simply to an insensitivity of the ovarian tissue itself, we performed the following experiments.

Five female rats, 21 days of age at the beginning of the experiment, have been injected daily with 100 R.U. of A.P.L. subcutaneously for a period of 8 months. Then they and 7 untreated females of the same size received 100 R.U. of A.P.L. intravenously. All animals were killed 1 hour after the injection. The blood of each animal was collected separately and injected in 3 doses of approximately 1.5 cc. each on 3 consecutive days into an immature rat 21 days of age, and thus tested for its A.P.L. content. The blood of all 7 previously untreated rats led to corpus luteum formation in the immature females, while the blood of the A.P.L.-insensitive animals was ineffective with one exception. The odds against obtaining such a result by chance greatly exceed 100 to 1. From this experiment we conclude that A.P.L. disappears more rapidly from the blood of the A.P.L-insensitive rat than from the blood of the normal animal.

Since this experiment does not show conclusively that the inactivation of A.P.L. has taken place in the blood itself we performed a second experiment, in which 3 units of A.P.L. were injected once a day on 3 consecutive days into each of eight 21-day-old female rats. Five of these animals received daily injections of the blood of A.P.L.-insensitive rats 3 days before and during A.P.L. administration. While all the control animals showed oestrous vaginal smears and corpus luteum formation following the injections, all the rats treated with the blood of the insensitive animals remained anoestrous and only one of them showed corpus luteum formation.

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1 Hertz, R., and Hisaw, F. L., *Am. J. Physiol.*, 1934, **108**.

2 Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 487.

3 Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 566.

4 Collip, J. B., Mount Sinai Hospital Reports, 1934 (in press).

These experiments show that the blood of animals which have become insensitive to the anterior pituitary-like substance of pregnancy urine is able to inactivate this hormone.

7461 P

Prolonged Pregnancy in Albino Rat following Injection of Pregnancy Urine Extract.

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Previous observers have found marked alterations in the termination of pregnancy in the rat, following (1) injections of extracts of the corpus luteum (Nelson, *et al.*[1]), (2) injections of extracts of the anterior pituitary (Teel,[2] Hain[3]), and implants of the anterior pituitary (Hain[3]), (3) injections of the urine of pregnant women (Levin, *et al.*,[4] Hain[3]), and (4) hypophysectomy (Pencharz and Long[5]). The results of these investigations are difficult to evaluate in view of the differences in the substances administered, in their preparation and dosage, and in the parity and stage of pregnancy of the animals when treated.

In the present experiments the attempt was made to observe the duration of pregnancy in rats injected near term with a uniform dose of urine extract. The investigation was planned to take into account the factors which were found to be involved in prolongation of pregnancy in the rabbit (Snyder[6]). A satisfactory method for the prolongation of pregnancy was found to be the subcutaneous injection of 75 R.U. of pregnancy urine (Antuitrin-S of Parke, Davis Co.) 19 days after mating. The day of recovery of spermatozoa from the vagina was designated day 1. The appearance of the placental sign, usually on the 14th day, also aided in estimating the duration of pregnancy.

In a consecutive series of 19 animals, prolongation of pregnancy occurred in 16. Usually hysterotomy was performed a few days

[1] Nelson, W. O., Pfiffner, J. J., and Haterius, H. O., *Am. J. Physiol.*, 1930, **91**, 690.

[2] Teel, H. M., *Am. J. Physiol.*, 1926, **79**, 170.

[3] Hain, A. M., *Quart. J. Exp. Physiol.*, 1932, **22**, 249.

[4] Levin, L., Katzman, P. A., and Doisy, E. A., *Endocrinology*, 1931, **15**, 207.

[5] Pencharz, R. I., and Long, J. A., *Am. J. Anat.*, 1933, **53**, 117.

[6] Snyder, F. F., *Bull. Johns Hopkins Hospital*, 1934, **54**, 1.