its effect upon the hyaluronidase-hyaluronic acid reaction in the pericapillary sheath rather than to an effect upon the intercellular cement substance. This is suggested because the action is rapid and does not take place in the 18- to 24-hour period characteristic of action on the cement substance. Ascorbic acid either inhibits hyaluronidase directly, or it alters the hyaluronic acid in the sheath so as to make it more resistant to hyaluronidase.

Summary. Both *in vitro* and *in vivo* experiments show that ascorbic acid exerts an inhibitory effect upon the hyaluronidase-hyaluronic acid reaction. The inhibition is more marked in the animal than in the test tube.

The components of the capillary wall and their relation to capillary permeability have been discussed.

These studies suggest that in some cases where capillary fragility is corrected by ascorbic acid, the effect could be due to the inhibitory effect of ascorbic acid upon a hyaluronidase-hyaluronic acid system, rather than to an effect of vit. C on the intercellular cement.

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Some Hormones Involved in Elaboration or Activation of the Mammary Spreading Factor.* (18765)

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In a previous paper(1) observations were presented indicating the presence of a spreading factor in the mammary glands of pregnant albino rats. This factor, which increased in potency during the first half of pregnancy, is thought to make possible the rapid advance of the mammary gland into the subcutaneous tissue. The close relation of mammary gland growth to the amount of active spreading factor suggested that the hormones regulating mammary gland growth might also regulate elaboration or activation of the spreading factor.

Hormones involved in mammary gland growth have been adequately reviewed by Turner(2), Riddle(3), Folley and Mallpress (4), and Trentin and Turner(5), so no attempt at review will be made.

Methods and materials. Castrate male and female albino rats weighing from 150 to 250 g were employed. If only castrate animals were to be used treatment was started on the eleventh day postoperatively, however, some castrate-adrenalectomized rats were used. In these cases the castrate animals were adrenalectomized on the eleventh day and treatment was started about 5 days later. The castrate-adrenalectomized animals were maintained on 1% sodium chloride drinking water. The estradiol benzoate and progesterone used in the treatment were dissolved in olive oil, the testosterone was dissolved in 60% alcohol, while the anterior pituitary extract† was made up as a suspension in distilled water. The treatment involved subcutaneous injections daily for 10 days. On the eleventh day the animals were killed, the mammary glands re-

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1. Elliott, J. R. and Turner, C W., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 384.

2. Turner, C. W., *The Mammary Glands.* In Allen, E., *Sex and Internal Secretions,* 1939, The Williams and Wilkins Co., Baltimore.

3. Riddle, O., *Ann. Rev. Physiol.,* 1941, v3, 573.

4. Folley, S. J. and Mallpress, F. H., Chap. IV in Pincus and Thimann. *The Hormones,* vI, 1948, Academic Press, Inc., New York.

5. Trentin, J. J., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.* 418, 1948.

† The preparation employed was an ant. pituitary extr. rich in lactogenic hormone. This extract was generously supplied by United Research Laboratories, Philadelphia, Pa.

TABLE I. Effects of Hormone Treatment on Amount of Spreading Factor.

Controls & treatment	No. of animals and sex	Assay of spreading factors* Avg initial bleb, mm	Avg final bleb, mm	Area of spread, mm²
pH 6 buffer solution	34 trials	14 × 14	16 × 17	59.7
Testicular extract	34 "	"	24 × 25	317.4
12 days pregnant	27 ♀	13.9 × 14	22.6 × 23.5	264.3
1 μg estradiol benzoate	5 ♀ †	14 × 14	16 × 18	72.3
3	5 ♀ †	13 × 14	19 × 20	155.6
5	5 ♀ †	14 × 14	20 × 21	176
5	5 ♂ †	"	20 × 21	176
5	5 ♀ † adren.	"	16 × 17	59.7
1 mg progesterone	5 ♀ †	14 × 14	16 × 18	72.3
3	5 ♀ †	13 × 14	17 × 18	97.4
5	5 ♀ †	14 × 14	18 × 19	114.7
5	5 ♂ †	"	18 × 18	100.6
5	5 ♀ † "	"	18 × 19	114.7
10	5 ♀ †	"	21 × 21	192.5
10	5 ♂ †	13 × 14	20 × 21	187
10	5 ♀ † "	14 × 14	21 × 21	192.5
1 μg E. B. + 1 mg prog.	5 ♀ †	13 × 14	16 × 16	58.1
1 3	5 ♀ †	14 × 14	20 × 21	176
1 5	5 ♀ †	"	21 × 22	209
1 5	5 ♀ † adren.	"	21 × 22	209
3 1	5 ♀ †	"	17 × 17	73.1
3 3	5 ♀ †	"	20 × 21	176
3 5	5 ♀ †	13 × 14	20 × 21	187
5 1	5 ♀ †	14 × 14	18 × 19	114.7
5 3	5 ♀ †	"	18 × 18	100.6
5 5	5 ♀ †	"	19 × 19	129.6
5 5	5 ♀ † adren.	"	18 × 19	114.7
1 mg testosterone	5 ♀ †	"	19 × 20	144.6
1	5 ♂ †	"	19 × 21	159.5
1	5 ♂ † adren.	"	19 × 20	144.6
10 mg A. P. extract	5 ♀ †	"	18 × 19	114.7
10 " " " + 1 μg E. B.	10 ♀ †	"	21 × 21	192.5

* .2 extract + .1 cc Evans blue dye. † Castrate animal. Prog. = progesterone. Adren. = adrenalectomized. E. B. = Estradiol benzoate. A. P. = Ant. pituitary.

moved, extracts made and the amount of spreading factor in the extracts determined by a method previously described(1).

Experimental. The first experiments involved the injections of 1, 3 and 5 μg of estradiol benzoate. The second experiment employed the injection of 1, 3, 5 and 10 mg of progesterone. Experiments were also completed with various combinations of 1, 3 and 5 μg of estradiol benzoate and 1, 3 and 5 mg of progesterone. One mg of testosterone was injected into 5 castrate females, 5 castrate males and 5 castrate-adrenalectomized males. A 10 mg level of anterior pituitary extract was injected alone into five castrate females and together with 1 μg of estradiol benzoate into 10 castrate females.

Results. The spreading properties of the mammary gland extracts from the hormone injected rats were compared to testicular extracts and pH 6.0 buffer solutions (Table I).

The extracts from the 1 μg estradiol benzoate injected castrate females showed the presence of very little spreading factor. When the estradiol benzoate level was raised to 3 μg the mammary gland extracts indicated good elaboration or activation of the spreading factor. Mammary gland extracts from castrate males and females treated with 5 μg of estradiol benzoate indicated the presence of slightly more spreading factors. However, the mammary gland extracts from castrate-adrenalectomized females injected with the above level of hormone indicated the presence of no active spreading factor.

With progesterone alone the amount of spreading factor in the mammary gland extracts was small at the 1 mg level. More

spreading factor was demonstrated with increasing amounts of hormone. The extracts from castrate males, castrate females and castrate-adrenalectomized females indicated the presence of fair amounts of the spreading factor at the 5 mg level and a further increase when the hormone level was doubled.

The combination of 1 μg of estradiol benzoate with 1, 3, and 5 mg of progesterone gave increasingly higher amounts of the spreading factor.

When the amount of estradiol benzoate was raised to 3 μg in combination with 1, 3 and 5 mg of progesterone the response was not quite as good but showed the same graded effect. When the estradiol benzoate level was raised to 5 μg with the above amounts of progesterone uniformly smaller amounts of the spreading factor were shown to be present in the mammary gland extracts of castrate animals. In a group of castrate-adrenalectomized females on this same level of hormone, the amount of spreading factor was about the same. Mammary gland extracts from the various groups of animals injected with 1 mg of testosterone indicated the presence of the spreading factor in only fair amounts.

The administration of 10 mg of anterior pituitary extract alone indicated only fair elaboration or activation of the spreading factor, while 10 mg of anterior pituitary extract plus 1 μg of estradiol benzoate gave a good response.

Discussion. Experiments employing estradiol benzoate indicated that increasing amounts of this hormone produced increasing amounts of the spreading factor, however, even the highest level of estradiol benzoate stimulated little or no spreading factor when injected into adrenalectomized animals. Trentin and Turner(5) found earlier that in the albino rat, mammary gland growth response to estrogen administration depended upon the presence of the adrenal. They postulated that response of the mammary gland to estrogen was due to the ability of this hormone to stimulate the secretion of an adrenal cortical steroid with which it synergized. It was suggested that this steroid was identical with or closely related to progesterone.

It is well known that progesterone is effective in stimulating mammary gland growth. Progesterone was found to be effective also in stimulating the mammary gland spreading factor. The extent of the elaboration or activation of the spreading factor in response to combinations of estradiol benzoate and progesterone showed that optimal synergism was attained when a high level of progesterone and a low level of estradiol benzoate was employed. These observations are similar to those reported upon the stimulus of mammary gland growth by these hormones. In contrast to our inability to obtain production of the spreading factor with estradiol benzoate in adrenalectomized animals, it was found possible to obtain stimulation in adrenalectomized animals when estradiol benzoate and progesterone were administered together, thus confirming the role of the adrenal in mammary gland physiology.

The production of the mammary gland spreading factor by the anterior pituitary extract and the increased response when small amounts of estradiol benzoate were added is consistent with the mammary gland growth produced by anterior pituitary extracts alone and in combination with estradiol benzoate. De Graff, de Jongh and Paesi(6) suggested that testosterone might produce better mammary gland growth than an estrogen-progesterone combination. The close relation between mammary gland growth and production of the spreading factor prompted the trial of testosterone. However, the results obtained with this hormone were distinctly poorer than those obtained with the estradiol benzoate-progesterone combination.

In the previous study(1) the maximum amount of spreading factor was observed in the mammary glands of rats pregnant 12 days. In none of the present experiments did the hormone treatment stimulate the elaboration or activation of an amount of spreading factor equal to the 12th day of pregnancy. However, it will be seen that with certain hormone combinations, the amount of spreading factor extracted equaled that obtained at 10 days of pregnancy. The observations presented in

6. De Graff, H. J., de Jongh, S. E., and Paesi, F. J. A., *Acta. Physiol. Pharmacol. Neerl.*, 1950, v1, 290.

this paper suggest that the hormones regulating mammary gland growth are responsible for the elaboration or activation of the spreading factor. Even the amounts of hormone and the hormone combinations which have produced the greatest mammary gland growth were observed to produce the largest amounts of the spreading factor. Thus the amount of spreading factor present and extracted from the glands could well be related to the number of mammary gland cells present.

Summary. 1. Hormones which stimulate mammary gland growth cause elaboration or activation of the mammary gland spreading factor. 2. Increasing amounts of estradiol benzoate caused increasing amounts of the spreading factor to be elaborated or activated in the mammary glands of castrate animals, but little or no spreading factor was found to be present in the mammary gland extracts of estradiol-benzoate treated, castrate-adrenalectomized animals. 3. Increased amounts of progesterone alone caused increased production of the spreading factor, however, the largest amount of progesterone employed did not give as good response as half the level of progesterone plus a little estrogen. 4. Best elaboration or activation of the spreading factor in response to treatment was with a combination of 1 μg estradiol benzoate and 5 mg progesterone. Other combinations of estrogen and progesterone gave graded responses. The castrate-adrenalectomized animals showed as good production of the spreading factor in response to estradiol benzoate-progesterone as castrate animals. 5. An anterior pituitary extract alone produced only fair amounts of the mammary spreading factor. When a low level of estrogen was added much better response was obtained. 6. One mg level of testosterone caused production of fair amounts of the spreading factor. However, the estrogen-progesterone combination gave a better response. 7. Ten days' treatment with some combinations of hormones caused as much elaboration or activation of the mammary gland spreading factor as was extracted from rats pregnant 10 days.

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Localization of a "Feeding Center" in the Hypothalamus of the Rat. (18766)

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Development of obesity in animals following destructive lesions placed in or ventrolateral to the ventromedial nucleus of the hypothalamus is a well-established fact(1-6). This obesity has been shown to arise primarily from a marked increase in food intake, or from what has been termed "hyperphagia."

In an experimental investigation being carried out to discover the effect on food intake, etc. of small electrolytic lesions placed by the Horsley-Clarke instrument in other areas of the hypothalamus of the albino rat, a well-localized area has been found in the lateral hypothalamus, bilateral destruction of which is followed by a complete absence of eating. This area is situated in the extreme lateral portion of the lateral hypothalamus at the same rostro-caudal level (coordinate) as the ventromedial nucleus (Fig. 1). Small, bilateral, electrolytic lesions produced in this area by a current of 1 milliampere used for 15 sec, completely inhibit food intake to the point where the rat dies of starvation. On the other hand, after a unilateral lesion in this area, the animal goes on eating normally; when another

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1. Hetherington, A. W., *Am. J. Physiol.*, 1941, v133, 326.
2. Hetherington, A. W., *J. Comp. Neurol.*, 1944, v80, 33.
3. Hetherington, A. W., and Ranson, S. W., *J. Comp. Neurol.*, 1942, v76, 475.
4. Brobeck, J. R., Tepperman, J., and Long, C. N. H., *Yale J. Biol. Med.*, 1943, v15, 831.
5. Brooks, C. McC., *Fed. Proc.*, 1945, v4, 9.
6. Brooks, C. McC., *Fed. Proc.*, 1946, v5, 12.