

formation. The mechanisms probably are not identical. In addition to exerting lateral pressure and limiting the blood supply, tannic acid and gentian violet exert chemical inhibition to the growth of the nerve for a varying period following the injection which further favors the encasement of the resting nerve

fibers by a connective tissue envelope.

This method of preventing the formation of end-bulb neuromata may aid in solving the problem of painful amputation stumps and the phenomenon of the phantom limb. No contraindications to injecting the nerve ends in amputation stumps have been observed.

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Vaginal Response to Adrenal Stimulation.*

JOSEPH SCHILLER.[†] (Introduced by F. H. Pratt.)

From the Department of Physiology, Boston University School of Medicine.

The participation of the adrenal cortex in the estrous cycle, established by Wyman,¹ has been confirmed since under various experimental and natural conditions. However, the results of those experiments do not necessarily imply that the adrenal cortex influences the estrous cycle through the release of sex hormones rather than through hormones acting on the general metabolism. The isolation of both estrone (Beall²) and of progesterone (Beall and Reichstein³) from the adrenal cortex points to the former possibility as a primary factor. In spite of the chemical findings the physiological evidence is scant, and limited to the experiments of Moon,⁴ Corey and Britton,⁵ Nelson,⁶ and Bourne and Zuckerman.⁷ The availability of adrenocorticotrophic hormone furnishes an additional technic for this type of investigation.

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[†] Present address: Carnegie Institution, Department of Embryology, Baltimore, Md.

¹ Wyman, L. C., *Am. J. Physiol.*, 1928, **85**, 414.

² Beall, D., *J. Endocrinol.*, 1940, **2**, 81.

³ Beall, D., and Reichstein, T., *Nature*, 1938, **142**, 479.

⁴ Moon, H. D., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 36.

⁵ Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 1931, **99**, 33.

⁶ Nelson, W. O., *Anat. Rec.*, Suppl., 1941, **81**, 97.

⁷ Bourne, G., and Zuckerman, S., *J. Endocrinol.*, 1941, **2**, 268.

Material and Methods. Young female rats of the Long-Evans strain were injected daily for 5 days (unless otherwise specified) with adrenocorticotrophic hormone (ACTH).[‡] Hepatectomy was performed by making an incision parallel to the ribs and extirpating the median and lateral left lobes. Mortality was 5%. Grafting of the adrenals was done by suturing both glands to the spleen and the "takes" were practically 100%. All sections were stained with hematoxylin-eosin and vaginal smears were taken by the lavage method.

Results. The administration of ACTH to normal females gave inconclusive results. The estrous phase seemed in some instances to be prolonged beyond normal; but since prolonged estrus may occur spontaneously, and since the diestrous phase was unaffected by ACTH, it was concluded that ACTH was ineffective in normal female rats. (Table I.)

On the assumption that the liver might inactivate part or all of the estrogenic substances released by the adrenal cortex

[‡] The ACTH was obtained through the courtesy of Dr. E. B. Astwood, and was prepared according to the technic described by R. Tyslowitz (*Science*, 1943, **98**, 225). In all probability the product contained some lactogenic hormone. However, ACTH prepared by us from hog pituitaries according to the technic of G. Sayers, A. White, and C. N. H. Long (*J. Biol. Chem.*, 1943, **149**, 425), which behaves like a pure protein, gave identical results in our hands.

TABLE I.
Vaginal Responses in Young Female Rats Injected Twice Daily with 2 mg of Adrenocorticotrophic Hormone.

No. of group	Type of animal	No. of rats	Vaginal reaction	
			Before ACTH	After ACTH
1	Normal	5	Normal cycle	Unaffected
2	Ovariectomized	5	Leucocytes	"
3	Ovariectomized-adrenalectomized	5	"	"
4	Partially hepatectomized	15	Constant subestrus with epithelial cells predominant	Full cornification
5	Ovariectomized—partially hepatectomized	12	Leucocytes. Rare occasional cornified	Presence of nucleated and mucous cells

(Schiller⁸), further experiments were performed on partially hepatectomized rats. After this procedure, 12 out of 15 animals (Group 4, Table I) went into the condition known as "constant subestrus" characterized by the predominance of epithelial cells in the vaginal smear. Three remained in diestrus throughout the experiment and Loeb's placental sign was negative in all 3. The injection of ACTH in the responsive ones induced full cornification, indicating that the stimulated adrenals can supplement the estrogenic substances sufficiently to induce full estrus.

In order to eliminate the possibility that the injected ACTH had stimulated the pituitary gland to release gonadotropins, five partially hepatectomized rats in constant subestrus were ovariectomized and injected without delay with 4 mg of ACTH daily for 10 days. The vaginal smear continued to show epithelial and cornified cells throughout the experiment. According to Hemmingsen,⁹ the estrous cycle once initiated will continue despite ovariectomy. We found this to be true for adrenalectomized and adrenalectomized-ovariectomized rats as well. Groups of 5 normal animals were used and operations were performed during proestrus. However, in no case was a positive smear detectable on the third day after operation, whereas the animals treated with ACTH remained in estrus for 10 days.

Supporting evidence was obtained from Group 5 (See Table I). Twelve partially hepatectomized rats were ovariectomized, permitted to reach anestrus and only then injected with ACTH. Eight only responded to the stimulation. The responsive ones showed in the vaginal smear a predominance of round, nucleated, and mucous cells, and serial sections of the vagina showed areas of stratification (Fig. 1, A) and mucification (Fig. 1, B). The stratification was unevenly distributed along the vaginal wall and full cornification never occurred. Apparently the amount of estrogenic substances released by the adrenal cortex is quantitatively insufficient to produce full cornification, but the evidence indicated the presence of subthreshold amounts.

In order to test the hypothesis that the inconclusive results obtained in normal animals were due to the inactivation of adrenal estrogen by the liver, 10 non-hepatectomized female rats were subjected to autoplasmic grafting of the adrenals into the spleen. If the liver does inactivate estrogens, then the vaginal smear ought to reflect the same condition as that of adrenalectomized animals. After 6 weeks of salt-water solution for drinking, tap water was substituted and the vaginal smear followed daily. Contrary to expectations 9 animals went into "constant estrus," the cornified cells being predominant. The 10th animal showed regular cycles and even became pregnant, but at autopsy one regenerated cortical mass was found near the left kidney. The spleen-grafted animals subjected to the "resistance to cold" test did not behave differently

⁸ Schiller, J., *Endocrinol.*, 1945, **36**, 7.

⁹ Hemmingsen, A. M., *Skand. Arch. Physiol.*, 1933, **65**, 97.

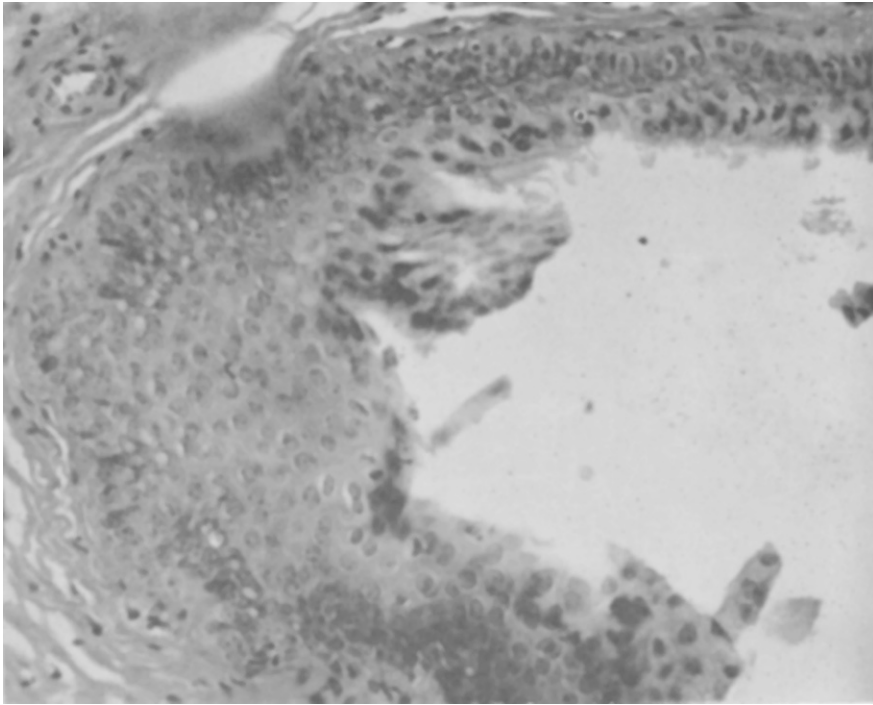


FIG. 1, A.

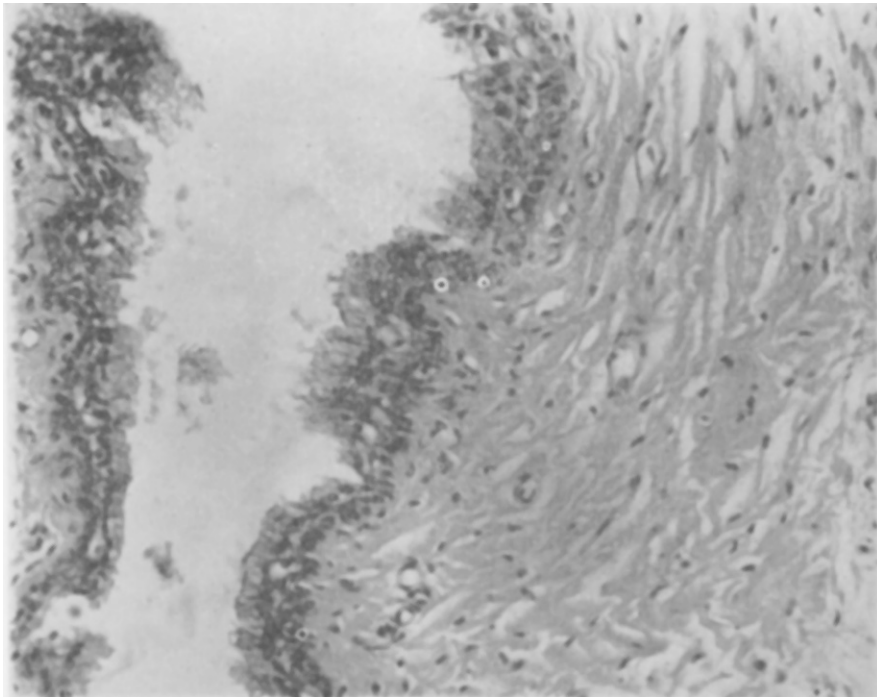


FIG. 1, B.

Stratification (A) or mucification (B) of the vaginal wall after injection of adrenocorticotrophic hormone, in ovariectomized rats with subtotal hepatectomy. $\times 300$.

from normal controls. They have been maintained alive without treatment for as long as 3 to 5 months preceding autopsy.

Discussion. The experiments raise several problems. The estrous cycle of normal female rats was not influenced by the administration of ACTH within the dosage used. This contrasts with the results obtained in immature rats. Moon⁴ under similar circumstances observed an earlier opening of the vagina, and Nelson⁶ an enlargement of the sex accessories. Although the opening of the vagina could be explained in terms of a lower threshold to estrogen stimulation, such would not be the case in Nelson's experiment. It is more plausible to assume either that the metabolism of estrogen assumes different patterns in immature and mature animals, or that the hormonal equilibrium in the normal adult rat cannot be disturbed by ACTH injection. At first sight, the fact that partial hepatectomy facilitates vaginal reactions in young mature rats injected with ACTH would indicate that the amount of estrogenic substances released by the adrenals is quantitatively insufficient to overcome the inactivating power of the liver. However, this is not supported by the fact that estrous manifestations are present in non-hepatectomized rats with spleen-grafted adrenals. It is more probable that the metabolism of estrogen follows different patterns in normal and partially hepatectomized rats with regenerating livers.

The stratification and mucification of the vaginal wall in responsive rats indicates that the amount of estrogenic substances released by the stimulated adrenal cortex is too small to induce full cornification. This is in agreement with the findings of Bourne and Zuckerman,⁷ who induced cyclic estrus in ovariectomized rats injected with subthreshold doses of estrone, the difference being supplemented

by the adrenal gland. Likewise Meyer and Allen¹⁰ obtained mucification of the vaginal epithelium in rodents by using subthreshold doses of estrone. Stratification and mucification of the vaginal epithelium was obtained by Jones and Astwood¹¹ by using proper amounts of both estrone and progesterone. Since Beall and Reichstein³ and Beall² have isolated these two hormones from the adrenal cortex it is not impossible that our animals were affected by both of them, and by androgens as well. As long as the substances which act on the vagina are not identified, it is impossible to decide which hormone or group of adrenal hormones is responsible for the induced vaginal reaction. If this hormone is a true estrogen, then it differs qualitatively from ovarian estrogen (Golden and Severinghaus¹²) and from exogenous estrogen, which are inactivated by the liver. Parallel conclusions were reached by Burrill and Greene¹³ for adrenal androgens and by Eversole, Edelman, and Gaunt¹⁴ for "life maintenance" and "resistance to water intoxication" hormones.

Summary. In partially hepatectomized young female rats the administration of ACTH induces the release of small amounts of hormone from the adrenal cortex, affecting the sex organs.

¹⁰ Meyer, R. K., and Allen, W. A., *Anat. Rec.*, 1933, **56**, 321.

¹¹ Jones, G. E. Seegar, and Astwood, E. B., *Endocrinol.*, 1942, **30**, 295.

¹² Golden, J. B., and Severinghaus, E. L., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 361.

¹³ Burrill, M. W., and Greene, R., *Endocrinol.*, 1941, **28**, 874.

¹⁴ Eversole, W. J., Edelman, A., and Gaunt, R., *Anat. Rec.*, 1940, **76**, 271.

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