Adenine and Adenosine Deaminase Activity of Rat Mammary Gland Homogenates Through Pregnancy and Lactation.* (22897)

W. F. WILLIAMS† AND C. W. TURNER
Department of Dairy Husbandry, University of Missouri, Columbia.

Various studies of mammary gland growth and milk secretion which have been carried out in this laboratory in the last few years have indicated the importance of nucleic acids, pentose nucleic acid and deoxypentose nucleic acid. Kirkham and Turner(1,2) have reported the changes in PNA and DNA content which occur in the rat mammary gland through pregnancy and lactation and in the gland stimulated to growth with estrogen and progesterone. These changes were described by Yamamoto and Turner(3) for the rabbit mammary gland stimulated to growth with various levels of estrogen and progesterone. These studies indicate an increase in total DNA as gland growth occurs and an increase in PNA content of the gland throughout pregnancy and lactation which probably corresponds roughly to the amount of synthesis occurring. Williams and Turner(4) have reported studies in the rabbit which implicate PNA in the action of lactogenic hormone on the mammary gland. Morgan *et al.*(5) reported the presence of adenine deaminase and xanthine oxidase in cow's milk, which would suggest the possible presence of these enzymes of purine metabolism in mammary tissue. Adenosine deaminase, another of the enzymes of purine metabolism, was reported present in many mammalian tissues by Conway and Cooke(6).

The presence of these enzymes in mammary gland tissue, which is suggested by the work cited, and their activity during gland growth and milk secretion might be indicative of various biochemical and physiological changes during growth and milk synthesis. The presence or absence of these enzymes would suggest the presence or absence of certain pathways of metabolism for adenine and its derivatives. Further, changes in level of enzyme activity if correlated with physiological processes being studied might indicate more completely the nature of these changes at the cellular level.

Methods and materials. Female albino rats weighing approximately 150 g were used at various stages of mammary gland development. These physiological states were nonpregnant, pregnant, lactating, and involuting animal. In all cases the animal was sacrificed, the mammary tissue rapidly dissected free, with as little connective tissue as possible, and chilled on ice. The mammary tissue was then homogenized for 3 minutes in Waring blendor, in the cold, with 9 volumes of isotonic NaCl. The homogenate was then strained through 4 layers of cheese cloth. The strained homogenate was assayed immediately for its deaminase activity toward adenine and adenosine. The *assay* of the rate of deamination of adenine and adenosine was carried out using manometric technic of Warburg as described by Zittle(7) for determination of liberated ammonia. The *reaction flask* contents for all determinations were as follows: 1.4 ml of 1.15 M $NaHCO_3$ gassed with CO_2, 1 ml of mammary gland homogenate, and 5 mg of substrate. The gas phase was 100% CO_2 and temperature 38°C. The final volume was 3.4 ml at pH 7. The Warburg flask contents were assayed for DNA content using the *p*-nitrophenyl hydrazine method of Webb and Levy(8) because of its high sensitivity.

Results. Under conditions of this study there was apparently no deamination of adenine by rat mammary gland homogenates. This was found to be the case at all stages of pregnancy, lactation and involution.

On the other hand, these mammary gland homogenates showed a marked ability to deaminate adenosine in all physiological states studied (Fig. 1).

* Contribution from the Dept. of Dairy Husbandry, Missouri Agri. Exp. Station, Journal Series No. 1676. Approved by the Director.

† Public Health Service Research Fellow of Natl. Cancer Institute.

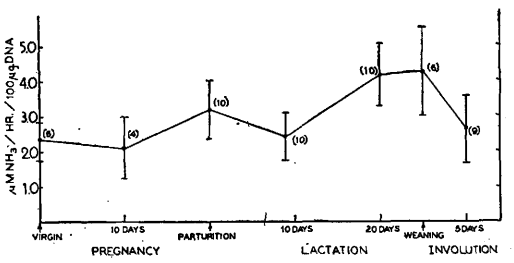

FIG. 1. Adenosine deaminase activity during pregnancy, lactation and involution in the rat mammary gland.

The changes which were noted in ability to deaminate adenosine under different physiological conditions studied are of interest. An apparent increase in adenosine deaminase activity was noted in the last third of pregnancy. However, this was not statistically significant. During early lactation deaminase activity was approximately the same as found in the gland of virgin and pregnant animals. During the last half of lactation there was a marked rise in adenosine deaminase activity from a mean of 2.41 μM/hour/100 μg DNA in early lactation to 4.17 μM/hour/100 μg DNA in late lactation. This increase was found to be highly significant ($P < .01$).

It appears, then, that the mammary gland of the rat contains no adenine deaminase and that adenosine deaminase is present and increases in the latter half of lactation.

Discussion. The lack of adenine deaminase activity in these rat mammary gland homogenates is in accord with previous studies of other tissues by Conway and Cooke(9), Richert and Westerfeld(10), and Block and Johnson(11). This would appear to indicate that the mammary gland does not catabolize adenine via deamination to hypoxanthine. Christman(12) and Brown(13) have reviewed the studies of nucleic acid precursors which indicate that in the rat adenine is utilized as a precursor of nucleic acid adenine and guanine. The apparent lack of adenine deamination may be related to its utilization in the pronounced synthesis of nucleic acids in the mammary gland which was demonstrated by the work of Kirkham and Turner(1,2) and Yamamoto and Turner(3) to occur during pregnancy and lactation.

From these studies it appears that adenosine is deaminated by mammary gland throughout pregnancy and lactation. This may indicate that adenosine as such is not utilized in the synthesis of the mammary gland nucleic acids. The presence of adenosine deaminase and the apparent lack of adenine deaminase would seem to indicate a pathway of catabolism for the adenine compounds which proceeds via deamination to inosine.

The rise in adenosine deaminase activity noted in the latter stages of lactation may be indicative of the onset of involution which would involve increased nucleic acid breakdown. This, however, may not be the answer as adenosine deaminase activity was not found to be increased in the involuting mammary gland.

Summary. Adenine deaminase activity and adenosine deaminase activity of rat mammary gland homogenates were studied through pregnancy, lactation and involution. Adenine deaminase activity was not apparent in the mammary gland. Adenosine deaminase activity was present throughout pregnancy and lactation, showing maximum activity in the latter stages of lactation.

1. Kirkham, W. R., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 123.

2. ———, *ibid.*, 1954, v87, 139.

3. Yamamoto, H., and Turner, C. W., *ibid.*, 1956, v92, 130.

4. Williams, W. F., and Turner, C. W., *ibid.*, 1955, v90, 531.

5. Morgan, E. J., Stewart, C. P., and Hopkins, F. G., *Proc. Roy. Soc. London,* 1922, vB94, 109.

6. Conway, E. J., and Cooke, R., *Biochem. J.,* 1939, v33, 479.

7. Zittle, C. A., *J. Biol. Chem.,* 1946, v166, 499.

8. Webb, J. M., and Levy, H. B., *ibid.,* 1955, v213, 107.

9. Conway, E. J., and Cooke, R., *Biochem. J.,* 1939, v33, 457.

10. Richert, D. A., and Westerfeld, W. W., *J. Biol. Chem.,* 1950, v184, 203.

11. Block, W. D., and Johnson, D. V., *ibid.,* 1955, v217, 43.

12. Christman, A. A., *Physiol. Revs.,* 1952, v32, 303.

13. Brown, G. B., *Ann. Rev. Biochem.,* 1953, v22, 141.

Received November 9, 1956. P.S.E.B.M., 1957, v94.