

clearly involved in carbohydrate metabolism it seems more than coincidence that this gland—particularly the cortex—responds especially well to injections of insulin. The present data, and also the observation that repeated injections of huge doses of insulin progressively lose their ability to depress the blood sugar and usually soon lead to a marked hyperglycemia,^{1,9} suggest that both cortical and medullary parts of the adrenal actively oppose the ability of insulin to decrease the blood sugar; and that this occurs in pigeons deprived of their anterior pituitary glands.⁹ In a recent review of pituitary-adrenal cortex relationships Swann¹⁰ concludes that in carbohydrate regulation the adrenal cortex is probably completely dependent upon pituitary stimulation. The present results seem to provide an exception.

Summary. In tests made on 41 normal young White Carneau pigeons daily injections of 1-60 units of insulin during 10 hours to 10 days enlarged the adrenals, produced mitosis and cellular activity. These cytological effects were more pronounced in cortical cells than in medullary cells. Wholly comparable effects were produced in the adrenals of 16 additional birds previously deprived of their anterior pituitary glands. The cytological changes in the cortex following insulin are identical with those induced by adrenotrophic pituitary extracts. Food consumption of the test animals and of 73 controls was regulated at different and definite levels. The relation of these results to the alarm reaction and to the rôle of the adrenals in carbohydrate regulation is discussed.

13172

Growth of the Lobule-Alveolar System of the Mammary Gland With Pregneninolone.*†

JOHN P. MIXNER AND CHARLES W. TURNER.

From the Department of Dairy Husbandry, University of Missouri, Columbia, Mo.

Turner and Frank¹ have shown that the growth of the lobule-alveolar system of the mammary gland which normally occurs dur-

⁹ Riddle, O., and Opdyke, D. F., unpublished data.

¹⁰ Swann, H. G., *Physiol. Rev.*, 1940, **20**, 493.

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 759.

† This study has been aided in part by a grant of the International Cancer Research Foundation.

¹ Turner, C. W., and Frank, A. H., *Missouri Agr. Exp. Sta. Res. Bul.* 174, 1932.

ing the first half of pregnancy could be stimulated experimentally in castrate animals by the simultaneous injection of estrogen and progesterin. Selye² reported that the administration of progesterone to spayed female rats, not pretreated with estrin, at the rate of 15 mg per day for 15 days caused a development of the mammary lobule system similar to that seen in late pregnancy. It has been shown that the action of the ovarian hormones upon the growth of the mammary gland is mediated by the AP for these hormones are ineffective in the hypophysectomized animal³ and the AP of pregnant animals contains a factor which will stimulate lobule-alveolar growth.⁴

Recently the writers⁵ have developed a technic for the assay of the mammogenic lobule-alveolar growth factor in pituitary materials, which technic may also be used to assay progesterone and progesterone-like substances that stimulate the secretion of the mammogenic factor by the AP of the assay animal. The ovariectomized virgin female mouse is used as the assay animal, and a minimal development of the lobule-alveolar system of the mammary gland is required for a positive response. A mouse unit of the lobule-alveolar growth promoting substance according to this assay is defined as the total amount of material required per mouse, injected subcutaneously daily over a period of 10 days, to obtain definite lobule-alveolar development in $50 \pm 10\%$ of 10 or more castrate, nulliparous, female mice weighing between 12 and 18 g. By this assay method 1 mg of progesterone (plus 133 i. u. of estrone) gave 60% positive responses in 10 mice.

A new synthetic hormone, pregneninolone (see 6 for references) known also as anhydro-hydroxy-progesterone and ethinyl testosterone, has been reported as having estrogenic, androgenic, and progestational properties,⁷ the latter property being most marked and as also being orally active. The assay technic described above was used to study the ability of pregneninolone† to stimulate mammary lobule-alveolar growth. As pregneninolone is reported to have both estrogenic and progesterin-like properties it was thought that it might not need estrogen as an accompanying hormone for mammary

² Selye, H., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 343.

³ Gomez, E. T., and Turner, C. W., *Missouri Agr. Exp. Sta. Res. Bul.* 259, 1937.

⁴ Mixner, J. P., Lewis, A. A., and Turner, C. W., *Endocrinology*, 1940, **28**, 888.

⁵ Mixner, J. P., and Turner, C. W., *Endocrinology*, in press.

⁶ Salmon, U. J., and Geist, S. H., *Proc. Soc. Exp. Biol. and Med.*, 1940, **45**, 522.

⁷ Emmens, C. W., and Parkes, A. S., *J. Endocrinology*, 1939, **1**, 332.

† Pregneninolone was kindly supplied by Dr. E. Schwenk, Schering Corporation.

TABLE I.
Lobule-Alveolar Responses of Castrate Female Mice to Pregneninolone Injected Subcutaneously for 10 Days.

No. of mice	Dosage in mg (total)	Responses		% positive
		Positive	Negative	
16	20-12.5	16	0	100
12	10.0	5	7	42
8	7.5	2	6	25
10	5.0-2.5	0	10	0

lobule growth. Table I presents the results secured with pregnenolone alone in promoting mammary lobule-alveolar growth.

In all injection groups propylene glycol was used as the carrier for the pregnenolone. In almost every case .2 cc per day of the propylene glycol with the pregnenolone was injected. From the table it may be seen that 10 mg of pregnenolone gave approximately a 50% response.

In another group of experiments (Table II), the effect of injecting a constant amount of an estrogen (estrone) with the pregnenolone was studied. It may be seen from a comparison of Tables I and II that approximately 1/5 as much pregnenolone is required to secure a 50% response when estrogen is administered simultaneously with the pregnenolone. Also 2.0 mg of pregnenolone (with estrone) gave 58.3% positive responses while 1.0 mg of progesterone (with estrone) as mentioned before gave 60% positive responses. Thus pregnenolone would seem to possess one-half the activity of progesterone in stimulating mammary lobule-alveolar growth when administered with a basic estrogen injection, and the activity of pregnenolone in stimulating mammary lobule-growth is enhanced 5 times by the simultaneous injection of estrogen. It is suggested that the enhancement of the growth effect is due in part to the hyperemia of genital tissues including mammary gland stroma by the estrogen, thus increasing the amount

TABLE II.
Lobule-Alveolar Responses of Castrate Female Mice to Pregneninolone Injected Subcutaneously with Estrone for 10 Days.

No. of mice	Pregneninolone dosage in mg (total)	Estrone dosage in i.u. (total)	Results		% positive
			Positive	Negative	
12	10.0-5.0	133	12	0	100
10	2.5	133	9	1	90
12	2.0	133	7	5	58
12	1.5	133	3	9	25
12	1.0	133	1	11	8

of blood and mammogenic lobule-alveolar growth hormone available to the growing gland tissue.

Summary. Pregneninolone alone and injected subcutaneously with estrone into castrate virgin female mice caused the development of the lobule-alveolar system of their mammary glands, having a property similar to progesterone in this respect. The injection of estrone with the pregnenolone enhanced this activity of the pregnenolone by five times. Pregnenolone has one-half the activity of progesterone in stimulating mammary lobule-alveolar growth when both are injected with estrone.

13173

Note Concerning the Mechanism of Increased Capillary Permeability in Inflammation.

VALY MENKIN

*From the Department of Pathology, Harvard University Medical School.**

The writer has demonstrated the presence and isolated a crystalline nitrogenous substance in inflammatory exudates which offers a reasonable explanation for the mechanism of both increased capillary permeability and migration of polymorphonuclear leukocytes at the site of inflammation.^{1, 2} This substance, termed leukotaxine, seems to belong to the group of relatively simple polypeptides. The possibility, however, of an additional prosthetic group is not yet precluded by the available data. Further studies on its chemical nature, including molecular weight, amino nitrogen content before and after hydrolysis, indole nucleus or perhaps tryptophane concentration, and a simpler method of purification will all form the subject of a separate future report.

Leukotaxine has been shown to have none of the physiological or chemical properties of histamine thus rendering difficult to accept the view that the latter plays a primary rôle in the mechanism of increased capillary permeability in inflammation.³⁻⁵ The various

* Aided by grants from the International Cancer Foundation and the Dazian Foundation for Medical Research.

¹ Menkin, V., *J. Exp. Med.*, 1936, **64**, 485.

² Menkin, V., *J. Exp. Med.*, 1938, **67**, 129, 145.

³ Menkin, V., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 103.

⁴ Menkin, V., *Physiol. Rev.*, 1938, **18**, 366.

⁵ Menkin, V., *Dynamics of Inflammation*, 1940, Macmillan Co., New York.