development of reticulum and collagen in treated as well as in control animals, at least as judged by histologic technics. There is some evidence that fibroblastic proliferation is inhibited by corticosteroids(12,13), and a failure of development of the fibroblast might be responsible for the lack of organization and continued inflammatory response noted in the treated nodules.

Regardless of the mechanism of action of hydrocortisone, in view of the rapid increase in nodule size on cessation of therapy, it would appear that the mouse is incapable of effectively "detoxifying" silica particles while on steroids. This failure to inactivate silica may account for the continued cellular exudate noted in treated lesions. Whether this continued toxicity is directly related to inhibition of fibrosis or is due to another effect of hydrocortisone cannot be determined from these experiments.

Summary. The effect of treatment with hydrocortisone upon growth and maturation of the silicotic nodule has been studied in mouse liver. Treatment slowed growth of lesions and development of collagen and reticulin regardless of the time at which therapy was instituted. When hydrocortisone was discontinued, however, the size of nodules returned close to control level. Histological differences were noted between control and treated nodules after 150 days. These differences (disorganization and cellularity of treated nodules) suggest a continuation of the early inflammatory response in hydrocortisone treated lesions.

1. Curran, R. C., *Brit. J. Exp. Path.,* 1952, v33, 82.

2. Magarey, F. R., Gough, J., *ibid.,* 1952, v33, 76.

3. ———, *ibid.,* 1952, v33, 510.

4. Harrison, C. V., King., E. J., Dale, J. C., Sichel, R., *Brit. J. Ind. Med.,* 1952, v9, 165.

5. King, E. J., Harrison, C. V., Attygalle, D., *ibid.,* 1955, v12, 228.

6. Stacy, B. D., King, E. J., *ibid.,* 1954, v11, 192.

7. Marenghi, B., Rota, L., *A.M.A. Arch. Ind. Hyg.,* 1954, v9, 315.

8. Schiller, E., *Brit. J. Ind. Med.,* 1953, v10, 1.

9. Vyskocil, J., *ibid.,* 1957, v14, 30.

10. Zaidi, S. H., King, E. J., Harrison, C. V., Nagelschmidt, G., *A.M.A. Arch. Ind. Health,* 1956, v13, 122.

11. Cummings, D. E., *J. Indust. Hyg.,* 1929, v11, 245.

12. Ragan, C., Howes, E. L., Plotz, C. M., Meyer, K., Blunt, J. W., Lattes, R., *N. Y. Acad. Med.,* 1950, v26, 251.

13. Scheinberg, S. R., Saltzstein, H. C., *A.M.A. Arch. Surg.,* 1951, v63, 413.

Received September 28, 1962. P.S.E.B.M., 1963, v112.

Mammary Gland Enzyme Systems Concerned with Synthesis of Monoiodotyrosine.* (27968)

E. P. REINEKE

Department of Physiology and Pharmacology, Michigan State University, East Lansing

Research on the secretion of administered I^{131} into milk has been reviewed by Brown-Grant(1). It is now well established that mammary tissue of man and the common laboratory and domestic animals has an active iodide concentrating mechanism capable of maintaining an elevated milk:plasma I^{131} ratio. Results in goats(2,3) and in rats(4,5) suggest that under conditions of limited iodine intake mammary tissue may actively compete with the thyroid for available iodine, resulting in reduced thyroxine formation. Iodine secreted into milk *in vivo* occurs in part as iodide and in part as monoiodotyrosine in peptide linkage in the milk protein(6).

Recently we reported(7) that lactating rat mammary tissue has the ability to concentrate I^{131} *in vitro* and defined some of the conditions influencing this process, but did not identify the iodinated compounds formed. It was of interest, therefore, to determine the

* Published with approval of the Director, Michigan Agri. Exp. Station as Journal Series No. 3063.

chemical nature of the iodine concentrated and to characterize further the enzyme systems involved.

Materials and methods. Whole mammary tissue. Whole mammary tissue from lactating and post-lactating rats (CFN strain) was prepared as described earlier(7) in 2 ml tissue culture (TC) medium 199(8) or Ringer-Phosphate (R-P) buffer at pH 7.4 containing an appropriate amount of carrier-free I^{131}†. Samples were incubated 6 hours with constant shaking at 38°C. After rinsing in distilled water and blotting on filter paper the tissues were weighed to 0.1 mg. Radioactivity of tissue (T) and an aliquot of medium (M) was measured, and T/M ratios were computed by dividing counts per minute per 100 mg tissue by counts per minute per 0.1 ml medium. The tissues were then placed in 0.5 ml M/15 phosphate buffer at pH 8.0. After addition of 10 mg pancreatin and 0.2 mg thiouracil they were digested 24 hours in a constant temperature water bath with constant shaking at 38°C. Paper strip chromatograms were run by the ascending method on 20 μl aliquots of digest. Developing reagent was 125 parts collidine and 44 parts H_2O in an NH_3-saturated chamber. Radioactive spots were outlined from radioautographs prepared with No Screen X-ray film. Individual spots were cut out and radioactivity measured in a scintillation well counter connected to a γ-ray spectrometer and scaler. The remainder of each strip was pooled and counted separately to determine "diffuse" activity. Preliminary tests established only 2 clearly defined radioactive components in the mammary tissue digests, their R.F. values corresponding to those of iodide and monoiodotyrosine run at the same time. Therefore, reference strips containing radioactive iodide and 3-iodo-L-tyrosine (MIT) were prepared for each experiment. The non-radioactive MIT was located on the reference strips with Pauly's reagent. Finally, the radioactivity of each component was computed as percent total radioactivity on the strip.

† Purchased from Oak Ridge National Laboratories, Oak Ridge, Tenn.

Tissue homogenates. Soluble system. The procedure was similar to that used by Cunningham and Kirkwood for salivary tissue (9). 0.2 g tissue was ground with 4 ml cold distilled H_2O in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 17,000 $\times$ *g* for 30 minutes at 0°C and the supernate was used in the subsequent experiments. Each 15 ml centrifuge tube was supplied with 1.0 ml tissue extract, 1 ml M/10 phosphate buffer at pH 7.4 and 0.1 ml carrier-free radioiodide containing 50 μc of activity and, unless otherwise noted, 0.2 ml 0.04 M Na-L-tyrosine. Metal ions were supplied where indicated by adding 0.1 ml of 0.04 molar $CuCl_2$ or $MnCl_2$. H_2O was added to bring the volume to 2.9 ml, and the samples were incubated aerobically with shaking at 38°C for 2 hours. At the end of this step, 0.1 ml of solution containing 0.2 mg of thiouracil was added and the samples refrigerated overnight; 20 μl of each reaction mixture were chromatogrammed directly as already described.

Mitochondrial system. Mitochondria were prepared by the procedure of Taurog *et al.* (10); 2 g tissue was ground with 18 ml ice-cold Krebs-Ringer-bicarbonate buffer, centrifuged at 600 $\times$ *g* for 10 minutes at 0° to remove cell debris and nuclei, and then at 25,000 $\times$ g at 0° for 30 minutes to bring down the mitochondria. It should be noted that this fraction contains some microsomes. The precipitate was resuspended in 0.75 ml of the above buffer and incubated with carrier-free radioiodide for 1 hour at 38°. The mixture was digested with pancreatin, chromatogrammed, and radioactivity measured as described for whole tissue.

Results and discussion. Lactating mammary tissue concentrated I^{131} when incubated in either TC medium or R-P buffer (Table I). T/M ratios were enhanced by cold storage of tissue, reduced to about unity by boiling or addition of thiouracil or thiocyanate, and decreased progressively during mammary involution.

In fresh lactating mammary tissue (Exp. I, II, III) 44.2 to 52.4% of the I^{131} taken up was found as MIT. The variable quantity of diffuse activity is probably due to

TABLE I. I^{131} Distribution in Pancreatin Digests of Rat Mammary Tissue Incubated with I^{131} Under Various Conditions.

Exp No.	T/M ratio	MIT (%)	Iodide (%)	diffuse (%)	Remarks
I	2.8	52.4	37.3	10.3	Fresh tissue, 17 days lactation.
Ia	6.8	55.9	17.5	26.6	Stored 6 days at 7°C.
Ib	8.2	48.4	26.7	24.8	*Idem.*
II	2.8	44.2	49.9	5.6	Fresh tissue, 15 days lactation.
II	.8	0	100.0	0	Tissue boiled 5 min.
II	1.1	0	100.0	0	Thiouracil, .04 mg/ml.
II	.9	1.9	98.1	0	KSCN, .1 mg/ml.
III	4.0	51.4	35.9	12.7	Fresh tissue, 21 days lactation.
III	2.2	17.1	69.9	12.9	10 days involution.
III	1.7	8.3	79.8	11.9	2 mo. "

Samples in Exp I and Ia were incubated in TC 199 Medium. The remainder were incubated in Ringer-Phosphate buffer. pH of both media was 7.4. Each sample was chromatogrammed in duplicate and results were averaged. Like Roman numerals indicate tissues from the same rat.

persistence of MIT peptides resulting from incomplete hydrolysis with pancreatin. Clearly defined spots for organic compounds other than MIT were not found. MIT formation was blocked by boiling or adding thiouracil, and reduced by thiocyanate.

Both T/M ratio and percent MIT formation decreased progressively during mammary involution (Exp. III), indicating that the enzyme systems for both iodide concentration and MIT formation are correlated with the lactation process.

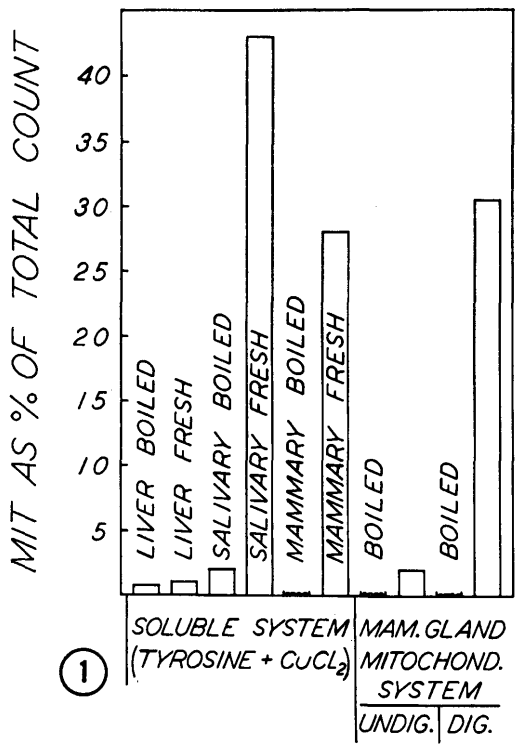
The 3-fold increase in I^{131} uptake of mammary tissue after 6 days cold storage was paralleled by MIT formation to about the same percent as in fresh tissue. Increase in activity under these conditions can perhaps be accounted for by (1) greater availability of iodine oxidizing enzyme accompanying declining respiration of tissues or (2) loss of an inhibitor during storage. Further research is needed to define the mechanisms involved.

Results with subcellular fractions (Fig. 1) demonstrate a soluble heat labile tyrosine iodinase in lactating mammary gland, but not in liver, comparable to that in salivary tissue. Confirming an earlier report(10), mammary gland mitochondria formed considerable MIT without addition of tyrosine or Cu^{++}. This remained at the origin in chromatograms of whole material, but was identified as MIT after pancreatin digestion, suggesting MIT was in peptide combination.

Soluble system from mammary tissue (Fig. 2) showed no MIT formation without added tyrosine. Formation of small amounts of MIT with either no extract or boiled extract was probably due to aerobic oxidation of part of the radioiodide. Although control extract showed appreciable activity, this was greatly enhanced by Cu^{++} and increased further by Cu^{++} plus Mn^{++}. Mn^{++} alone did not increase MIT formation appreciably above that in control extract.

Our results show that the soluble mammary system has substrate and metallic ion requirements, insofar as they have been tested, that are similar to those of the soluble systems in thyroid(11) and salivary glands(9,12). Considerably more evidence will be needed, however, to determine whether these systems are identical.

Summary. Lactating mammary tissue was incubated with I^{131}-iodide at 38°C in TC 199 Medium or Ringer-Phosphate buffer at pH 7.4 and digested overnight with pancreatin at pH 8.0. Iodinated compounds were separated by paper chromatography, radioautographed, and the radioactive spots counted separately. In fresh tissue 44 to 52% of I^{131} taken up was found in monoiodotyrosine. Mammary tissue stored at 7°C for 6 days prior to iodination showed a 3-fold increase in I^{131} accumulation, but proportion of MIT formed was similar to that in fresh tissue. Both I^{131} uptake and percentage MIT formation decreased progressively during mammary involution, indicating that mammary iodine metabolism is related to lactation. MIT formation was blocked by boil-



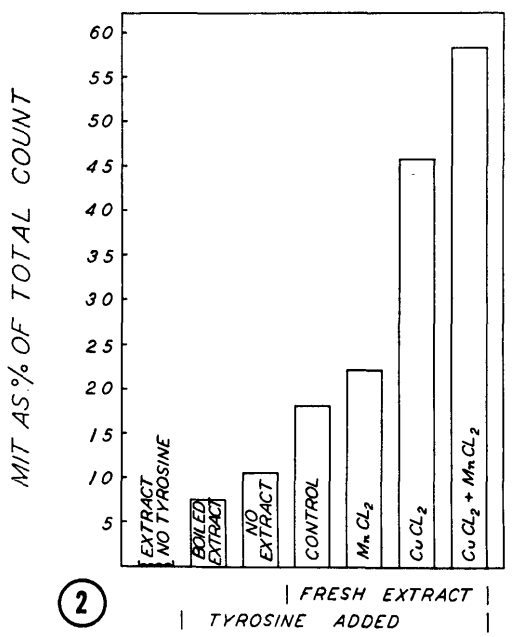


FIG. 1. MIT formation by subcellular fractions of lactating mammary and submaxillary salivary tissues. Tyrosine and Cu^{++} were added to soluble system but not to mitochondrial system.

FIG. 2. Influence of tyrosine substrate and metallic ions on MIT formation by soluble system from lactating mammary gland.

ing, by thiouracil (0.04 mg/ml) and reduced by KSCN (0.1 mg/ml). Tissue homogenates were separated into soluble and mitochondrial-microsomal fractions by centrifugation. Soluble fraction from parotid and lactating mammary glands, but not from liver, formed MIT from added tyrosine. MIT formation was catalyzed by Cu^{++} and increased further by $Cu^{++} + Mn^{++}$. Mitochondrial fraction from lactating mammary tissue formed MIT without addition of tyrosine substrate or metal catalyst. All except traces of MIT in this system was in combined form until released by hydrolysis with pancreatin.

The author wishes to acknowledge the technical assistance of Mr. H. W. Kaczkofsky.

1. Brown-Grant, K., *Physiol. Rev.*, 1961, v41, 189.

2. Flamboe, E. E., Reineke, E. P., *J. Animal Sci.*, 1959, v18, 1135.

3. Reineke, E. P., *J. Dairy Sci.*, 1961, v44, 1.

4. Grosvenor, C. E., *Am. J. Physiol.*, 1960, v199, 419.

5. ———, *Program of Endocrine Soc.*, 44th Meeting, June 1962, p58.

6. Potter, G. D., Chaikoff, I. L., *Biochem. Biophys. Acta*, 1956, v21, 400.

7. Maqsood, M., Reineke, E. P., *Am. J. Physiol.*, 1960, v199, 829.

8. Morgan, J. F., Morton, H. J., Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.

9. Cunningham, B. A., Kirkwood, S., *J. Biol. Chem.*, 1961, v236, 485.

10. Taurog, A., Potter, G. D., Tong, W., Chaikoff, I. L., *Endocrinol.*, 1956, v58, 132.

11. Weiss, B., *J. Biol. Chem.*, 1953, v201, 31.

12. Serif, G. J., Kirkwood, S., in *Trace Elements*, Acad. Press, Inc., New York, 1958, p257.

Received October 1, 1962. P.S.E.B.M., 1963, v112.