

tion were removed for titration with 2,6-dichloro-indophenol. The results obtained were compared with that of a control solution containing no tissue but similarly treated. It was found that the scorbutic tissues were able to take up more ascorbic acid from Ringer solution than the normal tissues. Among the tissues examined, adrenal and muscle took up most, next the skin, then the intestine and kidney. It is interesting to note that the adrenal took up to its full capacity during the first hour of incubation since nothing more was taken up during the next 4 hours of incubation. On the other hand, the intestine continued to take up more during the following four hours than during the first hour. The other tissues took up comparatively little during the second period.

8813 C

Effect of Small Doses of Oestrin upon Ovarian and Vaginal Cycles of Mature Rats.

LUCIUS L. BUTTON AND CHARLES I. MILLER. (Introduced by G. W. Corner.)

From the Department of Anatomy, The University of Rochester, School of Medicine and Dentistry.

Much work has been done to demonstrate the effect of large doses of oestrin upon the ovaries, but scarcely any to show the effects of threshold or subthreshold doses. Bialek-Laprida,¹ injecting rats with 2-25 r.u. (Coward-Burn units) daily for 30 days, showed that the oestrous cycle was interrupted and also that the ovaries contained many atretic follicles at the end of this period. Thirty days after the injections were stopped the ovaries were slightly degenerate, there were few corpora lutea, many atretic follicles and the average weight of the ovaries was decreased. However there were also a few mature follicles present. Sixty days after the injections had ceased the ovaries were normal.

Selye, Collip and Thomson² treated 6 adult rats with 20 gamma of oestrin (200 int.u.) daily and obtained almost continuous vaginal oestrus. At the end of 20 days, however, the ovaries were noticeably atrophic, the average weight being 25 mg. In only one case did they contain recent corpora lutea.

¹ Bialek-Laprida, Z., *Compt. Rend. Soc. Biol.*, 1934, **117**, 456.

² Selye, H., Collip, J. B., and Thomson, D. L., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1377.

De Castillo and Calatroni³ injected mature female rats with 0.1 to 0.25 Coward-Burn rat units and found that after a latent period the oestrous cycles gradually became prolonged up to 40 to 80 days and eventually returned to normal length. At autopsy the ovaries contained no mature follicles and no recent corpora lutea.

Wade and Doisy⁴ injected .65 to 13 gamma of oestrin (6.5-130 int.u.) daily for 60 days. The vaginal smears during this period showed prolonged periods of oestrus or dioestrus varying from 5 to 25 days interspaced with regular cycles.

In the present experiment young mature female rats were observed by the method of vaginal smears through a control period of 3 oestrous cycles, and were then treated with oestrin in the form of Progynon-B, kindly donated by the Schering Corporation. The hormone as diluted with sesame oil for use in these experiments was retested on castrated rats to insure correct potency. As controls, 10 rats were injected daily for 8 weeks with amounts of sesame oil equal to those used in the experimental injections. The ovaries of these rats showed no significant changes in appearance or weight and the vaginal cycles remained normal.

Groups of rats were injected subcutaneously with daily doses of .25, .50, 1.0, and 2.0 r.u. (approximately 1.25, 2.5, 5.0, and 10 int.u.) respectively for periods from 3 to 13 weeks. Another group of rats were used as untreated controls. Vaginal smears were made daily. At the end of the injection period the animals were killed, and the ovaries were dissected out, weighed, and examined under the binocular microscope. Serial sections of each ovary were made and the corpora lutea counted. The effects of the injections upon ovulation were determined by a comparison of the number of corpora lutea in the normal and experimental rats.

Effect on the cycle: Of the group of 5 rats receiving .25 r.u. per day, 3 exhibited no significant disturbance of cycles, and underwent a practically constant succession of oestrous cycles during the experimental period of $\frac{1}{2}$ to 3 months. The remaining 2 exhibited some disturbance. In No. 26, for example, there were 4 regular cycles, followed successively by anoestrus for 10 days, 5 approximately normal cycles, another 10-day period of anoestrus and again 5 normal cycles. In No. 29 the cycles occurred with practically normal rhythm for $2\frac{1}{2}$ months, after which there were 20 days of anoestrus (about 4 cycles omitted) followed by 2 oestrous smears 2

³ De Castillo, E.-B., and Calatroni, C.-J., *Compt. Rend. Soc. Biol.*, 1930, **104**, 1024.

⁴ Wade, N. J., and Doisy, E. A., *Endocrinol.*, 1935, **19**, 77.

days apart, after which the animal was killed. At autopsy an ovarian tumor was found, which may possibly have been concerned with the slight irregularity of the cycle.

Another group of 7 rats received 0.5 r.u. daily for 3 months except that one rat (No. 13) was killed after 6 weeks. The effect on the vaginal cycle was variable. One rat (No. 13) exhibited normal cycles throughout the period of injection. In general, the others of the group had their cycles interrupted and periods of anoestrus varying in length from a few days (one cycle omitted or delayed) to as much as a month. These anoestrous periods were in several cases followed by periods of normal rhythm. Finally, one animal of this group (No. 3) after 5 approximately normal cycles had 4 oestrous periods at intervals of about 2 days, and then remained in continuous oestrus for 10 days, until autopsy.

A 3rd group of 3 rats received 1 r.u. per day for 2 to 7 weeks. The cycles were interrupted almost immediately. No. 19 went into anoestrus after one cycle and remained in the anoestrous state for 15 days, until killed. No. 2 after one cycle went into anoestrus for 2 weeks, then had 4 cycles and became anoestrous for 15 days until autopsy. Rat No. 10 went into continuous oestrus after 2 cycles. The fourth and last group received 2 r.u. daily. All these went at once, or after one cycle, into continuous anoestrus which lasted from 10 to 22 days. One was killed in this state; the other 2 went into continuous oestrus, in one case for 9 days, and in the other for 2 periods of 7 days each, interrupted by one day of dioestrus. After this phase of continuous oestrus both the animals resumed cycles of rather rapid rhythm (intervals of about 2 days).

In summary, the effect of daily treatment with oestrin in the form of Progynon-B in doses of 0.5 to 2.0 r.u. was to produce irregularities of the vaginal cycles, chiefly characterized by cessation of the rhythm in the anoestrous phase, and sometimes by continuous oestrus. The time of onset of the anoestrous state varied roughly with the doses; the larger dosages (1 r.u. and 2 r.u. daily) produced anoestrus at once or after one cycle, while the 0.5 r.u. dosage permitted several cycles before the supervening of the anoestrous state. With the dose of 0.25 r.u. daily 3 of 5 rats showed no disturbance of the vaginal cycle.

Effect on the ovaries: In the group of rats receiving 0.25 r.u. daily the ovaries were found to weigh less than those of the normal controls and also in every case the number of corpora lutea was found to be reduced. Whereas the ovaries of the normal rats weighed 40 to 45 mg., those of the experimental animals weighed

only from 13 to 32 mg. Similarly, the control ovaries contained 75 corpora lutea while those of the experimental animals contained only $\frac{1}{4}$ to $\frac{1}{2}$ this number. For example, the ovaries of rat No. 28 contained 47 corpora lutea, which is a definite reduction, and the ovaries of rats 6 and 26 contained only 19 and 16 corpora lutea respectively, which is a rather striking reduction.

In the group receiving 0.5 r.u. daily there was a considerable reduction in the corpora lutea in all cases except 2 in which there was still, however, a slight reduction. These 2 (Nos. 13 and 32) contained 57 and 62 corpora lutea, as compared to the others in this group which contained 14 to 37 corpora lutea, as shown by rats Nos. 24 and 30. The ovaries of rat No. 3 contained only 1 corpus luteum.

Approximately the same effects were obtained in the 2 groups of 3 rats each receiving 1 and 2 r.u. daily. There was a large reduction of the corpora lutea in all cases, with the numbers of corpora lutea present varying from 3 to 24. The weight of the ovaries was also reduced, varying from 9 to 30 mg., and seemed to vary directly with the number of corpora lutea.

In some of the animals which were autopsied at the end of long periods of induced anoestrous smears, large recent-looking corpora lutea were found. Whether this may be taken to indicate ovulation without corresponding vaginal changes, or rather indicates the persistence of corpora lutea under the influence of the oestrin treatment, cannot definitely be answered. We think it probable, however, that the latter alternative is correct, and that the effect of the treatment is actually suppression of ovulation with persistence of old corpora lutea, because (1) in some of the rats the number of corpora became very small, and only old retrogressing corpora remained; (2) Corner⁵ found that proportionately comparable doses of oestrin caused suppression of ovulation in rhesus monkeys; (3) in a forthcoming note from this laboratory by Allen and Heckel, it will be shown that in rabbits the life of corpora lutea is prolonged by small doses of oestrin.

There is even evidence from the state of the ovaries that in some of our animals exhibiting regular vaginal cycles up to the time of autopsy ovulation was nearly or quite in abeyance.

These effects of the ovarian hormone upon the ovary itself are probably explainable as due to depression of the gonad-stimulating power of the hypophysis.

⁵ Corner, G. W., *Am. J. Physiol.*, 1935, **113**, 238.

It is evident from these results that the reproductive cycle cannot be determined accurately by means of the vaginal smear during periods of hormone treatment if small amounts of oestrin are present in the extracts administered. This must be taken into account, for example, in evaluating the results of those who have at various times attempted to inhibit the vaginal cycle with corpus luteum extracts, which when not fully purified contain small amounts of oestrin.

Conclusions: (1) Daily treatment with oestrin (Progynon-B) in very small doses ($\frac{1}{4}$ to 2 r.u.) disturbs the vaginal cycle by inducing periods of continued anoestrous smears, or sometimes continued oestrous smears. (2) The ovarian cycle is also disturbed, the incidence of ovulation being reduced; and the weight of the ovaries is reduced.

We wish to express our appreciation to Dr. George W. Corner for his many helpful suggestions and criticisms.

8814 P

Migration of Nuclei in Uterine Epithelium of Monkey Following Prolonged Estrin Injections.*

MILTON D. OVERHOLSER AND WARREN O. NELSON.

From the Department of Anatomy, University of Missouri.

Eight *Macacus rhesus* monkeys received subcutaneous injections of estrin in oil solution (Amniotin† or Theelin‡) over time periods ranging from 6 to 24½ months. The total estrin dosage ranged from 36,400 to 73,400 rat units. Two of the animals were ovariectomized, 5 were normal, and 1 was depancreatized. Two ovariectomized animals were used as uninjected controls.

The migration of nuclei in the cells of the uterine epithelium (Fig. 1) occurred in 6 of the 8 injected animals. Of these, 3 were normal, 2 were ovariectomized, and 1 was depancreatized. The 2 negative cases (animals 14 and 59) were animals receiving the lowest dosages (36,400 and 44,150 r.u.). The ovariectomized controls were also negative. The nuclear migration is brought

* Aided by a grant from the Committee on Scientific Research of the American Medical Association to Dr. M. D. Overholser.

† Supplied by Dr. J. A. Morrell of E. R. Squibb and Sons.

‡ Supplied by Dr. Oliver Kamm of Parke, Davis and Co.