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DISCLAIMER

DEMONSTRATION OF THE CONCENTRATION OF ASTATINE-211 IN THE MAMMARY TISSUE OF THE RAT[1]

C. Willet Asling, Patricia W. Durbin, Muriel E. Johnston, and Marshall W. Parrott

University of California Radiation Laboratory, and the Departments of Anatomy and Medical Physics, Berkeley, California

INTRODUCTION

When 55-day old female Sprague-Dawley rats were given 0.5 μc/gm. body weight of At^{211}[2] (1), 75% of the At-injected rats developed mammary tumors primarily of duct origin during the ensuing year. Only 17% of non-injected control rats developed mammary tumors during this same interval (2-3). In the elucidation of the cause of this augmented tumor incidence, it was of paramount importance to investigate the possible role of direct alpha-particle irradiation of the immature mammary tissue. There were indications from earlier work that At^{211} might concentrate in mammary tissue (4). The metabolism of At^{211} resembles that of I^{131} in many respects, and gross assays had shown that both halogens tended to concentrate in the skin and subcutaneous tissues (4). Further, Rugh (5) and Brown-Grant (6) have demonstrated the capacity of the mammary gland to concentrate I^{131} and to secrete it in milk.

Anatomically, the breast tissue of the virgin rat consists of a simple duct system terminating in tiny alveolar buds, embedded in, and surrounded by a copious amount of connective tissue and fat (7). Gross dissection of virginal mammary parenchyma is virtually impossible; thus radioactive assay was inconclusive for assessing the concentration of At^{211} in mammary tissue. The At^{211} found in the mammary region might merely be associated with blood vessels, lymphatic tissue, or fat. Autoradiographs, however, provide a means by which the presence of At^{211} in the mammary parenchyma can be demonstrated visually. Because of the possibility of movement of At^{211} from its site of

deposition in the living tissue during the processes of fixation and dehydration, it was also necessary to study the distribution of At^{211} in the lactating mammary gland and to determine whether At^{211}, like I^{131}, is secreted in the milk.

METHODS

At^{211} was prepared by the method of Parrott et al. (8). Three 43-day old virgin female Sprague-Dawley rats and two lactating Long-Evans rats each received 200 μc. of At^{211} intraperitoneally. After injection, the lactating rats were returned to their litters. Four hours later all the At-injected rats and the nurslings of the injected mothers were sacrificed with chloroform. This experiment was repeated with identical groups of virgin and lactating rats, except that the animals were sacrificed three hours after the At^{211} injection. Samples of mammary tissue were dissected from both inguinal regions of each of the injected rats, and were fixed in Bouin's fluid. One portion was dehydrated with dioxane, embedded in paraffin, sectioned at 6μ, and mounted on Eastman's 10μ NTA stripping film for autoradiographs. The remaining portions of the mammary tissue were weighed and assayed for At^{211} x-ray activity with a NaI-TII crystal scintillation counter.

The gastrointestinal tracts and thyroids of 4 nurslings from the litters in Experiment 1 were dissected and assayed, and when present, bladder urine was also assayed. To exclude the possibility that any radioactivity present was due to external contamination, the remaining nurslings were skinned, and carcasses were assayed separately. In the second experiment all the nurslings were skinned and the gastrointestinal tracts dissected. These samples, and the residual carcasses were assayed separately.

RESULTS

The measurements of At^{211} radioactivity in mammary tissue of virgin and lactating rats are shown in Table 1. The 1-hour difference in post-injection interval did not affect the At^{211} concentration, percent of injected dose per gram of wet tissue (%/gm.), in the virginal tissue. The mean for all animals was 0.38%/gm. On the other hand, the At^{211} concentration in the lactating mammary tissue was appreciably higher at the earlier interval. This difference is reflected in the amount of At^{211} transferred to the nurslings at the 2 intervals (See Table 2). The total passed to the litters was twice as much 4 hours after injection as after 3 hours. At the 3-hour interval the gastrointestinal tracts contained 35% of the At^{211} found in the young. One hour later, while the total At^{211} in the nurslings had doubled, the gastrointestinal concentration was reduced to 25%, indicating rapid absorption of At^{211} from the infant gastrointestinal tract. The relatively low skin concentrations indicated that contamination from such sources as the mother's urine was negligible, and that nearly all of the At^{211} found in the young had been transferred in the milk.

The distribution of At^{211} in the mammary glands was studied autoradiographically. In the glands from the virginal rats the tissue was preponderantly adipose. Scattered about within this stroma were small ducts and alveolar buds of epithelial structure. Small aggregates of lymphoid tissue were sometimes seen. The autoradiographs showed that the alpha-particle tracks most commonly arose from the epithelium, whether ductal (Fig. 1) or alveolar (Fig. 2). Tracks were very rare in the lumens of these structures. The adipose tissue also showed some At^{211}, but tracks were only about one-fifth as frequent. They arose from within the fat-containing space of the adipose cells. Tracks were also seen arising directly from the fibroblasts of the stroma. Blood vessels were scanty in this non-

secreting gland, but tracks could sometimes be seen arising in the lumen of small vessels. The lymphatic tissue showed no tracks.

In a typical autoradiograph from a lactating rat (Fig. 3) the tissue was predominantly glandular; the stroma contained an abundant vasculature and scanty adipose tissue. The acini of the glands were in varying stages of secretion, some being composed of heightened epithelium and a relatively small lumen, while in others the cells were lower, and the acini were dilated with secretion. In some acini tall cells were seen whose distal portion (i.e., toward the lumen) was distintegrating in the typical fashion of apocrine secretion. Lymphatic tissue could also be seen in these glands.

The autoradiographs showed tracks originating in the acinar epithelium. In the cells showing disintegration of the distal portion, tracks were often found originating in this part of the cell. Correspondingly, some tracks were seen in the lumen when it contained secretion. Tracks could also be seen arising from cells which were not yet actually releasing secretion. The blood vessels often showed a few tracks originating in the lumen and occasionally from the tissue of the walls. Tracks were rarely found in fat cells. The lack of tracks arising from lymphatic tissue of either group provided evidence that no appreciable translocation of the At^{211} had occurred during the technical manipulations.

DISCUSSION

The localization of At^{211} in lactating glandular tissue and its transfer to nursing young via the milk parallels demonstrations of radioiodine in milk. It is, of course, well established that casein may be iodinated *in vitro*, and it is possible that At^{211} is bound in comparable fashion. However, the possibility that it is associated with the fat of milk should not be neglected.

The epithelial localization in the non-lactating, virginal gland is of substantial interest in view of the frequency of occurrence of neoplasms of duct origin when such animals receive At^{211}. Of no less interest is the occasional uptake by fibroblasts in such animals, for fibromata and sarcomata are not infrequently seen, and tumors showing both epithelial and connective tissue hyperplasia are very common.

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FOOTNOTES

[1] This work was performed under the auspices of the U. S. Atomic Energy Commission.

[2] At^{211} is an isotope of the heaviest halogen with a half-life of 7.5 hours. It decays by emission of alpha particles with a mean energy of 6.3 Mev and by electron capture (1). In the latter process 80 Kev x-rays are emitted allowing the detection of this isotope with photosensitive scintillation equipment.

Table 1. DEPOSITION OF At^{211} IN TISSUE FROM THE MAMMARY LINE OF IMMATURE VIRGIN FEMALE RATS AND LACTATING RATS 3 TO 4 HOURS AFTER INTRAPERITONEAL INJECTION

Animal	Postinjection Interval	Sample Wt., Gm.	% Injected At^{211}	At^{211} Concentration %/gm.
Virgin rats				
a	3 hours	.25	.08	.31
b	" "	.25	.14	.57
c	" "	.68	.27	.40
d	4 hours	.35	.13	.36
e	" "	.33	.10	.29
f	" "	.49	.17	.34
Mean				.38
Lactating rats				
a	3 hours	1.69	1.47	.87
b	" "	1.34	1.01	.75
c	4 hours	.95	.48	.50
d	" "	.82	.32	.40

Table 2. MATERNAL TRANSFER OF At^{211} BY WAY OF THE MILK

Litter	Experiment 2 (3 hours) No. of Specimens	Experiment 2 (3 hours) % of Mother's At^{211} dose	Experiment 1 (4 hours) No. of Specimens	Experiment 1 (4 hours) % of Mother's At^{211} dose
a./			c./	
Whole nurslings	6	1.21	2	2.63
Skin only	6	.12	2	.32
GI Tract	6	.78	4	.90
Thyroid			4	<.01
Bladder urine			4	.02
Total passed to litter	(6)	7.3		15.8
b./			d./	
Whole nurslings	6	.58	8	.84
Skin only	6	.07	8	.11
GI Tract	6	.33	4	.35
Thyroid			4	<.01
Bladder urine			4	<.01
Total passed to litter	(6)	3.5	(12)	10.1

ABSTRACT

Concentration of At^{211} in the mammary tissue of virgin and lactating female rats has been demonstrated both by gross radioactive assay and by autoradiography. Secretion of At^{211} in the milk was shown by its presence in the gastrointestinal tracts and carcasses of nurslings and by the presence of alpha-particle tracks in glandular lumens in autoradiographs. In virgin mammary tissue At^{211} was chiefly associated with the parenchyma, although small amounts could also be found in the stroma.

FIGURE LEGENDS

Fig. 1 Stripping film autoradiograph (NTA) of At^{211} localization in mammary tissue of 55-day old virginal rat. H and E counterstain; magnification 300 X. Concentration in ducts and periductal tissue; sparse tracks in adipose connective tissue.

Fig. 2. Association of At^{211} with acinar tissue of mammary gland of 55-day old virginal rat. Technical data as in Figure 1.

Fig. 3. At^{211} localization in mammary tissue of lactating rat. Note tracks in stroma, epithelium and lumens. Technical data as in Figure 1.

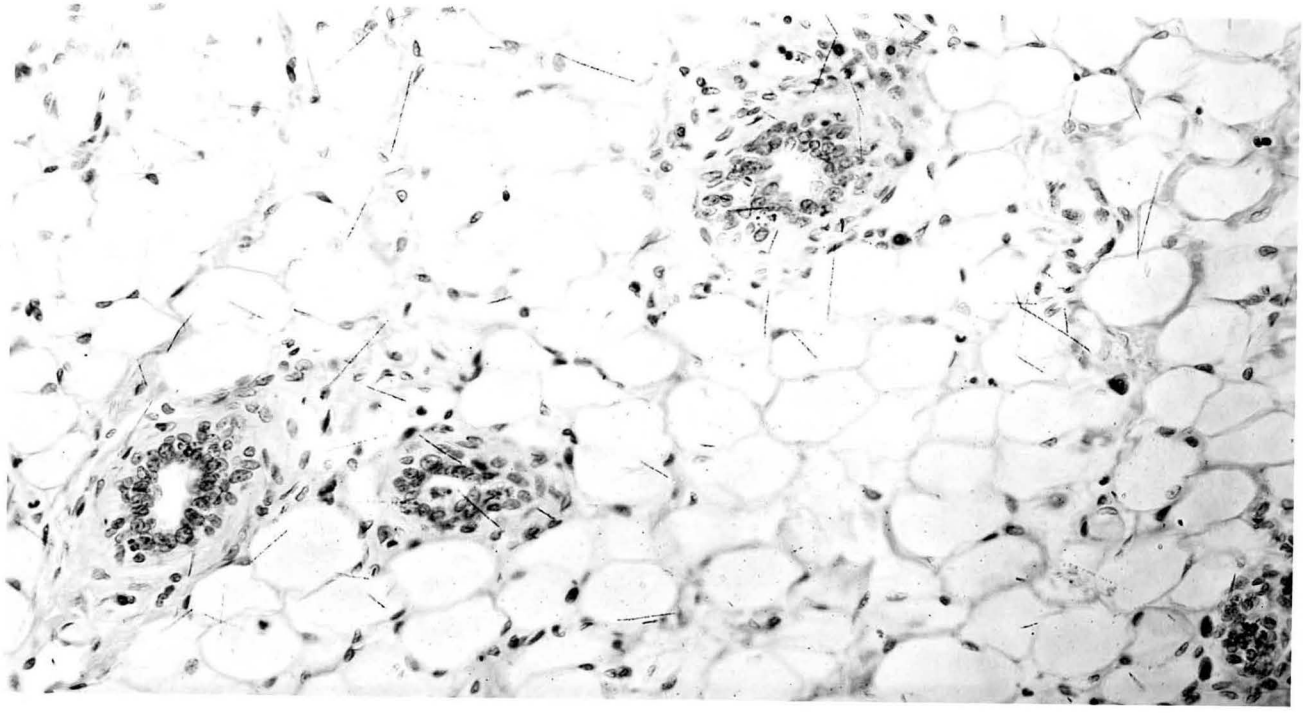

Fig. 1

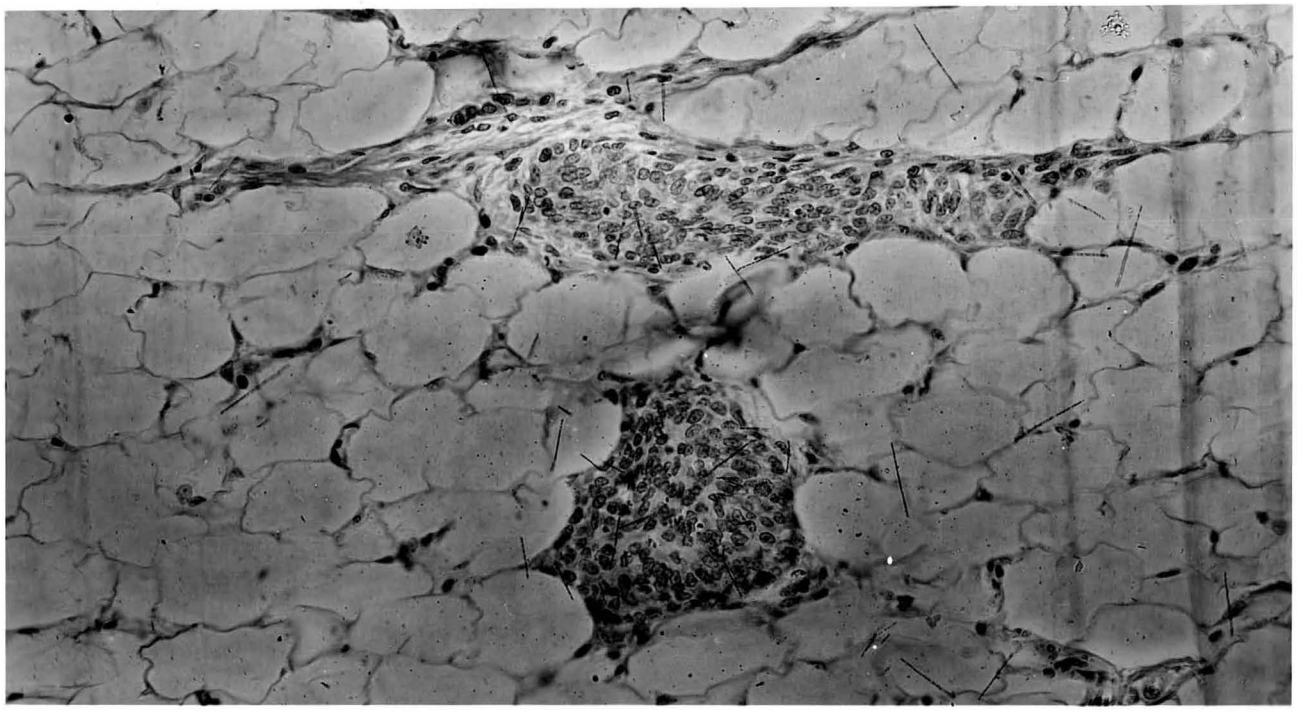

Fig. 2

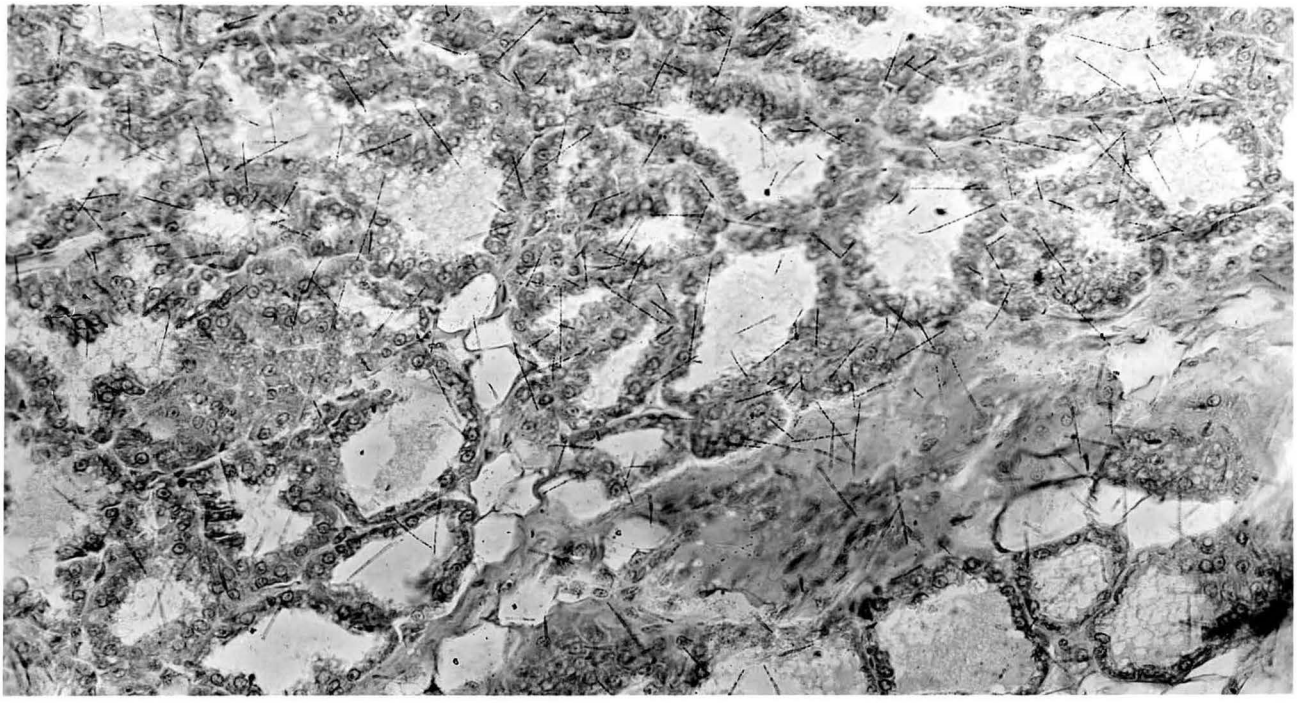

Fig. 3