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Preliminary Quantitative Studies on Action of Follicular Hormone in Spayed Monkeys.

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(Introduced by J. F. Anderson.)

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Since the various preparations of follicular hormone, which are commercially available for clinical use, are assayed only in terms of their estrus inducing effect in the white rat or mouse it seems highly desirable to obtain some information, if possible, regarding the quantitative effect of this hormone in spayed monkeys. The studies of Corner, 1923, and Allen, 1927, on the sex cycle of the common *macacus rhesus* monkey paved the way. Allen showed that in some monkeys, in some cases, menstruation could be produced by the injection of the follicular hormone. An attempt is under way at the present time to make a quantitative study of the action of the follicular hormone in monkeys. This is a preliminary report of the work.

Technique: Completely ovariectomized, sexually mature, healthy monkeys of the *macacus rhesus* species were used. The injections were made subcutaneously, using aseptic technique. Sterile, aqueous solutions of the hormone that had been previously standardized by the Allen and Doisy rat test were used. Equal injections were made at 8:00 a. m. and 4:00 p. m. daily for a period of 6 days. Vaginal smears were taken daily and the appearance of red blood cells in the smear taken as the criteria of menstruation.

Every one of our colony of 20 monkeys has menstruated at least once under the influence of injections of follicular hormone, leaving little doubt that if sufficient hormone be injected into a spayed monkey, menstruation will invariably result. Our present data are insufficient to definitely establish the relationship of weight and of the length of the interval since the last menses to dosage, but the indications are that weight is not an important factor. The interval since the last menses may, however, influence the reactivity of the animal.

As may be seen from the table there is apparently no relation between dosage and the amount of epithelial growth, the length of the menses, and the date of the beginning of menses with reference to the date of injection. The color and swelling of the sexual skin was found to be roughly proportional to the dosage for the higher dosages, however.

TABLE I.

Total No. of monkeys injected	Ave. No. of days of epithelial growth	No. of monkeys menstruating	Ave. No. of days of menses	No. of monkeys failing to menstruate	Ave. No. of days after last injection that menses started	Total units injected in each monkey
6	7	3	3 2/3	3	11	30
9	7 8/9	6	5 1/3	3	10 1/6	48
12	8 1/2	10	4 1/2	12	12 2/5	60
7	9 6/7	6	4 1/3	1	10 1/6	72
6	8 2/3	5	5 1/5	1	9 3/5	84
12	6 1/2	12	5 1/2	0	10 1/2	90
3	7 2/3	3	5 1/3	0	9 1/3	96
12	7 1/2	12	4	0	10 1/2	120
12	7	12	5 1/2	0	9 1/2	240

Thus far it is impossible to say just what the minimum effective subcutaneous dose is for a monkey. Failures are reported up to 84 units, but the largest number of failures occurred at 30 units and 48 units. At present we can only say that at 30 unit dosage about 50% of the monkeys responded and no failures occurred where over 84 units were injected.

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The Male Hormone: Its Standardization.

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Method of Extraction: The hog testes used was first carefully freed of all extraneous tissue, and then put through a sausage mill. The substance was then thoroughly washed with weakly acid ice-cold water, and filtered, to remove the spermatic element. Half the volume of ether was added, and the whole agitated in a pressure drum for 12 hours or more, and then filtered. Thus the lipoidal substance desired was obtained in the filtrate. The filtrate was then placed in a separating funnel and allowed to separate into layers for several hours. The upper 2 yellow layers of liquid retained. On experimentation it was found that the lower 2 layers contained nothing of value. The retained liquid was then evaporated to vaseline consistency and extracted with acetone, and again filtered. The filtrate was evaporated to dryness and then extracted with distilled