substance may interfere with activation of an enzyme system. Although EACA is an inhibitor of plasminogen activation(5,6,7), it may have other actions as well(8,9). Edema and hemorrhage are not produced in isolated perfused canine lung by administration of fibrinolysin which suggests that some mechanism other than activation of fibrinolysin is involved in venom action(10). This and other experiments to clarify the mechanism of venom action are in progress. As expected, EACA has no demonstrable effect on the intravascular coagulation produced by rattlesnake venom.

Summary. 1. The pulmonary vascular effects of edema and hemorrhage produced by *C. adamanteus* and *N. hannah* venoms are mediated through a heat labile non-cellular component of blood. 2. Venom of *C. adamanteus* also produces intravascular thrombosis not seen with venom of *N. hannah*. 3. The vascular effects of both but not the coagulating property of *C. adamanteus* are completely prevented by Epsilon Amino Caproic Acid. 4. The best explanation is that this compound prevents activation of an enzyme system which is responsible for the vascular changes.

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Endocrine Stimulating Effects of Yohimbine-HCl. (28508)

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Many conflicting reports are found in literature concerning the effects of yohimbine on the reproductive cycle of rodents. Rumry(1) reported that 1 mg of yohimbine per animal per day for 8 months was without effect on the number of litters produced by white mice. Ludwig and Ries(2) showed 0.05 to 0.10 mg of yohimbine produced typical conditions of estrus in 6-8 g immature mice. D'Amour(3) demonstrated hyperemia of the genital organs of 21-day-old rats by daily injections of 0.4 mg of yohimbine per animal for 10 days, but was unable to produce vaginal opening. Four milligrams of yohimbine for 7 days did not produce cornified vaginal smears in ovariectomized rats. Fugo and Gross(4) reported that injections of 1 to 2 mg of yohimbine-HCl per animal per day cause the estrous cycles of the adult female rat to be delayed. Three milligrams per day produced a pseudopregnant condition and male-like behavior. Spayed or hypophysectomized female rats did not exhibit estrus after yohimbine treatment. Corpora lutea formed in ovaries transplanted to the eyes of male rats treated with yohimbine.

Sulman and Black(5) found daily injections of 1-4 mg of yohimbine-HCl for 5 weeks to be without effect on estrous cycles, ovarian weight, or body weight of 60- to 100-day-old female rats. They reported no effect with 0.3 to 3.0 mg of yohimbine in the Allen-Doisy test on spayed mice or infantile rats, no gonadotropic effects of 0.5 to 5.0 mg of yohimbine in infantile rats, and no difference in time of first estrus or in the periodicity of vaginal estrus with 0.1 to 3.0 mg of the drug.

The present study was undertaken to ascertain the effect of yohimbine-HCl on mammary glands and ovaries of mature rats. Preliminary work indicated that daily injections of 10, 20, and 40 mg of yohimbine per kg of body weight stimulated both these organs.

Materials and methods. In the first experiment, 28 Holtzman rats (average body weight —122 g) were divided into 4 groups of 7 rats each. Groups I and II received daily subcutaneous injections of 20 mg of yohimbine-HCl (Mallinckrodt Chemical Works, control RBY) in distilled water per kg body weight. Groups III and IV were control groups and received daily injections of distilled water. Animals of Group III were caged with vasectomized males and served as pseudopregnant controls. Group IV remained as normal cycling controls. Daily vaginal smears were followed in all animals and weights were obtained at weekly intervals. Yohimbine induced pseudopregnancy in all animals which were receiving the drug. Four days following termination of the first pseudopregnancy, each rat in Group II had 3 loops of surgical cotton placed in each uterine horn to determine if the corpora lutea of this yohimbine-induced pseudopregnancy were capable of supporting decidual reactions. The surgery was performed through a midventral incision under ether anesthesia. The rats were sacrificed 4 days following the operation. The animals of Group I were used to determine the effect of yohimbine on the weight and histology of various endocrine organs. This group of animals was killed the second day following the estrus ending the second pseudopregnancy. Animals of Group III (normal pseudopregnancy) were also sacrificed 2 days following pseudopregnancy. The cycling control rats (Group IV) were sacrificed 2 days after a normal estrus. Length of treatment with yohimbine was 26 to 33 days, depending on the length of the 2 pseudopregnancies.

Immediately following sacrifice, the rats were carefully skinned. The pelts were pinned out on cardboard and fixed with Bouin's fluid for 20 minutes. Excess Bouin's fluid was then rinsed off in running water and the subcutaneous fascia containing the mammary glands was removed by blunt dissection. Following 24 hours additional fixation, the tissue was stored in 70% ethanol. Portions of the mammary glands were prepared as whole mounts using Harris alum haematoxylin as the stain. Ovaries, adrenals, uteri and pituitaries were weighed, fixed in Bouin's fluid, and stored in 70% ethanol. Paraffin sections of these organs were cut at 10 μ and stained with haematoxylin and eosin.

In a second experiment, the effects of yohimbine were studied in a group of 15 male rats. Eight animals were started on daily subcutaneous injections of 7.5 mg of yohimbine-HCl per kg of body weight at 23 days of age. The quantity of drug was gradually increased with gain in body weight to maintain a constant dose per kg of body weight. The dosage was decreased in this experiment to reduce any toxicity which might have obscured any growth stimulation in the first experiment. The remaining 7 rats acted as controls. Weights of all animals were determined daily during the 40 days of treatment. The animals were all sacrificed the day following the last injection. Mammary glands, thyroids, testes, adrenals, pituitaries, ventral prostates, and seminal vesicles were weighed, fixed and prepared for histological study. Statistical analysis by the t-test was made of differences in organ weights of these two groups. The same analysis was applied between the normal pseudopregnant and treated group of the previous experiment.

In a third group of 14 sexually mature female rats, the effect of a single injection of yohimbine on the estrous cycle was studied. A single injection of 20 mg of yohimbine-HCl per kg of body weight was given subcutaneously on the afternoon of either the day of proestrus, estrus, 1 day postestrus or 2 days postestrus as determined by daily vaginal smears. Proestrus was taken as the day on which vaginal smears contained only epithelial cells, while estrus was the day of completely cornified vaginal smears. One or more normal estrous cycles were observed following the treatment cycle before again injecting the animals.

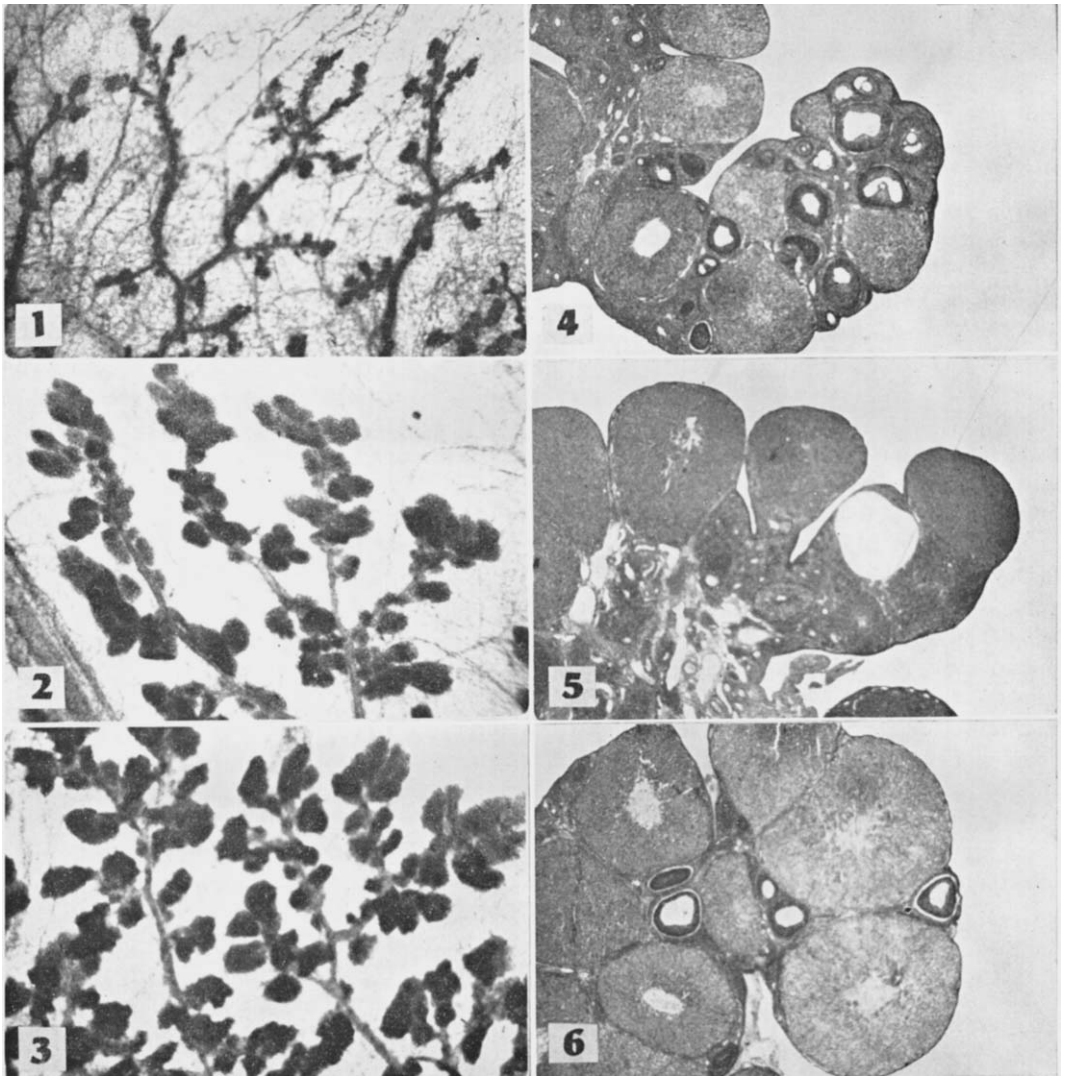

FIG. 1. Mammary gland of cycling control rat. (×18
FIG. 2. Mammary gland of pseudopregnant control rat. (×18)
FIG. 3. Mammary gland of yohimbine-treated rat. (×18)
FIG. 4. Ovary of cycling control rat. (×18)
FIG. 5. Ovary of pseudopregnant control rat. (×18)
FIG. 6. Ovary of yohimbine-treated rat. (×18)

Results. Indications from a preliminary experiment indicated that yohimbine-HCl caused development of the mammary glands and an increase in weight of the ovaries of female rats. This mammary stimulation has been confirmed by the present experiments. Mammary glands obtained on the 2nd day postestrus from cycling rats had only a duct system with some end bud formation, while pseudopregnant control rats had mammary glands with lobuloalveolar development (Fig. 1 & 2). Female rats treated with yohimbine-HCl had a more extensively developed lobulo-alveolar system than did those glands from pseudopregnant rats not receiving yohimbine (Fig. 3). Sections of mammary glands cut at 10 μ also show more complete development with the alveoli having larger lumina. Some alveoli from both treated and normal pseudopregnant rats contained secretions.

Yohimbine-HCl treatment of male rats had no apparent effect on mammary development.

All female rats in the groups treated with yohimbine were in estrus on the day treatment was started or within the following 5 days. Pseudopregnancy occurred in all these rats following the first estrus after initiation of treatment. This episode of pseudopregnancy was followed by a second pseudopregnancy without intervention of normal estrous cycles. The 21 pseudopregnancies in Groups I and II (yohimbine treated) varied from 11 to 15 days in length (average 13.1 days) as compared to 12 to 17 days (average 14.7 days) for pseudopregnancy following mating with vasectomized males. All estrous cycles in the cycling controls (Group IV) were 4 or 5 days in length (average 4.4 days for 41 cycles).

The ovaries of the yohimbine-HCl-treated group were heavier than those of control groups. The average weight of both ovaries at 2 days postestrus was 68, 73, and 80 mg for the cycling, pseudopregnant and yohimbine treated groups, respectively. However, this difference between the latter 2 groups was not statistically significant at the 5% level as determined by the t-test between group means.

Histological study showed a marked increase in size of the corpora lutea of the yohimbine treated animals when compared to those of the estrous cycle or normal pseudopregnancy (Fig. 4, 5, & 6). Corpora lutea formed during yohimbine treatment were active as shown by the formation of decidual tissue in the uterus of the 7 animals following uterine trauma.

The paired adrenal weights were significantly increased ($P<.01$) by yohimbine-HCl from an average of 58 mg in the normal pseudopregnant females to 73 mg in treated animals. In the male rats the adrenal weights were increased from an average of 39.7 mg in controls to 50.5 mg in treated groups ($P<.05$). Uterine weights were less ($P<.01$) in yohimbine treated animals than in pseudopregnant controls (264 mg *vs* 320 mg).

No significant differences were found in the weights of thyroids, testes, ventral prostates or seminal vesicles in the males or in pituitary weights of either male or female rats, nor were differences in growth rates between treated and control groups of either the male or female rats found.

In the group of female rats receiving single injections of 20 mg of yohimbine per kg of body weight, only those rats injected on the day of estrus consistently went into pseudopregnancy. Single injections of yohimbine on the day of estrus produced pseudopregnancy (average length of 14 days) in 10 of 13 animals. The number of pseudopregnancies resulting from injections on the day of proestrus, day 1 postestrus, and day 2 postestrus, were 4 of 12, 7 of 13, and 1 of 12 respectively.

Discussion. These results indicate that the mammary development resulting from yohimbine-HCl treatment is apparently due to increased progesterone secretion by the corpora lutea of the yohimbine-induced pseudopregnancy. The cause of the additional mammary development over that of normal pseudopregnancy might be caused by increased levels of secretions from the ovaries, pituitary or possibly the adrenals. Since injections of yohimbine in male rats caused no mammary stimulation, it is unlikely that yohimbine directly stimulates mammary growth. Induction of pseudopregnancy and increased size of the corpora lutea indicate an increase in pituitary output of luteotrophin. Yohimbine may cause luteotrophin release by acting directly on the pituitary, through the hypothalamus or possibly by stimulation of oxytocin release. Mares and Casida(6) reported an increase in progesterone content of the bovine corpus lutem following oxytocin injections.

The increase in adrenal weight may be due to either an increase in ACTH secretion or to stress from yohimbine-HCl injections. Decreased uterine weight produced by yohimbine probably results from changes in the quantity of gonadal hormones. The pathway for yohimbine stimulation of the ovaries and mammary glands remains unknown, but it is probably by way of the hypothalamus and pituitary.

Summary. Daily subcutaneous injections of 20 mg of yohimbine-HCl per kg of body weight for 26 to 33 days caused pseudopregnancy, increased ovarian and adrenal weights, and produced lobulo-alveolar development in female rats. The corpora lutea formed during this treatment were active as shown by their ability to produce decidual reactions in traumatized uteri. Induction of pseudopregnancy by single injections of 20 mg of yohimbine-HCl per kg of body weight was successful when given on the day of estrus. Daily injections of yohimbine had no effect on mammary development in male rats.

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Sodium and Potassium Content of a Small Artery.* (28509)

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Recently(1) we reported work in which we interposed a small open reservoir and constant volume pump between the arterial inflow and venous outflow of the rat tail and monitored (Na^+) and (K^+)† in the circulating medium with glass electrodes. When a sucrose-substituted, low Na^+ solution was perfused, resistance in the vascular bed declined quickly while a considerable amount of Na^+, apparently from cells, was added to the perfusing medium in this same time. These results led us to expect that the smooth muscle of the peripheral vascular tree would contain a large pool of Na^+. We now present substantiating data obtained directly by chemical analysis of the ventral tail artery, a small (500 μ), predominantly muscular vessel.

Large (400 g) male albino rats under pentobarbitone-phenobarbitone anesthesia(2) were used. In one group, the ventral tail artery and a companion vein were cannulated and the tail then perfused at 0.65 ml/min and 37°C. The venous effluent was discarded. The system was allowed to run freely for 3 minutes using a basal solution of the following composition in mM/1: NaCl, 115.0; KCl, 5.0; $NAHCO_3$, 25.0; NaH_2PO_4, 1.2; $CaCl_2$, 2.1; $MgSO_4$, 1.2; dextrose, 11.0; calcium disodium versenate, 0.026. To this was added 4% Plasdone (polyvinyl pyrrolidine) as plasma colloid substitute. The base of the tail was then ligated and the perfusate changed for one modified by the addition of 400 mg% inulin (about 8 mM). This was then perfused without interruption for 30 minutes, the time necessary for inulin equilibration in this system.

In a second group, the renal pedicles were ligated and a carefully measured amount of inulin was injected into the tail vein and allowed to equilibrate for 2 hours. At the end of the procedure in both groups, a length of about 13 cm of tail artery was excised in less than 2 minutes (by stopwatch), then quickly dissected free of adventitia and placed in a pre-weighed stoppered bottle for immediate weighing. Inulin, Na and K in the tissue, in the venous effluent collected in the terminal minute of perfusion, or in an arterial sample were analyzed by conventional methods(2) modified for the small samples available here. The findings are

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† Na or K = sodium or potassium, not necessarily ionized Na^+ or K^+ = ions;
() = activity of the ions relative to the calibrating solution; [] = concentration relative to water.