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THE INDUCTION OF TUMORS IN THE RAT BY ASTATINE-211[1]

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INTRODUCTION

During the course of experiments on the longevity of female rats that had been given sublethal amounts of At^{211},[3] Hamilton et al. (1) found a striking incidence of mammary tumors. Shellabarger et al. (3) and Binhammer et al. (4) subsequently demonstrated increased incidence of mammary tumors in female rats of several strains after either sublethal doses of whole-body x-ray or lethal x-ray doses in animals protected from acute irradiation death by parabiosis. Cronkite et al. (5) have recently shown for dosages from 0 to 500 r that mammary tumor induction is proportional to the x-ray dose, and that immature irradiated rats develop more mammary tumors than rats irradiated at maturity. Their data further suggest that ovarian function is another important factor in mammary tumor production in irradiated rats. This report augments previous observations on mammary tumor incidence and, in addition, describes other neoplasms encountered in female rats injected with At^{211}.

The thyroid gland accumulates At^{211} in amounts sufficient to produce marked radiation damage, and the ability of the gland to recover is roughly proportional to the dose administered (1, 6-9). The complex metabolic and endocrine changes of hypothyroidism that follow thyroid damage by At^{211} vary in severity with the extent of the damage (6, 9-11). The general bodily distribution of At^{211} is such that tissues other than the thyroid gland suffer radiation injury in varying degrees (6,7,12).

It was therefore of interest to re-examine Hamilton's original observation, and to assess the possible relationships of irradiation and endocrine function to mammary tumor production.

These experiments were designed to compare (with normal rats as a base) the number and types of tumors that develop in virgin female rats

(a) partially "thyroidectomized" with 0.5 μC of At^{211} per gram of body weight (9,13), (b) similarly injected, but partially protected from excessive irradiation of the thyroid gland by blocking with thyroxine (14), and (c) maintained on exogenous thyroxine after the At^{211} injection, at a thyroxine level previously described as sufficient to maintain normal pituitary structure in partially thyroidectomized rats (15).

METHODS

The animals were female Sprague-Dawley rats obtained from the original colony. When the animals were 40 days old they were ear-marked, weighed, and distributed at random into groups. The At^{211} solutions were prepared by methods described previously (16,17). At 55 days of age three groups of rats, 120 in all, were given At^{211} intravenously at a level of 0.5 μC per gram of body weight. The three experimental groups were as follows: At^{211}-injected, untreated (At-U)--60 rats that received only the injection of At^{211}; At^{211}-injected, thyroxine-pretreated (At-TP)--40 rats that were given eight daily subcutaneous injections of 230 μg per kg. of l-thyroxine prior to the administration of At^{211} to reduce the thyroidal uptake of the At^{211} (14); and At^{211}-injected, thyroxine-therapy (At-TT)--20 rats that received approximately 1 μg per day of l-thyroxine in their drinking water from the day after the At^{211} injection until sacrifice. Sixty normal rats served as controls.

Five At-TP rats and 5 At-U rats were sacrificed 18 hours after the At^{211} injection. The thyroid glands were dissected, weighed, and assayed for At^{211} content according to methods described previously (14).

All animals were housed in groups of five in stock cages on wood

shavings and were fed Purina Lab Chow and tap water (the water of the thyroxine therapy group contained 0.1 μg/ml l-thyroxine). All animals were weighed and examined for tumors every 2 weeks for the first 6 months after the At^{211} injection, and monthly thereafter.

At 7 months of age (5 months after the At^{211} injection), white and red blood cell counts, hemoglobin, and hematocrit levels were determined for 17 rats in the At-U group, 18 rats from the At-TP group, and 45 normal control rats. Standard metabolic rates (SMR) were measured according to the method described by Watts (10) for several animals from each group when the animals were 13.5 months old. Vaginal smears were taken for ten consecutive days on these same rats immediately after the SMR determination.

The experiment was terminated 1 year postinjection (the animals were 14 months old) in an attempt to avoid confusion with the normal tumor incidence of this strain, which begins to be significant at about 14 months of age (18). During the year tumor-bearing animals were sacrificed in batches of no less than five regardless of the sizes of the tumors or the time elapsed since their appearance. Five control rats were sacrificed each time tumor rats were autopsied. The sacrifice procedures were the same for all animals. Complete autopsies were performed on all rats except for six At^{211}-injected and six control rats that died of pneumonia.

Twenty-four hours prior to autopsy each rat received 5 to 10 μC of I^{131} intraperitoneally. Sacrifice was performed with chloroform. The animals were weighed, and the gross appearances of the animals and their internal organs were recorded. Thyroid remnants, liver, spleen, kidney, ovaries, adrenals, pituitary, and tumors were dissected and weighed. Specimens were also taken of lung, lymph nodes, and uterus. Thyroid,

uterus, and ovary were fixed in Bouin's fluid, the pituitary was fixed in Zenker-formol, and the remaining tissue specimens were fixed in 10% neutral formalin. The thyroid remnants (in the fixative) were assayed for I^{131} gamma activity with a well-type scintillation counter. The fixed tissues were dehydrated with dioxane, embedded in paraffin, and sectioned at 6 μ (pituitaries at 4 μ), and all were stained with hematoxylin and eosin except the pituitaries, which were stained by the Mallory-Azan technique.

RESULTS

The standard metabolic rates of the experimental and control groups are shown in Table I. Watts (10) reported complete thyroid destruction after injection of 0.8 μC/g of At^{211}, with a reduction of the SMR to 67% of normal. The SMR's of the At-U group were 75% of normal, indicating that 0.5 μC/g of At^{211} effected a greater degree of thyroid destruction, and consequently of functional loss, than had been expected. The Fisher t-test (19) indicated that the SMR's of both the At-U and the At-TT groups were significantly lowered from the control mean. The difference between the SMR's of At-TP group and the normal controls was significant only to the 5% level. There was no correlation between SMR and the presence or absence of a mammary tumor.

There were few, if any differences in the measurements shown in Table II (I^{131} uptakes and body and organ weights) between tumorous and nontumorous rats within a particular treatment group; therefore, each treatment group is discussed as a unit without reference to the presence or absence of a mammary tumor.

The thyroids of the At-U and At-TT groups were small--only 35% of the control weight. The I^{131} uptakes were reduced to a similar degree; however, the ability of the remaining thyroid tissue to concentrate I^{131}

remained relatively high on the average--78% of normal. These results are in accord with previous findings (13).

Although thyroxine pretreatment effected a six-fold reduction in the thyroidal uptake of At^{211} (see Table III), enough At^{211} entered the gland to produce significant radiation damage. The animals in the At-TP group more closely resembled the controls in many respects, but pretreatment with thyroxine as a method of protection of the thyroid gland from irradiation injury by At^{211} was less than satisfactory. Six months to one year after the At^{211} injection the concentration of the I^{131} tracer in the thyroid remnants was within normal limits, but the glands were only one-half normal size, and the I^{131} uptakes were also only one-half of normal. The somewhat milder degree of thyroid deficiency was reflected by the SMR which was only one standard deviation below the normal mean.

Rats that were called severely thyroid deficient (see section on pathological findings) tended to have low thyroidal concentrations of the I^{131} tracer, but there was no statistical correlation between degree of thyroid deficiency (based on pituitary cytology or lowering of SMR) and the I^{131} concentration of the thyroid remnants. Thirty-five of the 46 rats (76%) of the At-injected group that showed "thyroidectomy" or severe thyroid deficiency changes in the pituitary, had thyroidal I^{131} concentrations well above the lower limit of the normal controls. Nineteen of the 55 normal controls (35%), all with normal pituitary cytology, had thyroidal I^{131} concentrations below the mean for the At-injected rats. These observations indicate that I^{131} concentration by the thyroid gland cannot be used alone to assess the ability of the gland to synthesize and secrete thyroid hormone.

The body weights, and the weights of the spleen and adrenals of the At-U and the At-TT groups were less than the corresponding control values, although not statistically different from them. The body and organ weights (except for the ovary) of the At-TP group were within normal limits. The weights of the pituitaries of the At-injected rats were within normal limits; the pituitaries of rats whose thyroids have been completely destroyed with I^{131} are only 17% smaller than those of normal controls (22).

A 60% to 65% weight reduction was noted in the ovaries of all At-injected rats regardless of their degree of thyroid deficiency. This striking uniformity in the extent of ovarian atrophy suggests some degree of radiation damage in the ovaries themselves. From Hamilton's (6) data for the distribution of At^{211} in the tissues of rats, the radiation dosage to the ovaries (see Table III) was calculated as 145 rad. If the relative biological effectiveness (RBE) of alpha particles compared to x-ray is close to four, the ovaries received 570 rem. Although the permanently sterilizing dose of whole-body x-ray for the rat ovary is still the subject of considerable controversy (23), recent experiments indicate much irreparable damage following x-ray doses greater than 400 r (24,25).

Further judgments of the relatively low level of ovarian function were obtained from the vaginal smears, the gross appearance of the uteri, and the microscopic appearance of the ovaries (see summaries of microscopic findings which appear in a later section). A series of vaginal smears taken shortly before autopsy of some of the At-injected animals showed slightly prolonged but normal estrus cycles in 7 of the 9 controls; normal cycling was absent in the At-injected rats, and even prolonged cycles were rare. The 9 At-TT rats tested showed a preponderance of

cornified cells, as did 5 of the 8 At-U rats examined. No smears were taken from rats in the At-TP group. Uterine weights were considered unreliable because of the large numbers of variable-sized polyps present. Grossly, the uteri were either very small and thread-like or were large, thin-walled sacs filled with clear watery fluid. Preliminary experiments in this laboratory demonstrated that young female rats given 0.65 μC At^{211}/g failed to breed, even when supplemental thyroid therapy was given (26).

The blood counts of some of the At-injected rats and normal controls are shown in Table IV. The normal red blood counts and hemoglobin levels of the At-injected rats were unexpected in view of the thyroid deficiency of these animals (27,28). The significantly low white cell counts of the At-U group indicated incomplete repair of the lymphatic tissue (6,28).

The number and sites of tumors observed in the At^{211}-injected and control rats are shown in Table V. The incidence of mammary tumors, adenomas of the anterior pituitary and adrenal glands, and uterine polyps was striking in all three groups of rats that received At^{211}. Pretreatment with thyroxine appeared to have some influence on the development of adrenal and pituitary adenomas and uterine growths (fewer of these tumors were observed), but did not affect the development of mammary neoplasms.

The major emphasis is here placed upon the occurrence of mammary tumors because they were grossly detectable, and because the age at which they first appeared and their growth rates were easily determined. All other tumors were detected only at autopsy or on microscopic examination of tissue specimens.

Rats were classified according to the method of Davis et al. (18), as either tumorous or nontumorous, regardless of the number of individual

tumors any one rat possessed. A single rat in which two or more benign tumors were found was tabulated as a single entry under the classification of the largest of the tumors. An animal that had more than one malignant tumor was classed as a single entry according to the most anaplastic of the tumors present. In a few cases rats possessed both benign and malignant tumors; these animals were classified as malignant tumor-bearing animals.

Figure 1 shows the incidence of mammary tumors as a function of the age of the animals. The first tumors appeared in the experimental groups just prior to the 150th day of life; no tumors were found in the control rats before the 210th day. The age at which the mammary tumors occurred, and the percentages of animals developing these tumors were comparable for all three experimental groups, and a composite curve is shown in the figure.

The classifications of the mammary tumors are given in Table VI. It is apparent from the Table, and from Fig. 1, that the administration of At^{211} to young female rats of this strain resulted in a four-fold increase in mammary tumor incidence regardless of their degree of thyroid deficiency. However, the proportion of the tumors that were diagnosed as malignant was greater in the At-U and At-TT groups (the majority of these animals were suffering from severe thyroid deficiency) than in either the normal controls or the rats whose thyroid glands had been protected by pretreatment with thyroxine. The difference between the percentage of malignancies in the At-U and the At-TP groups was highly significant ($P < .01$) when the chi-square test was applied for a 2-by-2 contingency table (19). Because of the small number of tumor bearers in the normal control group, the apparent increase in the proportion of malignancies in the At-U group

compared with the controls was not significant when the same statistical test was applied.

Although the number of mammary tumors classified as either benign or malignant varied slightly from group to group, the same types of tumors were encountered throughout the study. The histological characteristics of these tumors have been adequately illustrated by Shellabarger et al. (3), and no further description is necessary here.

In order to assess the possible contribution of the endocrine deficiency to induction of the mammary tumors, the microscopic structure of the endocrine glands and of several other tissues was examined.

The cytology of the anterior pituitary has been found to be a very sensitive indicator of thyroid function (15,29,30). When there is a slight thyroxine deficiency, as little as 0.1 μg per 100 g body weight per day, the percentage of basophilic elements rises, and the granulation of the acidophilic cells becomes sparse. When functional thyroid tissue is completely absent, the number of basophilic cells may increase as much as eight-fold, and the morphology of these cells is altered into a vacuolated signet-ring type. A further distinguishing feature of total thyroidectomy is the almost complete absence of granulated acidophils. Thus, even a rough cell count can distinguish between a complete lack of thyroid hormone and the presence of very slight amounts of this hormone.

In our experience the pituitaries of animals whose thyroid glands had been destroyed showed almost completely degranulated acidophils and markedly increased numbers of basophils, many of the signet-ring type. Those animals designated as markedly thyroid deficient showed partial acidophil degranulation and increased numbers of basophils, but no "thyroidectomy cells". Mild thyroid deficiency was indicated in the pituitaries that

showed partial degranulation of acidophils and relatively normal numbers of basophils.

A summary of the microscopic findings is given below; detailed reports have appeared (31) and are available from the authors upon request.

<u>At^{211}-injected, untreated</u>:

The microscopic appearance of the rat thyroid gland one year after the administration of 0.5 μC/g of At^{211} has been illustrated in a previous publication (13) and can be briefly described as follows:

The thyroid glands of the majority of animals possessed little functional tissue. The follicles were very small, with cuboidal epithelium. The colloid within the follicles was somewhat basophilic centrally and more eosinophilic at the periphery; it appeared dense. The vacuolation associated with colloid resorption was seldom seen. A few glands possessed scattered giant cells with pale cytoplasm and vacuolated nuclei (sometimes multilobular); these resembled Hürthle cells. The abundant stroma showed reduced vascularity and occasionally edema. The parathyroid was apparently undamaged.

Pituitary cytology, although variable within the group, showed more or less completely the picture associated with substantial thyroid deficiency. Acidophils were fewer than normal and were usually degranulated. Basophils were increased in number; they were often enlarged and contained droplets of colloid. The changes following very nearly complete thyroid destruction were seen in 28 of 53 of these rats (53%)--18 specimens (34%) showed severe thyroid deficiency, and 7 (13%) showed changes indicating only a mild degree of thyroid deficiency; there were no normal pituitaries in this group. Chromophobe adenomas of microscopic dimensions were found in 15 of the animals in this group.

Adrenal cortical atrophy was pronounced, especially in the fasciculate and reticular zones. Alignment of cells into columns and, deeper, into a network, was lost. Vascularity was usually much reduced, although occasionally the sinusoidal vessels were distended with blood. Cortical adenomas, hemorrhage, degeneration, or cysts were variably encountered.

The ovaries usually showed evidence of markedly disturbed function, including deficient intersitial tissue and reduced vascularity. Follicles, when present, were small to medium in size; corpora lutea usually degenerating. These changes reflected the severe functional and gross anatomical ovarian deficiency described earlier. Ovarian cysts and tumors were seen in a few animals.

The uterus was usually small and thin-walled with a hypoplastic endometrium. Polyps were seen frequently in the lumen.

The lungs, liver, and kidneys showed no damage directly attributable to the At^{211} irradiation. Varying degrees of pneumonitis were seen; in the animals suffering from more severe infection (such as lung abscesses), parenchymatous or vacuolar degeneration was seen in the liver. There were two pulmonary metastases of mammary carcinomas.

Lymph nodes from the mesenteric root varied in structure from normal to markedly atrophic. The lymphatic tissue of the spleen was similarly variable. The thymus showed a reduced amount of lymphatic tissue; in part, this was replaced by developing fat, and probably represented the involution commonly seen in older animals. A large proportion of the thymi in this group contained epithelium recognizable as, or reminiscent of, thyroid tissue (32).

At^{211}-injected, thyroxine-pretreated:

Although the thyroid glands of this group were somewhat less damaged than in the other At-injected groups, no normal glands were seen. Evidences of secretory activity such as colloid vacuolation and heightened epithelium were more frequently seen. The cells resembling Hürthle cells were less common. The stromal changes were like those in the At-U group, although they were usually less severe.

Pituitary cytology reflected the slightly better condition of the thyroid glands; in some instances, the glands were virtually within normal limits. Changes associated with complete thyroid destruction were seen in 6 of 36 specimens (17%); severe thyroid deficiency was diagnosed in 11 (31%); mild deficiency was found in 2 specimens (5%); and 17 (47%) were within normal limits. The adrenals also appeared healthier than in the preceding group, although individual variability was such that the groups overlapped in evidence of damage. Ovarian and uterine structure, although less severely affected than in the preceding group, was below normal functional limits.

Generally, the structure of the lungs, liver, and kidneys indicated the likelihood of adequate function. One lung contained a carcinoma for which no primary site could be found; its structure suggested a primary bronchial epithelial origin.

Lymph nodes, spleen, and thymus were normal or only slightly atrophic. Although many thymi contained epithelial remnants, they were seldom so organized as to suggest thyroid follicles.

At^{211}-injected, thyroxine-therapy:

The thyroid glands were more severely disorganized than in the At-U animals. Often they were preponderantly fibrous tissue, with scanty epithelium arranged in cell clusters or tiny follicles. Most of the specimens showed the giant, "Hürthle-type" cells, although they were seldom in large numbers.

Pituitary cytologic changes were, for the most part, intermediate between those seen in the two preceding groups. Complete loss of thyroid tissue was diagnosed in 4 of 16 animals (25%), severe deficiency in 9 rats (56%), and a mild degree of deficiency in 3 specimens (19%); there were no normals in this group. In evaluating the pituitary-thyroid relationship in this group it is noteworthy that when exogenous thyroxine was given, the impetus to even partial thyroid repair was not as great as in the At-U group. On the pituitary the action of thyroxine supplement was at least partially supportive, although at too low a level to maintain normal pituitary structure.

Adrenal cortical structure was highly variable, and ovarian structure was consistently defective.

The lungs, liver, and kidneys were much like those of other At^{211}-injected animals. One kidney bore a tumor which appeared to be a metastasis of a milignant mammary tumor.

The lymph nodes and the lymphatic tissue of the spleen and thymus were depleted. The thymi often contained epithelium of recognizable thyroid type. In fact, when found, this tissue was often more representative of thyroid structure than the thyroid glands in this group.

The presence of adenomata of the adrenal cortex and anterior pituitary gland has not been reported heretofore in rats that received whole-body irradiation, and hence is illustrated here. Figs. 2 and 3 are typical of the adrenal cortical adenomas encountered. A chromophobe adenoma of the anterior pituitary of microscopic dimensions is shown in Fig. 4. Figure 5 shows a portion of a pituitary adenoma that weighed 28 mg taken from an animal in the At-U group one year after injection.

DISCUSSION

These experiments corroborate the observation of Hamilton, et al. (6) that a sublethal dose of At^{211} markedly increases the number of early-appearing mammary tumors in female Sprague-Dawley rats.

There is reasonably broad acceptance of the hypothesis that sublethal amounts of radiation cause premature death by accelerating biological aging processes. Such premature death may often be due to the earlier onset of certain specific diseases to which the species is highly susceptible that are normally associated with or promoted by the aging process (33). Tumors of the breast (34) and adenomata of the pituitary (35,36) are seldom encountered except in rats of advanced age. Although adrenal cortical hyperplasia has been demonstrated in aging female Long-Evans rats (37), we are not aware of any report of the spontaneous occurrence of adrenal cortical adenomas in this species. In man these growths are associated with aging (38).

Davis et al. (18) reported a 57% incidence of spontaneous mammary tumors in female Sprague-Dawley rats; 87% of these tumors developed after the animals were 540 days old. Pathologic classification was made on about one-third of the total macroscopically observed tumors. They noted that 12% of all tumors examined were malignant. The spontaneous tumor incidence in the same strain of rat may differ under varying conditions of diet and laboratory care (18,39). Nevertheless, it seemed worthwhile to test the significance of the apparent difference between the incidence of mammary tumors in our young At-injected rats (less than 420 days old), and the spontaneous tumor incidence as observed over the life span of the same strain in Davis' colony. The percentages of tumors in the three At-injected groups were almost identical, and the groups were combined for this comparison. In the At-injected groups 78 of 107 rats (73%) bore mammary

tumors by the end of the 12-month observation period. In Davis' group of 150 normal rats, 85 (57%) developed mammary tumors by the end of their life-spans. As a measure of signifigance of the At^{211}-mammary-tumor association, the chi-square test was applied (2-by-2 contingency table) (19). The difference in tumor incidence was significantly above chance relationship, $x^2 = 7.1$, $P = 0.01$. We cannot rule out the possibility that the net effect of treatment with At^{211} may be that its radiation accelerates the normal aging processes of the strain. The total spontaneous incidence, however, is smaller than the sum of tumors in the irradiated animals. This question is being examined for a larger group of animals.

In two of the three At-injected groups--At-U and At-TT--31 of 51 rats were classified as malignant tumor bearers. Of the 71 spontaneous tumors examined by Davis et al. (18), six were diagnosed as malignant. The chi-square test for the independence of these two sets of data indicated a highly significant difference, $x^2 = 12.2$ and $P < 0.01$. This difference in the percentage of malignancies in spontaneous tumors and those arising after an injection of At^{211}, although probably real, is perhaps not as great as the above statistical test implies. The pathological material was examined by separate investigators, and in spite of efforts by both groups to adhere to standard criteria for the diagnosis of malignancy, there are bound to be differences of opinion on some points.

The number of chromophobe adenomas of the anterior pituitary and adenomas of the adrenal cortex was surprisingly large in the At-injected rats. Large numbers of pituitary tumors have been found in mice many months after exposure to the neutrons and gamma rays of a nuclear detonation (40) and after partial or complete thyroid destruction with I^{131} (41,42). This is, to the best of our knowledge, the first report of pituitary adenomas in irradiated rats. Furth and his co-workers (40,42,43)

have implicated thyroid deficiency as well as irradiation in the genesis of these tumors in mice.

There appeared to be a relationship between the number of pituitary tumors in an At-injected group and the degree of hypothyroidism. In the most nearly normal group--At-TP--5.6% of the animals had pituitary tumors; in the moderately deficient group--At-TT--19% of the rats bore tumors; and in the severely hypothyroid group--At-U--pituitary tumors were found in 27% of the animals.

Factors to be considered in mammary tumor induction, in what we believe to be the most likely order of importance, are as follows: (a) irradiation of the immature mammary ducts and alveolar buds; (b) irradiation of the ovaries; (c) whole-body irradiation; (d) the substantial reduction in thyroid hormone following thyroid gland destruction; and (e) secondary functional changes in other endocrines resulting from thyroid deficiency.

The diffuse distribution of the mammary ducts and alveolar buds in the young virgin rat limits calculation of radiation dosage from radioactive material deposited in this tissue to a rough approximation. Autoradiographic studies now in progress indicate a concentration of At^{211} in the immature mammary structures which is substantially greater than in the surrounding connective tissue, lymph nodes, and fat (20). The concentration of At^{211} was measured in dissected subcutaneous tissues taken from the inguinal region; the average concentration of At^{211} was also measured in the intact pelt and subcutaneous structures in earlier experiments (6). The dosages calculated from these two values are shown in Table III. The best estimate--65 rad--was based on an average weighted in favor of the At^{211} concentration in dissected subcutaneous tissues.

The whole-body radiation dose also can only be approximated because of the wide variations in the At^{211} concentrations in the different tissues

(6). An estimate of 64 rad was obtained on the basis of uniform distribution of 0.5 μC/g of At^{211} throughout the body with excretion of 15% of the injected material in the first 24 hours (see Table III). On the basis of these calculations, a dosage of 0.5 μC/g of At^{211} delivers the same amount of radiation to the mammary tissue as to the whole animal. Cronkite et al. (5) found that 400 r of x-irradiation induced nearly the same percentage of mammary tumors in young Sprague-Dawley rats--79%--as we observed following At^{211}. On the basis of radiation dosage alone, it would appear then that the alpha particles of At^{211} are about six times more effective than 250 kvp x-rays in increasing the number of mammary tumors in young female rats. It should be pointed out that the whole-body radiation dosage calculated above applies to all of the At-injected groups, not only those who received At^{211} before any other treatment, but also to those whose thyroid glands had been partially blocked with thyroxine. The uptake of At^{211} in the normal thyroids 18 hours after injection was 0.45% of the administered dose; the uptake of At^{211} in the thyroxine-blocked glands was 0.08% of the dose. Thus only an additional 0.37% of the injected At^{211} was available for irradiation of tissues other than the thyroid in the At-TP rats.

As has been pointed out, the ovaries sustained severe damage, and at the time of sacrifice the majority consisted of deficient interstitial tissue, a few very small follicles, and occasional old corpora lutea. Most of the ovaries appeared wholly incapable of reproduction and of sustaining any but low levels of hormone secretion. The results of Parrott et al. (26) suggest that the ovarian atrophy following At^{211} is chiefly due to irradiation damage and only secondarily to thyroid deficiency. They found that the ovaries of sexually mature rats whose thyroids had been removed either surgically or with I^{131} maintained a normal weight for many months, and

that over half of these animals were capable of conceiving and sustaining fetuses to normal term. Uptake of I^{131} in thyroid remnants and SMR measurement indicated that these rats were severely thyroid deficient. Microscopically, the ovaries of these surgically thyroidectomized and I^{131} thyroid-ablated rats consisted predominantly of resorbing corpora lutea, but follicles in all stages of development were present. The germinal epithelium was intact and appeared active. The vasculature was within normal limits, and the interstitial tissue was sufficiently abundant. On the other hand, At-injected rats in the same experiment possessed only ovarian remnants of less than one-third normal size, just as in the present experiments. Supplemental thyroid substance which was adequate to maintain normal ovarian weight, and to improve littering in surgically thyroidectomized and radioiodine thyroid-ablated rats, and to sustain nearly normal SMR's in At-injected rats, only slightly increased the size of the ovaries of the At-injected rats, and did not repair their structure.

The extent of thyroid deficiency did not appear to be a major factor in determining the percentage of mammary tumors developed. The animals that received the partially protective thyroxine pretreatment had a more nearly normal thyroid status than did the At-U or At-TT groups as evidenced by pituitary cytology, thyroidal uptake of a radioiodine tracer, and mean SMR within the normal range. Yet this group developed the same percentage of mammary tumors as did the notably thyroid deficient groups. Chen _et al._ (44) have shown that rats from which the thyroid, adrenals, ovaries, and pituitaries have been removed are able to sustain normal mammary development and even lactate when given replacement ovarian, pituitary and adrenal hormones. They concluded that thyroid hormone _per se_ has little if any influence on the mammary tissue.

Evidence relating the ovaries and estrogens to mammary neoplasms has been accumulated from clinical experience and animal experimentation. Much of this work has been reviewed by Burrows and Horning (45). These authors sum up their opinions on the relationship between estrogens and breast tumors as follows: "....the neoplastic effects of estrogen appear to depend less upon its amount than upon its uninterrupted supply and though an excess of estrogen is perhaps more likely than a normal quantity to cause neoplasia, yet the excess need not be great. The ordinary production of estrogen by the ovary is rhythmical, and time is available between each period of abundance for the breast to revert more or less to its resting condition. When deprived of the interval of rest, it seems that chronic hyperplasia, innocent tumors, and ultimately cancer are apt to follow."

The vast majority of spontaneous breast tumors occur in rats long after the active reproductive period, in apparent contradiction to the above hypothesis. However, as Wolfe et al. (35) point out, the rat does not seem to undergo a true menopause with ovarian atrophy and a decline in blood estrogen levels. Most of their very old female rats continued to cycle sporadically every two to three months. The ovaries of these rats were small and consisted chiefly of interstitial tissue, but a few old corpora lutea and some large follicles were usually present. Occasionally, animals were found that continued to cycle normally until death, and other animals were seen in which the ovaries contained persistent atretic follicles; vaginal smears from these latter animals consistently showed only cornified cells.

The studies of Cronkite et al. (5) clearly implicate the ovaries in the early development of breast tumors in young x-irradiated female Sprague-Dawley rats; mammary tumors were found in 79% of their animals at

12.5 months of age. Fewer breast tumors--19%--developed in animals ovariectomized prior to the irradiation. If unexposed ovaries were implanted after irradiation, or if during the irradiation the ovaries were exteriorized and shielded, nearly the same mammary tumor incidence was achieved as intact irradiated rats--91% and 62% respectively. X-irradiation of the exteriorized ovaries alone was without effect in inducing mammary tumors.

The constant cornification found in the vaginal smears of some of the At-injected rats indicated a sustained estrogen stimulation of unknown degree. The ovaries, although without follicles and incapable of reproduction, may still have been elaborating some estrogen. Other tissues, e.g., the adrenal glands, have been found to supply estrogens to gonadectomized animals, often in large amounts (45). The sizable number of glandular uterine polyps in the At-injected rats is also strongly indicative of prolonged estrogen stimulation (45).

In conclusion, exposure of rats to sublethal amounts of At^{211} results in the early appearance of large numbers of mammary tumors, many of them malignant, and in the production of an altered functional state simulating menopause. It is evident that the tumor induction is not yet tested for its association with radiation exposure separately from the endocrine disturbance.

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Table I

Standard metabolic rates 10.5 months after an injection of 0.5 μC/g of astatine-211. Mean is shown with standard error[a].

Group	No. of determinations	No. with mammary tumors	BMR $Cal/m^2/hr$
Normal controls	8	0	50.9 ± 1.3
At^{211}-injected, untreated	14	7	37.9 ± .95[b]
At^{211}-injected, thyroxine-pretreated	7	4	44.1 ± 3.4
At^{211}-injected, thyroxine-therapy	4	2	39.6 ± 2.4

[a] Standard error, $S.E. = \sqrt{\frac{\Sigma\, dev^2}{n(n-1)}}$

[b] Underlined means were compared to the control mean by the t- test of Fisher (19), and the P value was beyond the 1% level of confidence.

Table II

Comparison of body weights, organ weights, and thyroidal uptake of I^{131} by mammary-tumor-bearing and non-tumor-bearing female rats 3 to 12 months following the administration of 0.5 μC/g of At^{211}.

Experiment			Thyroid analysis			Weight of organ (mg)			
	No. rats autopsied	Body wt (g)	wt (mg)	$\%I^{131}$	$\%I^{131}/g$	Spleen	Adrenal (1 only)	Pituitary	Ovary[a] (1 only)
At-injected--tumorous	36	261	8.5	3.41	377	390	27.4	12.7	15.8 (17)
At-injected--nontumorous	21	257	8.4	2.71	305	370	26.1	12.3	17.7 (10)
At + thyroxine pretreatment--tumorous	28	282	11.2	5.39	469	514	29.3	13.0	14.7 (9)
At + thyroxine pretreatment--nontumorous	8	270	13.4	7.64	534	408	30.9	13.2	---
At + thyroxine therapy	13 tumorous[a] 3 non-tumorous	253	8.0	3.72	447	383	26.8	13.2	16.8 (9)
Normal controls	5 tumorous 41 non-tumorous	283	23.2[b]	10.97[b]	487[b]	544	32.6	13.6	45.9 (10)

[a] Parenthesized number is the number of specimens weighed.

[b] Data available for 9 additional rats for a total of 55 individual measurements.

Table III

Calculated tissue dosages from astatine-211[a,b]

Tissue	Mean concentration of At^{211} from 0 to 15 hours	Accumulated radiation dose, rad	Remarks
Thyroid:			
Normal	27.4%/g	3,340	
Thyroxine pre-treated	4.6%/g	560	
Ovary	1.2%/g	145	
Pituitary	$(8.3\%e^{-1.08t}+1.68\%e^{-0.12t})$%/g	240	Concentration changes rapidly with time. This expression is substituted for the last two terms in the dosage equation, and the whole is integrated. Time is in hours.
Mammary tissue:			
Dissected	0.43%/g	52	Samples of subcutaneous tissue include mammary tissue, fat, lymph nodes, and connective tissue.
Skin	0.75%/g	91	Pelt and subcutaneous tissues sampled as a unit.
Whole body	0.63%/g x 0.85	64	Calculation based on uniform distribution of 0.5 μC/g of At^{211} in whole rat and 15% excretion in 24 hours.

a Data for ovary, pituitary and skin from Hamilton et al. (6). Thyroid concentrations from this paper. Concentration of At^{211} in dissected mammary tissue from current unpublished data (20).

b The mean energy of the two alpha particles emitted in the decay of At^{211} is 6.5 Mev. The mean life of At^{211} is 7.5/0.693 hours. The average concentration of At^{211} in the ovary for example is 1.2%/g for the first 10 hours. Assuming a uniform distribution in the ovaries, one finds the radiation dosage is:

$$\text{rad} = (2.13\ \text{rad/Mev/}\mu\text{C/g/hour})(6.5\ \text{Mev})(80\ \mu\text{C})(1.2 \times 10^{-2}/\text{g})(10.8\ \text{hours}) = 145\ \text{rad}\ (21).$$

Table IV

The number and site of tumors induced in female Sprague-Dawley rats following 0.5 μC/g of astatine-211 administered at 55 days of age. The experiment was terminated when the rats were 420 days old.

	Untreated controls	At-injected, untreated	At-injected, thyroxine pretreated	At-injected, thyroxine therapy
No. of rats autopsied	46	55	36	16
Total tumors	10	107	47	28
Mammary tumor-bearers(total)	8	39	27	12
Pituitary adenoma[a]	-	15	2	3
Adrenal cortical[a] adenoma	-	8	-	2
Endometrial polyps	2	42	17	11
Bronchiolar carcinoma	-	-	1	-
Ovarian granulosa cell tumor	-	1	-	-
Ovarian endometrioma	-	1	-	-
Uterine squamous cell carcinoma	-	1	-	-

[a] Most of the adenomata were very small and only detectable microscopically.

Table V

The classification of mammary tumors induced by astatine-211

	Untreated controls	At^{211}-injected	At^{211}-injected, thyroxine-pretreated	At^{211}-injected, thyroxine therapy
No. of rats autopsied	46	55	36	16
Mammary tumor incidence(%)	8(17.5)	39(71)	27(75)	12(75)
Total benign tumors(%)	5(62)[a]	14(35)	18(67)	6(50)
Adenoma and fibroadenoma	3	8	14	6
Adenofibroma	1	6	4	-
Total malignant tumors(%)	3(38)	25(64)	9(37)	6(50)
Adenocarcinoma	2	10	5	3
Miscellaneous carcinoma	1	11	2	3
Sarcoma	-	4	2	-

[a] Includes one fibroma

Table VI

The classification of mammary tumors induced by astatine-211

	Untreated controls	At^{211}-injected	At^{211}-injected, thyroxine-pretreated	At^{211}-injected, thyroxine therapy
No. of rats autopsied	46	55	36	16
Mammary tumor incidence and (%)	8 (17.5)	39 (71)	27 (75)	12 (75)
Total benign tumors and (%)	5 (62)[a]	14 (35)	18 (67)	6 (50)
Adenoma and fibroadenoma	3	8	14	6
Adenofibroma	1	6	4	-
Total malignant tumors and (%)	3 (38)	25 (64)	9 (37)	6 (50)
Adenocarcinoma	2	10	5	3
Miscellaneous carcinoma	1	11	2	3
Sarcoma	-	4	2	-

[a] Includes one fibroma

FIGURE LEGENDS

1. Incidence of mammary tumors in female Sprague-Dawley rats as a function of age after an intravenous injection of 0.5 μC of At^{211} per gram of body weight. Data are also shown for intact controls. Solid line is the mean incidence for 114 At-injected rats.

2. Portion of large adrenal cortical tumor. Degeneration; colloid. H and E, X 70.

3. Small adrenal cortical tumor showing compression and distortion of adjacent cortical tissue. H and E, X 70.

4. Small chromophobe adenoma of the anterior lobe of the pituitary gland. Mallory-Azan, X 110.

5. Portion of a large chromophobic adenoma of the anterior pituitary gland, showing vascular dilatation and hemorrhage. Tissue of the anterior lobe proper has been compressed to a thin rim on the surface of the tumor. Mallory-Azan, X 110.

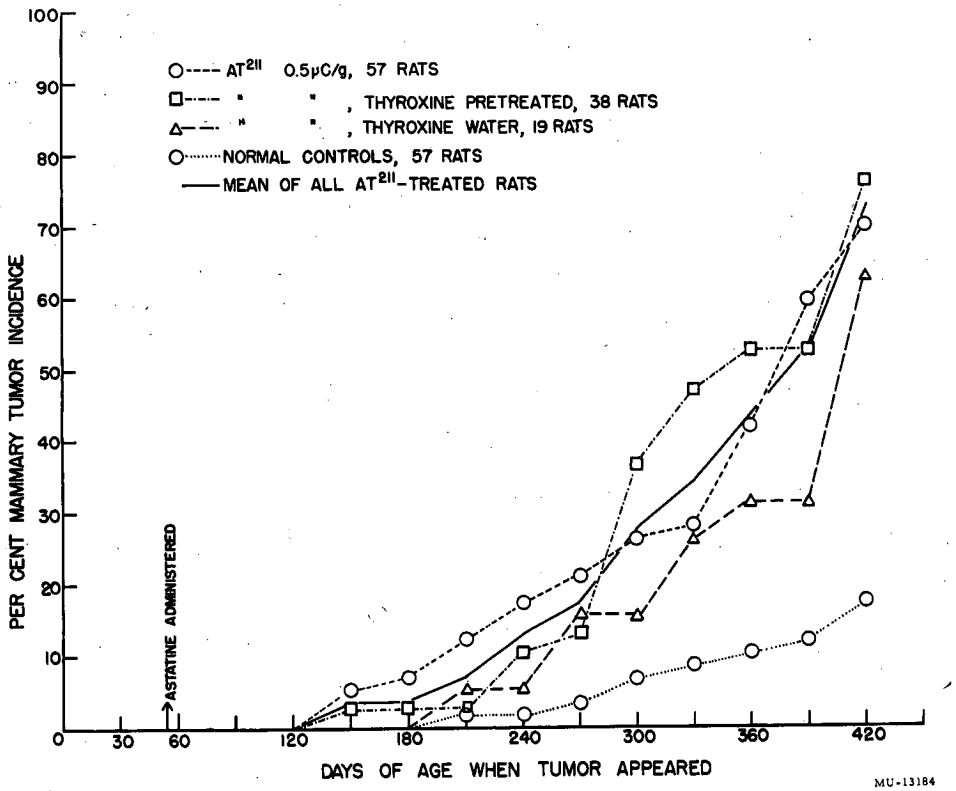
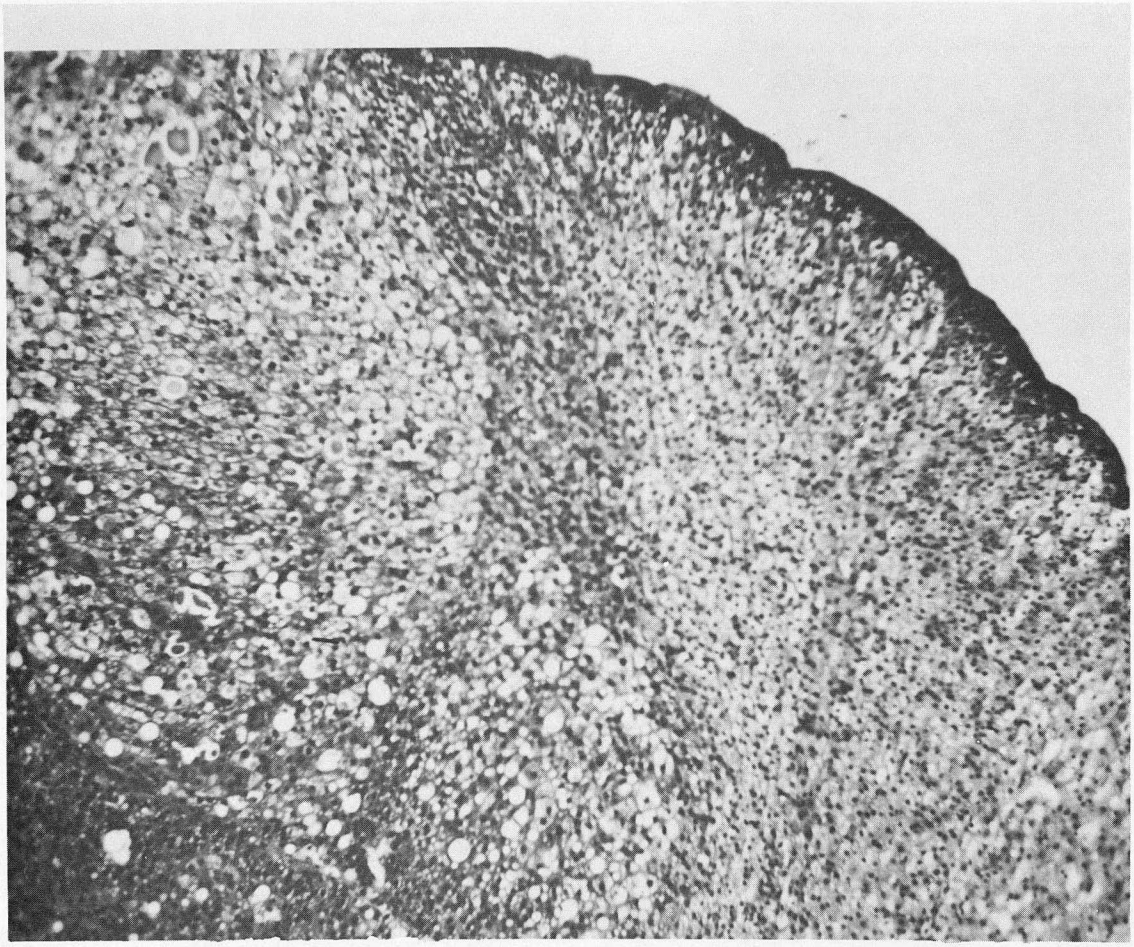
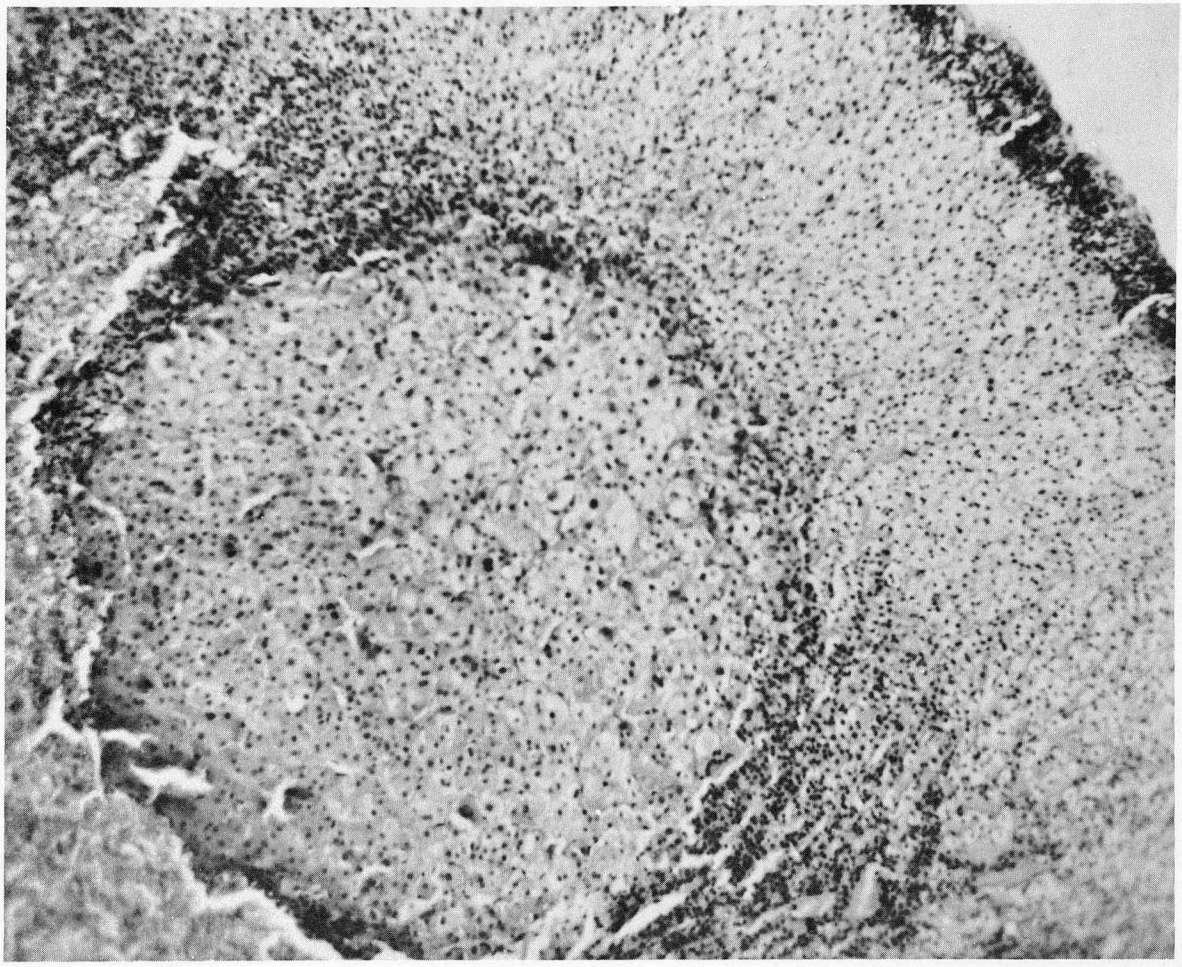

O---- AT211 0.5μC/g, 57 RATS
□-·-· " " , THYROXINE PRETREATED, 38 RATS
△— — " " , THYROXINE WATER, 19 RATS
O·····NORMAL CONTROLS, 57 RATS
—— MEAN OF ALL AT211-TREATED RATS
PER CENT MAMMARY TUMOR INCIDENCE
ASTATINE ADMINISTERED
DAYS OF AGE WHEN TUMOR APPEARED
0
30
60
120
180
240
300
360
420
10
20
30
40
50
60
70
80
90
100
MU-13184

Fig. 1



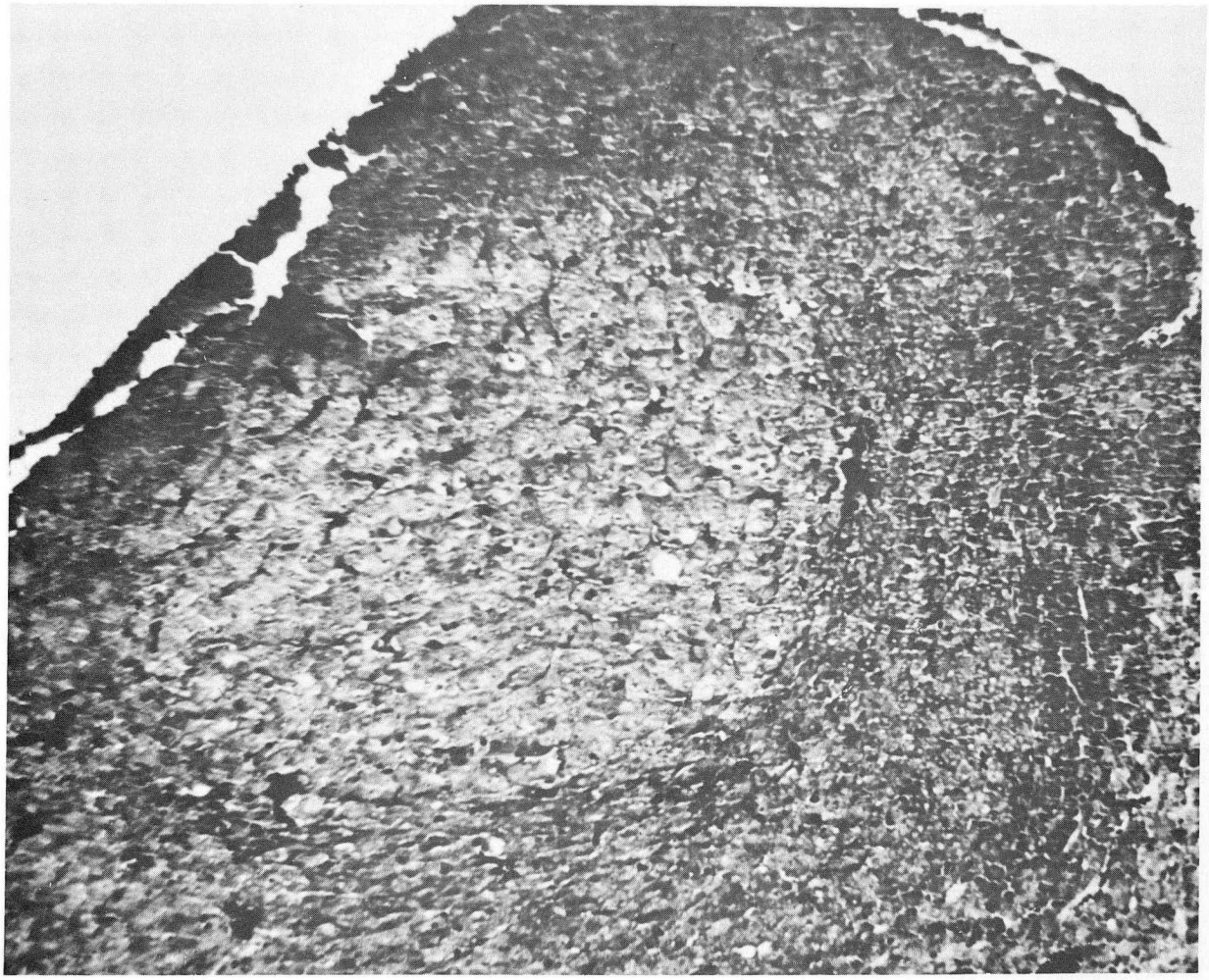
ZN-1920

Fig. 2



ZN-1921

Fig. 3



ZN-1922

Fig. 4

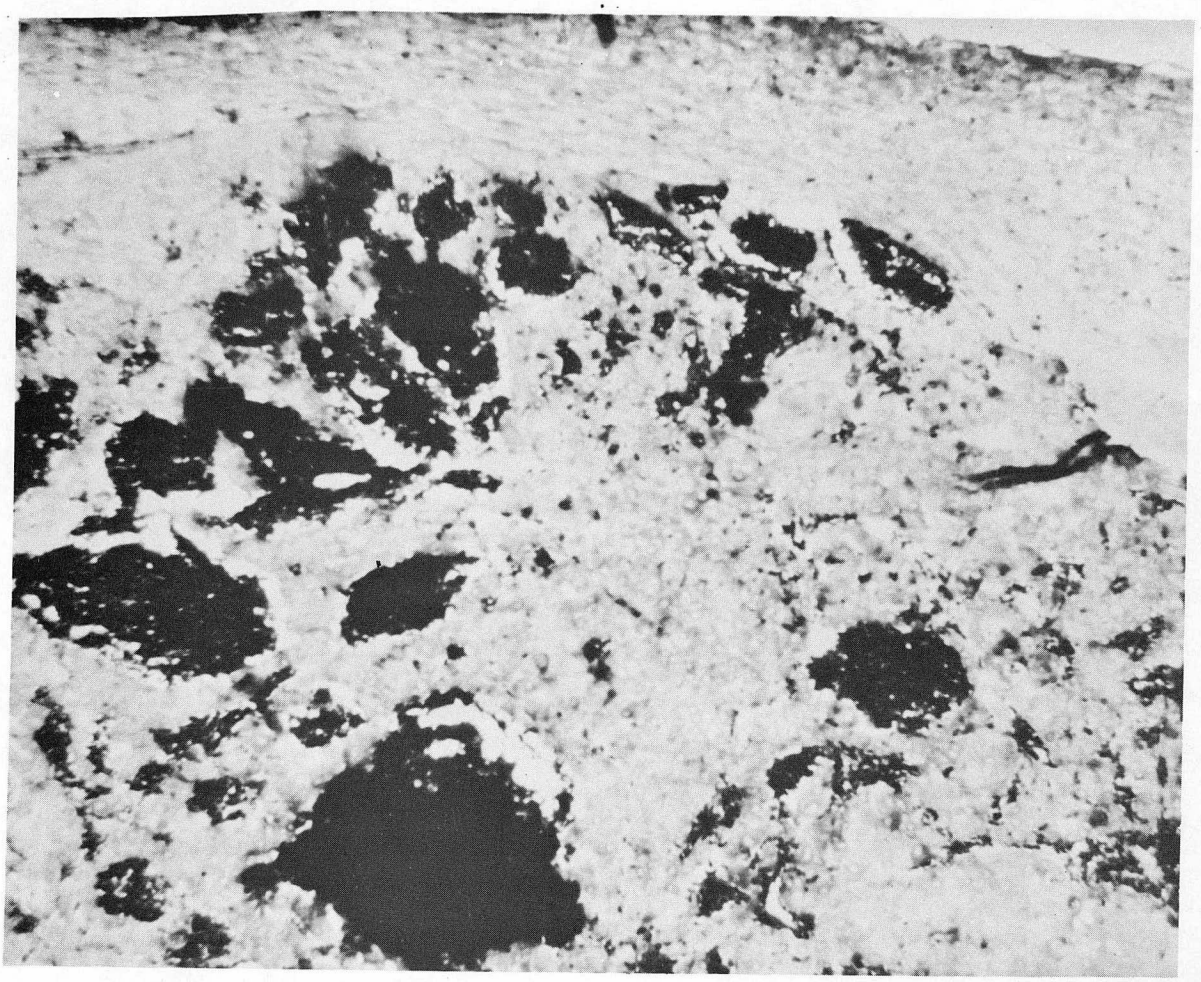


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Fig. 5.