with organisms studied by Kutscher(5) in rats and mice, by Bongert(6) in these and other animals, and Reed(7) in mice. A corynebacterium has been recently encountered by Le Maistre(8) in cortisone treated rats whose lesions bear a resemblance to those of our animals.

The situation described in this report offers an unusually favorable opportunity for the study of mechanisms of natural resistance. Through control of diet, resistance and susceptibility can be produced at will in the same species, even within the same litter. The organism is easily recognizable on smears and is readily cultured. Its virulence may be verified in the naturally susceptible mouse. The disease itself may be studied as it occurs spontaneously, or as an experimentally induced infection.

*Summary.* A corynebacterium has been isolated from spontaneous "pseudotuberculous" lesions of pantothenate-deficient rats. In inoculation experiments the organism is not pathogenic for rats on a fully supplemented diet in doses which produce progressive disease in deficient animals. This break in resistance can be demonstrated by inoculation of the organism sometimes as early as 16 days after the rats have been placed on the deficient diet, before overt deterioration in physical condition has developed.

1. Zucker, T. F., and Zucker, L. M., PROC. SOC. EXP. BIOL. AND MED., 1954, v85,

2. Jensen, H. L., *Ann. Rev. Microbiol.*, 1952, v6, 77.

3. Merchant, I. H., *J. Bact.*, 1935, v30, 95.

4. Brooks, R. F., and Hucker, G. J., *ibid.*, 1944, v48, 295.

5. Kutscher, *Z. f. Hyg. u. Infektionskr.*, 1894, v18, 327.

6. Bongert, *ibid.*, 1901, v37, 449.

7. Reed, D. M., *Johns Hopkins Hosp. Rep.*, 1902, v9, 525.

8. LeMaistre, C., and Thompsett, R., *J. Exp. Med.*, 1952, v95, 393.

Received March 10, 1954. P.S.E.B.M., 1954, v85.

## Intracellular Localization of $I^{131}$-Tagged Lactogenic Hormone in Mammary Gland of the Rabbit.* (20939)

W. F. WILLIAMS AND C. W. TURNER.
*From the Department of Dairy Husbandry, University of Missouri, Columbia, Mo.*

The lactogenic hormone is believed to function. primarily, in the epithelial cell of the mammary gland in initiating and maintaining the synthetic activity of these cells. Its activity in this connection would involve the activation and maintenance of the enzyme systems involved in the synthesis of the various constituents of milk. The question arises as to the role of the various cell components in milk secretion(1). If it could be shown that the lactogenic hormone becomes associated in greater concentration with certain cell particulates, it would be presumptive evidence of the synthetic activity of such particulates and of the site of hormone activity in the cell.

Cox(2) and Sonenberg *et al.*(3) demonstrated that the lactogenic hormone tagged with $I^{131}$ lost little biological activity and following intravenous injection could be found localized in various tissues. Stadie, Haugaard, and Vaughan(4) demonstrated the binding of $I^{131}$ tagged insulin in diaphragm muscle. Crampton and Haurowitz(5) used tagged antigens in their study of antibody formation and used the procedure of centrifugal fractionation of tissue homogenates to determine the point of intracellular localization of the antigens. These methods have been applied to a study of the intracellular localization of lactogenic hormone in the mammary gland.

*Methods and materials.* Essentially, the method of Stadie, Haugaard, and Vaughn(4) was followed for the preparation of lactogenic-

* Contribution from the Department of Dairy Husbandry, Mo. Agric. Exp. Station, Journal Series No. 1417. Approved by the Director.

This investigation was supported in part by research grant from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service.

TABLE I. Intracellular Localization of $I^{131}$-Tagged Lactogenic Hormone.

| Cell fraction | Mean total activity, % | Range of activity, % |
|---|---|---|
| Nuclei | 5.2 | 3.0- 6.8 |
| Mitochondria | 51.9 | 41.0-65.0 |
| Microsomes | 30.9 | 23.0-45.0 |
| Total particulates | 82.8 | 72.8-89.2 |
| Supernatant | 11.1 | 5.1-21.6 |

$I^{131}$. The lactogenic hormone preparation used assayed 20-30 I.U./mg, and contained less than 1% each of ACTH, growth hormone, FSH, and thyrotropin. Fifteen-day pseudo-pregnant rabbits were injected intraductally with approximately 5 I.U. of the tagged lactogenic preparation according to the procedure used by Lyons(6). The animals were sacrificed 45 to 95 minutes post-injection, the mammary glands were removed and all subsequent fractionations carried out in the cold. Mammary gland cell fractions were obtained essentially by the method of Schneider and Hogeboom(7), modified for the extraction of nuclei by the method of Wilbur and Anderson(8). Previous to cellular fractionation, the mammary tissue was ground, washed 5 times with isotonic sucrose, and then homogenized in isotonic sucrose. All samples were digested with KOH at 100°C, taken to faint alkalinity (phenolphthalin), dried on planchets, counted in a scintillation counter and corrected for sample self-absorption. The data reported includes the results of 6 experiments in which the localization of the $I^{131}$ tagged lactogenic hormone was determined for the several components of the cells of the mammary gland.

*Results.* It will be seen that the $I^{131}$-tagged lactogenic hormone was observed to become associated to a great extent in the particulate fractions of the mammary gland cells (Table I). The mitochondria contain over half the radioactivity and the microsomes almost one-third of the remainder. There was little activity in the nuclei and only nominal amounts in the supernatant fraction of the cells.

*Discussion.* The pseudo-pregnant rabbit mammary gland is very sensitive to the action of the lactogenic hormone. It was shown by Lyons(6) that the action of the lactogenic hormone could be localized in individual areas of a single gland by the injection of the hormone directly into the ducts at the end of the teat. Lactation was induced only in the area of a single gland where the hormone was injected. These experiments prove that intraductally injected lactogenic hormone is locally absorbed by the mammary gland cells. It was shown in the present work that within a period of 1½ hours, the hormone was largely localized in association with the particulate fractions of the cells. These data suggest that the site of activity of the hormone is in association with the mitochondria and microsomes of the cells and further, that the particulates of the mammary gland cells play important roles in the synthetic activity associated with milk secretion.

*Summary.* The lactogenic-$I^{131}$-tagged hormone was injected intraductally into the mammary glands of rabbits at the end of pseudo-pregnancy. After 45 to 95 minutes, the glands were removed and the components of the cells were separated. It was shown that the radioactive lactogenic hormone was localized, to a great extent, in the particulate fractions of the mammary cells. The nuclei and supernatant portions of the cell contained little hormone. These data suggest that the site of activity of the hormone is primarily in association with the mitochondria and microsomes of the cells and, therefore, that the particulates of the mammary gland cells play important roles in the synthetic activity of the cells.

1. Turner, C. W., *The Mammary Gland,* Chapter 11, Pub. Lucas Bros., Columbia, Missouri. 1952.

2. Cox, *Anat. Rec.,* 1951, v109, 285.

3. Sonenberg, *et al., Endo.,* 1951, v49, 709.

4. Stadie, Haugaard, and Vaughan, *J. Biol. Chem.,* 1952, v199, 729.

5. Crampton and Haurowitz, *Science,* 1950, v112, 300.

6. Lyons, PROC. SOC. EXP. BIOL. AND MED., 1942, v51, 308.

7. Schneider, and Hogeboom, *J. Biol. Chem.,* 1950, v183, 123.

8. Wilbur and Anderson, *Exp. Cell. Res.,* 1951, v2, 47.

Received March 10, 1954. P.S.E.B.M., 1954, v85.