TABLE II. Weight Responses of the Ovary and Uterus to 20 i.u. of PMS at Each Stage of the Estrous Cycle.

| | | | | | | Vaginal smear | | |
|---|---|---|---|---|---|---|---|---|
| | O | $M_1$ | $M_2$ | D | P | Epithelial cells only | Epithelial cells and leucocytes | All stages |
| No. of mice | 9 | 14 | 2 | 15 | 3 | 26 | 17 | 43 |
| Avg wt of right ovary (mg) | 3.6 | 3.8 | 3.2 | 3.6 | 3.5 | 3.7 | 3.5 | 3.6 |
| " " " left " " | 6.2 | 6.6 | 4.8 | 6.4 | 7.4 | 6.7 | 6.2 | 6.4 |
| Increase of ovarian wt (%) | 73 | 75 | 50 | 79 | 108 | 78 | 76 | 77 |
| Avg wt of right uterine horn (mg) | 46.0 | 44.9 | 36.7 | 31.0 | 26.3 | 43.1 | 35.9 | 38.9 |
| " " " left " " " | 43.1 | 51.1 | 36.5 | 43.5 | 42.0 | 47.3 | 42.6 | 45.5 |

trophin is not influenced by the stage of the cycle and hence that ovarian responsiveness is not a factor involved in the cyclic phenomena. The similarity of uterine weights after stimulation at different stages of the cycle suggests that ovarian hormone secretion capacity is not influenced by the stage of the cycle either.

These data indicate that adult animals of the same age can be used to bioassay gonadotrophin by ovarian weight increase if great accuracy is not necessary. Furthermore, the stage of the cycle can be disregarded as a factor that influences the outcome of the assay.

*Summary.* 1) The average weight of the right ovary of C57BL/6JAX mice 90 to 120 days old was about the same in mice killed in estrus, early metestrus, and diestrus. The average uterine weight varied according to the stage of the cycle. 2) The average ovarian weight increase after 20 i.u. of equine serum gonadotrophin was the same when the gonadotrophin was administered in estrus, early metestrus, and diestrus. Also the average uterine weight after ovarian stimulation was about the same, regardless of the stage of the cycle in which the stimulus was begun.

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## Radioactivity in the Uteri of Rats Following Injection of Estrone-16-C$^{14}$* (23269)

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The distribution and localization of gonadal steroids appear to be more closely related to their metabolism and excretion than to organs having sites of physiological action (1,2,3,4). In this report an attempt has been made to define the nature of the radioactivity in the uteri of rats injected with estrone-16-C$^{14}$.‡

*Materials and methods.* Adult ovariectomized rats (7 days) were injected subcutaneously with 5 μg of estrone-16-C$^{14}$ (12,000 c.p.m.) daily for 3 days. Uterine luminal fluid was withdrawn from the distended uteri

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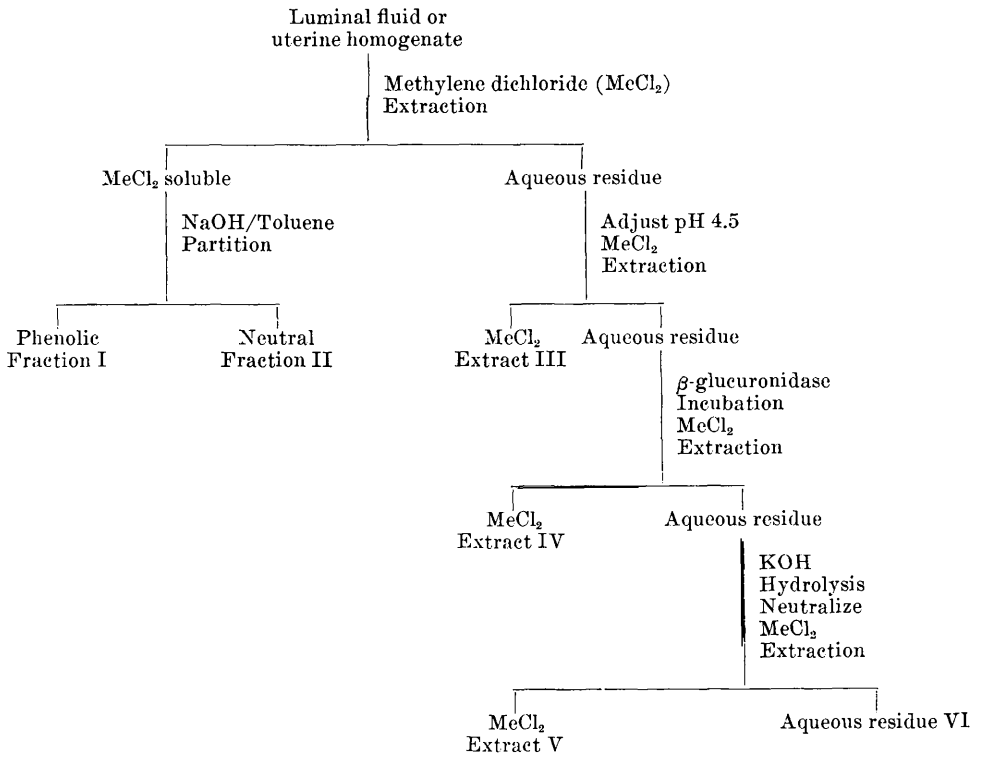


FIG. 1. Flow sheet for separation of radioactivity.

by syringe to avert contamination with blood. The uteri were then stripped of mesentery, washed in distilled water and immediately frozen on dry ice. The pooled samples of luminal fluid and uteri from 410 animals were 169 ml and 77.3 g, respectively. The uteri, in 500 ml distilled water, were homogenized in a Waring blendor for 5 minutes. The extraction procedure for uterine fluid and the uterine homogenate is outlined in Fig. 1. Both uterine luminal fluid and uteri were extracted with dichloromethane ($MeCl_2$) and the extract partitioned between 1N NaOH and toluene. The aqueous residue was adjusted to pH 4.5 with glacial acetic acid and extracted with $MeCl_2$. β-glucuronidase (200,000 units) was added to the acid residue and the mixture was incubated for 48 hours at 37°C. Following extraction with $MeCl_2$, the aqueous residue was adjusted to 4N with KOH, hydrolyzed for 2 hours at 90°C, neutralized and extracted with $MeCl_2$. All samples were counted in a windowless gas-flow counter for a period to insure ±5% accuracy, and corrected for background radiation.

*Results.* A tabulation of radioactivity in rat uterine luminal fluid and uteri is summarized in Table I. The recovered radioactivity in the phenolic fractions from both luminal fluid and uteri (79 c.p.m.) is approximately .005% of the total injected radioactivity. Bioassay of the phenolic fraction by the technic of Astwood(5) proved negative. No steroidal radioactivity was present as either a glucuronide-conjugate or bound to protein. The only appreciable radioactivity was present in the aqueous residue. A similar

TABLE I. Distribution of Radioactivity in Uterine Luminal Fluid and Uteri.

| Fraction | Radioactivity (c.p.m.) Uterine luminal fluid | Uteri |
|---|---|---|
| I | 19 | 60 |
| II | 0 | 0 |
| III | 0 | 0 |
| IV | 0 | 0 |
| V | 0 | 0 |
| VI | 210 | 36,000 |

radioactive component(s) in the aqueous phase has been noted in plasma and liver from estrone-16-$C^{14}$ treated rats. These results indicate that the rat is capable of degrading the steroid nucleus at ring D. Heard, *et al.*(6) has reported the presence of radioactive $CO_2$ after administration of isotopic estrone. however, other workers have been unable to detect any respiratory $C^{14}O_2$(2,7). A more definite identification of the radioactive compound(s) in the aqueous residue is now in progress.

The lack of any appreciable steroidal radioactivity or biological activity in either luminal fluid or uteri seems to indicate that once the steroid has produced its physiological effect in the uterus, it either leaves the uterus to enter the general circulation or is degraded.

*Summary.* The partitioning of radioactivity in uterine luminal fluid and uteri from rats injected with estrone-16-$C^{14}$ is described. Though a minute amount of radioactivity is present in the phenolic fraction, the major portion is present as a water soluble component. No steroidal radioactivity was present as either a conjugated or protein-bound form.

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## Adaptation of Equine Abortion Virus to HeLa Cells.* (23270)

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Goodpasture(1) has presented histological evidence that equine abortion virus (EAV) is infectious for the human amnion. The agent has been propagated in tissues obtained from the natural host(2). This reference contains a suitable review of the literature. A search for readily susceptible cells to investigate further the nature of chemical changes induced in hamster liver cells infected with EAV(3) led to the use of HeLa cultures. In the present communication the propagation of the virus is reported.

*Materials and methods.* The tissue culture cells used have been adapted to horse serum in this laboratory for over 3 years. Stock cultures were maintained in a mixture of 40% horse serum, 2% chick embryo extract, and 58% Earle's balanced salt solution (BSS), and were subcultured approximately every 10 days. The virus strain used in this study deserves some comment. Preliminary experiments indicated that "native" virus from infected horse tissue could not be propagated in HeLa cells. Therefore, it seemed advisable to employ a strain modified to another host. The virus (strain F)(4) used to initiate this study had been adapted to hamster liver through 86 passages. Subsequently, the agent had been subjected to 2 alternate passages in young hamsters and HeLa cells maintained in ascitic fluid, followed by 12 successive passages in HeLa cells. Material from the last trial was reinoculated into hamsters. The infected liver used as inoculum was frozen and ground, diluted 1:10 in physiological saline and centrifuged for 5 minutes at 5,000 rpm.

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