In an effort to find the exact linkage hydrolyzed by the enzyme, substances that are known to contain the foregoing phosphate-hydroxyl or phosphate-amino linkages were synthesized and subjected to the action of the enzyme. The studies were conducted in the same manner as those in which the action on ribonucleic acid was noted.[5] Sodium β-glycerophosphate, disodium phenyl phosphate, sodium phenyl anilino phosphate, sodium phenyl amido phosphate, potassium diphenyl phosphate, and phenyl phosphoryl guanine were found to be unattacked by the enzyme. Thus, it is clear that the enzyme is not to be classified as any of the non-specific phosphatases, *i. e.*, phosphomonoesterase, phosphodiesterase, or phosphoamidase.

Evidence for positive enzymic action on allonucleic acid, isolated from pancreas, and upon the Hammarsten nucleoprotein of the pancreas was noted. In both of these cases, the liberation of a group characteristic of a secondary phosphate dissociation is obtained. It appears from these data that the action of the enzyme is that of a specific phosphatase. The specificity is satisfied by two conditions that are met in the molecules of ribonucleic and allonucleic acids: (a) the specific arrangement of the mononucleotides in the nucleic acid structures, and (b) the existence of a certain as yet unidentified phosphate-hydroxyl or phosphate-amino linkage.

The nucleoprotein of Hammarsten cannot be said definitely to be an homogeneous substance. It cannot be stated at this time whether or not the action of the enzyme is to hydrolyze the linkage between nucleic acid and protein or one of the components of the nucleic acid while the latter is still combined as nucleoprotein.

12019

Influence of Local Applications of Turpentine on Mammary Gland Growth and Involution.*†

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Evidence has been presented indicating that the anterior pituitary of pregnant cattle contains a factor which will stimulate the complete

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growth of the alveolar system of the mammary glands of castrate virgin female mice.[1] Whether this same factor is involved in the maintenance of the gland during the lactation period is an open question. Recently, Hooker and Williams[2] reported that the application of spirits of turpentine to the nipples and surrounding skin caused a retardation in the rate of involution of the mammary alveolar system in mice from which the young had been removed. These observations suggested that turpentine might also stimulate the growth of the mammary alveolar system as well.

The object of this paper is to present evidence in confirmation of the work of Hooker and Williams and to report the failure of turpentine to stimulate the growth of the alveolar system of the mammary glands of normal and ovariectomized female mice by similar applications.

Lactating female mice: These animals were allowed to suckle their young for four days after parturition. The young were then removed, and the lactating mothers were divided into 4 groups, 2 of which received applications of turpentine to the teats and surrounding skin twice daily.

In all of the lactating female groups some involution of the mammary glands had taken place as compared with mammary glands of mice which had suckled their young for 11 days. Group II, turpentine treated, had a maintenance of the lobule-alveolar system which was superior to that of Group I, untreated. The alveoli were better preserved, 8 of the 12 animals showing evidence of secretion. Only 2 animals of the 8 in Group I showed any evidence of secretion. In Groups III and IV the process of involution had proceeded much further than that in either Group I or II. The alveolar development of the treated group was only slightly superior to that of the untreated group and that not significantly so. Thus as time proceeded the turpentine treatment became less effective in inhibiting the normal progress of involution.

If the action of turpentine in maintaining the alveolar system was

TABLE I.

Group	No. of animals	Duration of observation (days)	Treatment	Results
I	8	7	None	Involution advanced
II	12	7	Turpentine twice daily	Alveolar system well maintained
III	4	14	None	Involution far advanced
IV	4	14	Turpentine twice daily	Involution quite similar to III

[1] Mixner, J. P., Lewis, A. A., and Turner, C. W., *Endocrinology*, 1940, **27**, 888.

[2] Hooker, C. W., and Williams, W. L., *Yale J. Biol. and Med.*, 1940, **12**, 559.

mediated by a stimulus of the lobule-alveolar mammogenic hormone, it would be expected that the application of turpentine to the teats of normal and castrate virgin female mice would stimulate the growth of the alveoli of such animals.

Normal and ovariectomized virgin female mice: Twelve virgin females weighing from 12-20 g were treated twice daily with turpentine for periods varying from 4 to 8 days. In no case was any sign of lobule-alveolar development observed. This treatment was ineffective in stimulating pseudo-pregnancy.

Similar negative results were obtained with a group of 28 ovariectomized mice which received treatment twice daily for periods varying from 4 to 10 days.

Discussion. It has been shown[3] that the stimulus of nursing causes a release of the lactogenic hormone by the AP. Whether the same stimulus also influences the maintenance of the lobule-alveolar system (which rapidly degenerates after the cessation of nursing) is difficult to determine. We have been able to confirm the interesting observation of Hooker and Williams concerning the influence of local application of turpentine upon the involution process in the mammary gland. Our observations with castrate and normal virgin mice are interpreted to indicate that turpentine does not retard alveolar involution by stimulating the secretion of mammogen in the AP. Neither does turpentine applied to the teats and adjacent skin produce pseudo-pregnancy in normal females.

It is believed that the application of turpentine to the nipples and skin causes a reduction in the rate of involution of the gland through its effect of producing a local hyperemia rather than any effect through the central nervous system. By maintaining a large blood supply around the glands a condition more nearly comparable to that present during active lactation is maintained. By care in preventing the spread of turpentine from one row of glands to the other side, it is possible to show a great difference in the hyperemia of the two rows of glands. Experiments are now in progress to determine if a differential rate of involution can be obtained under such conditions.

Summary. The application of spirits of turpentine for 7 days to the nipples and adjoining skin of lactating mice weaned on the 4th day after parturition was shown to retard the rate of involution of the mammary lobule-alveolar systems. Similar applications of turpentine to castrate and normal females failed to stimulate the growth of alveoli. Pseudo-pregnancy was not stimulated in the

[3] Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 266, 1937.

normal female. It is suggested that the retardation in the involution process is due to the great subcutaneous hyperemia produced by the turpentine applications.

12020

Qualitative Progesterone Assay of Pregnant Cattle AP and Extracts Having Mammary Growth Activity.*†

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It was shown in this laboratory[1, 2] that the duct system of the mammary gland could be experimentally stimulated in castrate male or female animals by the injection of estrogen while a simultaneous injection of estrogen and progestin was necessary to secure complete mammary gland growth. Since this growth could not be secured in hypophysectomized animals, it was suggested that the ovarian hormones might produce their action by stimulating the secretion of mammogenic hormones in the anterior pituitary.[3] It has since been shown that lipid extracts[4] of the AP will stimulate the growth of the mammary duct system and further, that fresh pregnant cattle pituitaries will stimulate the growth of the lobule-alveolar system.[5]

Since Gardner and Hill[6] have shown that progesterone alone will stimulate the growth of the duct system, it seemed desirable to determine whether progesterone was present in the lipid AP extracts of the pituitary in which the mammogen duct factor is present as well as in the fresh pregnant cattle AP which stimulates the growth of the lobule-alveolar system.

In previous studies of this question Corner[7] and Callow and

* Aided in part by a grant from the International Cancer Research Foundation.
† Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 728.

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2 Turner, C. W., and Frank, A. H., *Science*, 1931, **73**, 295.

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6 Gardner, W. U., and Hill, R. T., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 718.

7 Corner, G. W., *Am. J. Physiol.*, 1930, **95**, 43.