of dried venom ranging from 1 to 5 μg were added to 3 U of hypertensin in a medium buffered to pH 7.4 where the venom shows maximum hypertensinase activity.

The results obtained are summarized in Table II. There was no difference in the rate of inactivation whether oxygen was present or absent. The effect of 4-nitrotoluene sulphonic acid and of salicylic acid was studied by addition of 10 mg of the first compound or 20 mg of the second (adjusted to pH 7.4) for every 3 U of hypertensin. Since there was no change in the course of the inactivation, this process is probably not due to the action of the *l*-amino-acid oxidase, as Zeller *et al.*[7] have reported inhibition of this enzyme by these 2 substances.

There are further differences between the inactivation of hypertensin and the deamination of amino acids by *l*-amino-acid oxidase extract. The hypertensinase activity is not affected by absence of oxygen or presence of ammonium sulfate. The inactivation of hypertensin is at a maximum at pH 7.5 and does not decrease sharply when the pH is decreased or increased. According to Blanchard *et al.* the rate of oxygen uptake and ammonia production from amino acids has a pH maximum about 10. Above and below this pH the velocity of oxidation declines sharply, being negligible at pH 7.

Conclusion. The hypertensinase activity shown in the same preparation of *l*-amino-acid oxidase used by Blanchard *et al.* may be attributed to another enzyme, associated as an impurity. The results obtained with the venom of *Bothrops neuwidii* support our previous view that the hypertensinase activity of this venom is due to the presence of a proteolytic agent, and do not support the hypothesis that the *l*-amino-acid oxidase participates in the inactivation of hypertensin by the same venom.

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Effect of Steroid Sex Hormones on Distribution of Alkaline Phosphatase in Uterus of Mouse.*†

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The distribution of alkaline phosphatase in the uterus during the reproductive cycle and pregnancy has been described in several species[1-3] following the development by Gomori[1,4] of methods for demonstrating the presence of this enzyme in tissue sections. It is generally agreed that alkaline phosphatase may be found in any of the component tissues of the endometrium, the exact distribution varying with the physiological state and the species studied. Although the specific function of phosphatase in the physiology of reproduction is not clear, there is reason to believe that

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1 Gomori, G., *J. Cell. and Comp. Physiol.*, 1941, **17**, 71.

2 Kabat, E. A., and Furth, J., *Am. J. Path.*, 1941, **17**, 303.

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TABLE I.
Amount and Distribution of Alkaline Phosphatase in the Uteri of Ovariectomized Mice Treated Various Steroid Sex Hormones.

Group	Treatment (dose/day for 3 days)	No. of animals	Myometrium		Endometrium		Stroma
			Outer long.	Inner circ.	Uterine epith.	Glands	
1.	Untreated	6	+++	+	±	±	0
2.	1.0 μ estradiol benzoate	5	+++	++	+++	+++	0
3.	1 mg progesterone	5	+++	0	±	±	0
4.	1.0 μ estradiol benzoate 1 mg progesterone	5	+++	+	+++	+++	0
5.	0.5 mg testosterone propionate	5	+++	±	±	±	0

it plays an integral part. Many phases of the reproductive process have proven to be under the influence of the gonadal hormones. The following experiments were designed to ascertain whether the administration of steroid sex hormones influences the distribution and amount of alkaline phosphatase in the uterus of the mouse.

Experimental. Twenty-six young adult mice of the Swiss albino strain were bilaterally ovariectomized. A control group of 6 animals received no further treatment and was killed 7 to 14 days after operation. The remaining mice were divided into 4 groups of 5 each (Table I). Daily subcutaneous injections of several steroid sex hormones were begun 3 to 14 days after ovariectomy. Crystalline hormones were used; the daily dose was dissolved in 0.05 to 0.1 cc of sesame oil. The animals in the second and third groups received 1.0 μg of estradiol benzoate and 1 mg of progesterone respectively each day. The fourth group were injected daily with both 1.0 μg of estradiol benzoate and 1 mg of progesterone. The last group received 0.5 mg of testosterone propionate daily. Treatment was continued for 3 consecutive days and the animals were killed by illuminating gas 24 hours after the last injection. The animals were autopsied immediately and the uteri were fixed in chilled 80% ethyl alcohol for 24 hours. After dehydrating and imbedding in paraffin, sections were cut 5 micra in thickness. The tissue sections were incubated in a 1% sodium glycerophosphate substrate for 3 hours at 35° to 39°C and treated according to the technic of Gomori.[1,4] This results in a dark deposit of cobalt sulfide at the sites of alkaline phosphatase activity. The sections were counterstained with Ehrlich's hematoxylin and were mounted permanently for subsequent study.

In the untreated ovariectomized mice deposits of cobalt sulfide, indicating phosphatase activity, were dense in the muscle cells of the longitudinal layer of the myometrium and were very light in the circular muscle. (Table I). Occasional light deposits of sulfide were also present in the uterine glands and epithelium. No evidence of cytoplasmic enzyme activity was present in the endometrial stroma. On the other hand, in the animals injected with estrogen there was a marked increase in the enzyme activity in the uterine glands and epithelium and in the circular layer of the myometrium. The sulfide deposits in the cells of the epithelium and glands tended to lie apical to the nuclei, being particularly dense at the free borders of the cells. Frequently deposits were evident in the connective tissue cells immediately adjacent to the glands. The remainder of the stroma exhibited no enzyme activity.

The distribution of phosphatase in the uteri of the animals treated with progesterone and androgen was essentially the same as in the untreated controls. The only consistent exception was the absence of detectable sulfide deposition in the circular muscle in the progesterone-treated group. In the mice receiving estrogen and progesterone concurrently the sites of phosphatase activity appeared to be the same as those described in the animals

receiving estrogen alone.

Discussion. It has been shown clearly in the present experiments that the injection of estrogen into the ovariectomized mouse is followed by a marked increase of alkaline phosphatase in the lumenal epithelium and that of the uterine glands and in the circular layer of muscle. It is also evident that neither progesterone nor androgen have this effect. Progesterone given concurrently with estrogen does not modify the effect of estrogen on uterine phosphatase.

The distribution of alkaline phosphatase in the endometrium of the ovariectomized mouse treated with estrogen is similar to that described during the reproductive cycle of the dog, rabbit and guinea pig[1] and the human.[2] Like the human, however, no widespread enzyme activity is evident in the endometrial stroma. Since the uterine specimens used in these previous studies were not timed as to the stage of the cycle, it is impossible to determine whether the phosphatase distribution could be related to estrogenic activity. In the cases where the endometrial specimen was obtained during advanced pregnancy, it is possible to assume that in some of the species the level of estrogen secretion had been high. In the pregnant rat and guinea pig[3] the distribution is similar to that in our estrogen-treated mice. However, in the human the enzyme is found only in the endothelium of the blood vessels during early pregnancy and later disappears entirely. In the pregnant sow the phosphatase is confined to the endothelial cells and to the endometrial stroma. It is obvious that no generalization can be made from such divergent observations until further data on the hormone-enzyme interrelation are available in these species.

The presence of alkaline phosphatase in the myometrium and the variations in its distribution after hormone treatment present problems which await explanation. Earlier studies have indicated that the myometrium of the human uterus does not contain phosphatase during the menstrual cycle.[2]

The significance of phosphatase in metabolism is due in part to its participation in the phosphorylation and dephosphorylation of many biologically important compounds. It is interesting to note that estrogen causes an increase in the glycogen content of the rat uterus.[5] It is possible that there is a relationship between alkaline phosphatase and the appearance of glycogen under these circumstances. In addition, estrogen causes the disappearance of the lipids in the uterine glands and epithelium of the rat,[6] an event which might also be related to phosphatase activity.

Summary. The distribution of alkaline phosphatase was studied in the uteri of untreated ovariectomized mice and in castrates injected with 3 steroid sex hormones. In the untreated castrate large amounts of the enzyme are present in the longitudinal muscle and smaller amounts are found in the circular muscle. Occasional small amounts are also present in the uterine glands and epithelium. Injection of estrogen is followed by marked increase of phosphatase in the uterine glands and epithelium and in the circular muscle. Progesterone and androgen do not have this effect. Progesterone given concurrently with estrogen does not alter the enzyme response to estrogen.

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[5] Boettiger, E. G., *J. Cell. and Comp. Physiol.*, 1946, **27**, 9.

[6] Alden, R. H., *Anat. Rec.*, 1946, **94**, 445.