

immature female rat in 30 hours. This material was given in a single injection of 0.25 cc within 40 minutes from the time it passed the cervix. Its toxicity would seem to rule out bacterial activity or any protein decomposition other than what might occur in the uterus. The strongest indication of a specific toxin lies in the repeatedly confirmed observation that 1 to 2 cc twice daily of specimens of whole discharge or "plasma," which alone in these amounts are non-toxic to normal mature female rats, are lethal within 72 hours from the first injection when estrogen is simultaneously administered, whereas such is not the case with venous whole blood or serum.

Conclusion. The menstrual discharge of women with normal cycles is highly toxic to rats through the production of vascular damage. The possibility that this toxicity is accountable to protein decomposition has not been conclusively ruled out, although the marked effect of hormonal conditions upon resistance appears to argue against this. The greatest susceptibility requires the presence of the ovaries. Functional corpora lutea afford partial protection, but the administration of estrogen overrides this action. Protection may be rendered by pretreatment with large amounts of progesterone, adrenal cortical extract or "immune" rabbit serum. The toxin appears to be intimately associated with a large moleculed protein material.

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Menstrual Discharge of Women. II. Its Progesterone-Stimulating Effect in Mature Rats.*

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Early in the study of the toxicity of the catamenial discharge¹ it was noted that mature female rats, with previously regular cycles, which survived the first 2 or 3 days of injections went, within 72 hours, into constant diestrus on continued injections and reverted

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¹ Smith, O. W., and Smith, G. V., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **44**, 100.

to normal cycles when injections were discontinued. One to 2 cc twice daily of either whole menstrual discharge or "plasma" produce this effect, but "plasma" (without estrogen) is less likely to be lethal and is not as irritating to the subcutaneous tissues. Exploration revealed large corpora lutea which were functional, as demonstrated by the finding of deciduomata on the ninth day of injections following needling of the uterus on the sixth day.

Such a progesterone-stimulating action suggested that the discharge might contain either a known gonadotropic hormone or an estrogen, although the familiar estrogens in amounts sufficient to stimulate demonstrable hyperactivity of the corpora would first produce estrous vaginal smears. In searching for estrogenic potency, we have extracted large amounts of whole menstrual discharge, "plasma" and dried whole discharge with ethyl alcohol, ether, acetone, butyl alcohol and benzene. We have also attempted to recover any combined estrogen by performing acid hydrolysis upon wet and dried whole discharge and butyl extracts of the same.† None of these preparations, in olive oil solution or saline suspension, has given estrus in spayed mature female rats, standardized for estrogen assay, even when tested upon a number of primed animals for as little as 1 r.u. in 20 cc of material.‡ Although Frank³ has reported estrogenic activity in menstrual discharge, our results demonstrate that, if present at all, there is not enough to account for the increased production of progesterone in normal mature rats. These lipid extracts have neither been toxic nor had any effect upon the cycles of rats.

In searching for gonadotropic substance, whole discharge was shaken with 5 volumes of 95% ethyl alcohol and placed in the refrigerator overnight. After centrifugation, the precipitate was washed twice with ether, dried, powdered and taken up in water. After 24 hours in the refrigerator, with repeated shaking, the water-

† Crystalline estrone in water is rapidly destroyed by acid hydrolysis methods which may be used upon urine without loss of added estrone. A solution of high salt concentration (69 g NaH_2PO_4 and 179 g Na_2HPO_4 per L) has been found a satisfactory substitute for urine and used as the medium for hydrolysis in attempting to deconjugate any combined estrogens in menstrual discharge or extracts of it. The material to be tested has been diluted with 10 volumes of concentrated phosphate solution, boiled under a reflux for 10 minutes with 15 vol.% HCl^2 and extracted for 24 hours in a continuous benzene extractor.

² Smith, O. W., Smith, G. V., and Schiller, S., *Endocrinol.*, 1939, **25**, 509.

‡ A total of 22 lipoidal extracts of whole menstrual discharge or plasma have been tested, each representing from 40 to 270 cc of material.

³ Frank, R., *The Female Sex Hormones*, Charles C. Thomas, published 1929.

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insoluble residue was separated by centrifugation, washed 3 times with water and discarded, the watery extract (with washings) being made up to a measured volume and used for testing. Such extraction appears to yield a complete recovery of the progesterone-stimulating principle, since, with 12 specimens from 3 donors, the equivalent of 1 cc of discharge twice daily for 8 days was sufficient to increase and prolong the activity of corpora lutea in mature rats, with the production of deciduomata after needling of the uterus on the sixth day. These extracts have shown no toxicity other than a slight subcutaneous reaction. As tested on spayed rats, they contained no free or combined estrogen.† They have had no effect upon the genitalia of immature female rats in amounts equivalent to 6 cc of whole discharge. The combined injection of one of them and F.S.H. (from menopausal urine) into an immature female rat gave no luteinization. They have not enhanced the luteal response of immature rats to chorionic gonadotropin and have repeatedly failed to augment the weight of the seminal vesicles of immature males (the equivalent of 10 cc of material being given in 10 doses over 5 days). These findings rule out the presence of any known follicle-stimulating or luteinizing hormone. Furthermore, activity was not diminished by heating in a boiling water bath for 1 hour, which treatment is destructive of the known gonadotropic hormones.

After fractionation of the proteins of menstrual discharge with $(\text{NH}_4)_2\text{SO}_4$, the progesterone-stimulating activity is recovered in the water-insoluble portion (after dialysis) of the englobulin precipitate. The toxicity of menstrual discharge is also recovered in greatest concentration in this fraction.§ The toxin and progesterone-stimulating substance are not identical, however, since alcohol precipitation destroys the former but not the latter. Furthermore, the latter has been found in nonlethal materials, such as alcohol-ether precipitates of venous blood and urine at the time of menstruation and in a sample of catamenial discharge that had lost its toxicity on standing. None has been demonstrable in venous blood, in the amounts tested, during the luteal phase of the cycle or in blood from males. In testing for it, upon normal mature rats with previously regular cycles, the result is considered positive only when deciduomata, confirmed by microscopic section, are present on the eighth or ninth day of injections after needling of the uterus on the fifth or sixth day.

§ These findings suggest that the progesterone-stimulating factor occurs in menstrual discharge in a toxic protein conjugation which is split by alcohol but not by $(\text{NH}_4)_2\text{SO}_4$ precipitation.

Conclusion. The menstrual discharge of normally menstruating women contains a heat-stable factor, insoluble in lipid solvents, which stimulates increased luteal activity in mature rats. This factor has also been found in venous blood and urine at the time of menstruation. Its progesterone-stimulating effect is not accountable to any free or combined estrogen or to any known gonadotropic hormone.

So far as this work has progressed, the progesterone-stimulating factor of menstrual discharge resembles the substance reported by Astwood and Greep as occurring in the rat placenta.⁴ Our material has not yet been tested upon hypophysectomized rats but the fact that alcohol precipitation destroys toxicity without decreasing progesterone-stimulating activity makes it appear that the progesterone stimulation is not a non-specific toxic effect.

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Unsuccessful Therapy in Experimental Equine Encephalomyelitis with Salt Solutions of Varied Concentrations and Sulfanilamide Compounds.*

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The high mortality of Eastern equine encephalomyelitis in both man and animals has prompted interest in therapy. Specific antisera may have value in the treatment of horses if administered early, but its use after the disease is well established is ineffectual.¹ In horses the disease may be suspected and treated specifically; however, in man the diagnosis must usually await the appearance of neurological signs at which stage the lesions are too far advanced for antiserum therapy. This shortcoming suggested the investigation of other therapeutic procedures employing the highly susceptible rat and mouse.

The beneficial effect of hypertonic solutions, such as 10-25%

⁴ Astwood, E. B., and Greep, R. O., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 713.

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¹ Personal communication, Dr. B. M. Lyon, Assistant Director, Vet. Dept., Lederle Labs., New York.