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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},[2] 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 tumor 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 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 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 employed were female Sprague-Dawley rats obtained from the original colony. When the animals were 40 days old they were earmarked, 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 suocutaneous injections of 230 μg 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.

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 thyroidectomy 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} effects 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 the animals in the At-TP group more closely resembled the controls in many respects, pretreatment with

thyroxine as a method of protection of the thyroid gland from irradiation injury by At^{211} was less than satisfactory. The I^{131} concentration of the thyroid remnants was within normal limits, but the glands were only one-half normal size, and the I^{131} uptakes were also only 50% 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 completely thyroidectomized with I^{131} are only 17% smaller than those of normal controls (20).

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 was calculated as 580 rad (670 rep)[3]. Although the permanently sterilizing dose of whole body x-ray for the rat ovary is still the subject of considerable controversy (22), recent experiments indicate that a high degree of irreparable damage is produced by doses greater than 400 r (23,24).

Further judgments of the relatively low level of ovarian function were obtained from the vaginal smears and the gross appearance of the uteri. A series of vaginal smears taken shortly before autopsy of the animals showed slightly prolonged but normal estrus cycles in the controls; normal cycling was absent in the At-injected rats, and even very prolonged cycles were rare. Uterine weights were considered unreliable because of the large numbers of variably 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 (25).

The blood counts of some of the At-injected rats and normal controls are shown in Table III. The normal red blood counts and hemoglobin levels of the At-injected rats were somewhat surprising in view of the thyroid deficiency of these animals (26,27). The significantly low white cell counts of the At-U group indicated incomplete repair of the radiation-damaged lymphatic tissue (6,27).

The number and sites of tumors observed in the At^{211}-injected and control rats are shown in Table IV. 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 routine 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 V. 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 (28). 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 (15,30,31). 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 diagnosed as thyroidectomized 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 (29) and are available from the authors upon request.

At^{211}-injected, untreated:

The microscopic appearance of the rat thyroid gland 1 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 thyroidectomy 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 encountered with some frequency in this group.

The adrenal glands showed cortical atrophy and disorganized architecture, especially in the fasciculate and reticular zones. Cortical adenomas, hemorrhage, degeneration, or cysts were variably encountered.

The ovaries usually showed evidence of markedly disturbed function, including deficient interstitial tissue and reduced vascularity. Follicles, when present, were small to medium in size; corpora lutea were usually degenerating. 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. Thyroidectomy changes 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, suggesting that the exogenous thyroxine supplement was at least partially supportive, although at too low a level to maintain normal pituitary structure. Thyroidectomy 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. 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 malignant 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.

DISCUSSION

These experiments completely substantiate the observation by Hamilton et al. (1) that a sublethal dose of At^{211} results in a marked increase in early-appearing mammary tumors in female rats of the Sprague-Dawley strain. All the At-injected groups markedly exceeded normal in the incidence of these mammary tumors, and the percentage of mammary tumor-bearers in each treatment group was the same regardless of whether or not exogenous thyroxine was administered, either before or after the At^{211} injection. It is of interest to note that Chen et al. (33) have been able to sustain normal mammary development and even to induce lactation in thyroidless rats supplemented with pituitary, adrenal, and ovarian hormones. Their results indicate that thyroid hormone per se has little influence on the mammary tissue.

It was difficult to assess the separate contribution of either radiation injury or endocrine disturbance to the observed increase in tumor induction, because of the abnormal endocrine status of all the At-injected groups. Hamilton's (6) data on the bodily distribution of At^{211} in the rat shows that there are wide variations in the tissue concentrations and the relationships between the concentration of At^{211} in a particular tissue and the time after injection. Therefore, any estimate of the whole-body radiation dose must be considered only an approximation. Assuming a uniform distribution of At^{211} and 15% excretion in 24 hours, the whole-body radiation dose from 0.5 μC/g of At-211 is 270 rad (RBE = 4). This value is close to the 400r of x-ray found by Cronkite et al. (5) to induce the same percentage of mammary tumors in 43-day old rats of the same strain.

The work of the Brookhaven group (5) suggests that in addition to the radiation dose and the age at the time of irradiation, mammary tumor induction in irradiated female rats is also dependent on the presence of "functioning" ovaries. However, in our studies ovarian function and structure were so deranged, that any definitive relationship between the ovary and the incidence of mammary tumors in the At-injected rats was not clear.

As stated previously, the endocrine status of the different groups was not as completely separated as had been hoped. Nevertheless, qualitative differences were observed in the thyroid-pituitary relationships and in the endocrine organs dependent upon them. Adenomata of the anterior pituitary gland and adrenal cortex, and endometrial polyps, lesions which may be partially due to endocrine disturbances and which are found with some frequency in animals of advanced age (34), were more common in the At-injected, untreated rats whose endocrine state was highly deranged. A larger proportion of the mammary neoplasms was benign and the incidence of adenomata and endometrial polyps was lower (more nearly that in intact rats), when protective or supplemental thyroid

therapy was given. However, the endocrine status of all the At-injected animals was sufficiently complex that one could not say whether these findings were attributable to the improved function of a single gland (or more likely) to a slightly better balance of hormones.

SUMMARY

A total of 120 female Sprague-Dawley rats 55 days old were given 0.5 μC per gram of body weight of At^{211} intravenously. Sixty rats received only the At^{211} injection; 40 received eight daily injections of 230 μg of l-thyroxine prior to the At^{211} injection to reduce the thyroidal accumulation of At^{211}; and 20 rats were given 1 μg/day of l-thyroxine in their drinking water starting immediately after the At^{211} injection and continuing until sacrifice. Sixty noninjected rats of the same age served as controls. Between about 6 and 11 months after injection, standard metabolic rates were measured, and serial vaginal smears and complete blood counts were taken on representative lots of the three experimental groups and the controls to assess thyroid status, ovarian function, and residual radiation damage. Animals were sacrificed as mammary tumors appeared, and the experiment was terminated one year after the At^{211} injection. A tracer dose of I^{131} was given 24 hours before sacrifice. Complete autopsies were performed on all animals; tumors, major organs, lymphatic tissues, and endocrine glands were examined microscopically.

Study of the endocrine status revealed that (a) pretreatment with thyroxine (although affording some protection) did not completely prevent thyroid damage by the At^{211} alpha particles, and (b) the level of the thyroxine supplement given in the drinking water was not sufficient to maintain normal pituitary structure. Although in better condition than their untreated, At^{211}-injected counterparts, the above groups were definitely thyroid-deficient as judged by standard metabolic rate and pituitary cytology. The uptake of a tracer dose

of I^{131} by the remnants of the radiation-damaged thyroid glands was, by itself, an unreliable measure of the ability of these remnants to synthesize and secrete thyroid hormone.

The ovaries of all the At-injected rats were uniformly atrophic. At the time of sacrifice the very low level of ovarian function was indicated by (a) the small ovarian size, (b) the absence of follicles, (c) the absence of normal estrus cycles (on the basis of vaginal smears), and (d) the deficient state of the uteri.

Seventy-three percent of the At-injected rats developed a variety of mammary tumors (almost exclusively of duct origin and often multiple) prior to the 420th day of life; 17.5% of the controls had developed mammary tumors during the experimental period. The percentage incidence of mammary tumors and the age at which they appeared were the same for all three At-injected groups, with no apparent relationship between tumor incidence and the degree of thyroid deficiency. In the control rats 38% (3 of 8) of the mammary tumors were malignant, and in the At-injected, untreated group 64% (25 of 39) malignancies were observed. The injected rats that received thyroxine, either protective or supplemental, had fewer malignant tumors, more nearly the percentage found in the intact controls.

The At-injected, untreated rats possessed a high percentage of endometrial polyps (75%), of adenomata of the anterior pituitary (27%), and of adrenal cortex (15%). The percentage incidence of these lesions was lower in the thyroxine-treated groups.

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REFERENCES

1. J.G. Hamilton, P.W. Durbin, and M.W. Parrott, The accumulation and destructive action of astatine-211(eka-iodine) in the thyroid gland of rats and monkeys. J. Clin. Endocrind. and Metabolism 14, 1161-1178 (1954).

2. J.M. Hollander, I. Perlman, and G.T. Seaborg, Table of isotopes. Revs. Modern Phys. 25, 469-651 (1953).

3. C.J. Shellabarger, E.P. Cronkite, V.P. Bond, and S.W. Lippincott, The occurrence of mammary tumors in the rat after sublethal whole-body irradiation. Radiation Research 6, 501-512 (1957).

4. R.T. Binhammer, J.C. Finerty, M. Schneider, and A.W.B. Cunningham, Tumor induction in rats by single total-body x-irradiation. ibid. 339-348.

5. E.P. Cronkite, C.J. Shellabarger, V.P. Bond, and S.W. Lippincott, Studies of the mechanism of induction of radiation-induced tumors of the breast in the rat. ibid. 7, 311 (1957) abstract.

6. J.G. Hamilton, C.W. Asling, W. M. Garrison, and K.G. Scott, The accumulation, metabolism and biological effects of astatine in rats and monkeys. Univ. Calif. Publ. Pharmacol. 2, 283-344 (1953).

7. J.G. Hamilton, P. Wallace-Durbin, and M.W. Parrott, Comparison of acute and chronic changes produced in rats by iodine-131 and astatine -211 at lethal levels. Radioisotope Conf., Oxford, Eng., 1954 (Academic, New York, 1954) Vol. 1, Medical and Physiological Applications, p. 219-231.

8. J.V. Basson, and C.J. Shellabarger, Determination of the RBE of alpha-particles from astatine-211 as compared to beta-particles from iodine -132 in the rat thyroid. Radiation Research 5, 502-514 (1956).

9. J. G. Hamilton, P.W. Durbin, C.W. Asling, and M.J. Johnston, Physiological and biochemical studies of astatine-211 (eka-iodine). In Proc. of the International Conference on the Peaceful Uses of Atomic Energy, Geneva, Aug. 1955, Vol. 10 (United Nations, New York) p. 175-181.

10. R.W.E. Watts, Metabolic rate changes following thyroid destruction by At^{211} and I^{131} in the rat. Proc. Soc. Exptl. Biol. Med. 89, 220-222 (1955).

11. M. W. Parrott, and P.W. Durbin. The effect of surgical and radio-thyroidectomy-parathyroidectomy with I^{131} and At^{211} on breeding in the female Sprague-Dawley rat. In Medical Physics Quarterly Report, UCRL-3573, Nov. 1956, p. 5-6.

12. C. W. Asling, and J.G. Hamilton, Histological study of a monkey following alpha-irradiation. In Medical Physics Quarterly Report, UCRL-2243, June 1953, p. 35-58.

13. P.W. Durbin, C.W. Asling, M.E. Johnston, and J.G. Hamilton, The effect of propyl thiouracil on the recovery of rat thyroid gland from irradiation with astatine-211, Anat. Record. (In press.)

14. C.J. Shellabarger, P.W. Durbin, M.W. Parrott, and J.G. Hamilton, Effects of thyroxine and KSCN on capacity of rat thyroid to accumulate astatine-211. Proc. Soc. Exptl. Biol. Med. 87, 626-629 (1954).

15. E.S. Evans, Hormonal factors influencing metabolic rate in the rat (Thesis), Univ. Calif., 1956.

16. W.M. Garrison, J.D. Gile, R.D. Maxwell, and J.G. Hamilton, Procedure for Radiochemical determination of astatine in biological material. Anal. Chem. 23, 204-205 (1951).

17. M.W. Parrott, W.M. Garrison, P.W. Durbin, M.E. Johnston, H.S. Powell, and J.G. Hamilton, The production and isolation of astatine-211 for biological studies. UCRL-3065, July 1955, Univ. Calif. Radiation Laboratory.

18. R.K. Davis, G.T. Stevenson, and K.A. Busch, Tumor incidence in normal Sprague-Dawley female rats. Cancer Research 16, 194-197 (1956).

19. R.A. Fisher, Statistical Methods for Research Workers (Oliver and Boyd, Edinburgh, 1950).

20. P.W. Durbin, C.W. Asling, M.E. Johnston, N. Jeung, and J.G. Hamilton, Long term sequelae of massive doses of I^{131} in rats. Am. J. Roentgenol. Radium Therapy, Nuclear Med. (In press.)

21. J.J. Nine and G.L. Brownell, Radiation Dosimetry (Academic Press, New York, 1956) p. 823-830.

22. J.W. Casarett, The effects of ionizing radiations from external sources on gametogenesis and fertility in mammals, a review. Univ. of Rochester Atomic Energy Project, UR-441, May 1956.

23. W. Bloom, Histopathology of Irradiation (McGraw-Hill, New York, 1948) p. 598-673.

24. A. Glücksmann, The effects of radiation on reproductive organs, In Brit. J. Radiol., Suppl. 1, Certain Aspects of the Action of Radiation on Living Cells, p. 101-109 (1947).

25. M.W. Parrott and P.W. Durbin, The effect of early thyroidectomy by surgery, I^{131}, or At^{211} on breeding in the Sprague-Dawley rat. In preparation.

26. A.N. Contopolous, M.E. Simpson, D.C. Van Dyke, J.H. Lawrence, H.M. Evans, A comparison of the anemia produced by hypophysectomy with that resulting from combined thyroidectomy, adrenalectomy, and gonadectomy. Endocrinology 55, 509-515 (1954).

27. P.W. Durbin, N. Jeung, M.H. Williams, M.W. Parrott, and J.G. Hamilton, Relationships between radiation damage and endocrine deficiency after massive doses of iodine-131 in rats. Radiation Research 7, 313 (1957) (Abstract.)

28. D.S. Villars, Statistical Design and Analysis of Experiments for Development Research (Wm. C. Brown Co., Dubuque, Iowa, 1951).

29. P.W. Durbin, C.W. Asling, Induction of mammary tumors by astatine-211.II. In Medical Physics Quarterly Report UCRL-3880, July 1957.

30. R.C. Goldberg and I.L. Chaikoff, The cytological changes that occur in the anterior pituitary glands of rats injected with various doses of I^{131} and their significance in the estimation of thyroid function.

31. W.E. Griesbach, T.H. Kennedy and H.D. Purves, The physiological activities of the stereo-isomers of thyroxine. Endocrinology 44, 445-448 (1949).

32. C.W. Asling, P.W. Durbin, M.W. Parrott, M.E. Johnston, and J.G. Hamilton, Evidence for function of aberrant thyroid tissue in the thymus of rats. Proc. Soc. Exptl. Biol. Med. 94, 200-201 (1957).

33. T.T. Chen, R.E. Johnson, W.R. Lyons, C.H. Li, and R.D. Cole, Hormonally induced mammary growth and lactation in the absence of the thyroid, Endocrinology 57, 153-157 (1955).

34. W.A.D. Anderson, Pathology (Mosby, St. Louis, 1948).

FOOTNOTES

[1]This work was performed under the auspices of the United States Atomic Energy Commission.

[2]Astatine-211 is a radio isotope of the heaviest halogen. It emits alpha particles with a mean energy of 6.5 Mev and has a half life of 7.3 hours (2).

[3]Assuming a uniform distribution of At^{211} in the ovaries and a relative biological effectiveness of alpha particles equal to four, one finds the dosage to the ovaries is:

rad = (51.2/Mev/μC/g/day)(6.5 Mev)(80 μC)(1.2 x 10^{-2}/g)(0.45 day)(4)= 580 (21).

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 (20), and the P value was beyond the 1% level of confidence.

Table III

Formed elements of the blood 5 months after the administration of astatine-211 at a level of 0.5 μC/g body weight. Mean values are shown with standard errors.

	Normal Controls (45)	At^{211}-injected (17)	At^{211}-injected, thyroxine pre-treated (18)
White cell count (thousands/mm^3)	13.3 ± 0.6	8.43 ± 0.54	10.2 ± 0.6
Red cell count (millions/mm^3)	7.15 ± 0.08	7.04 ± 0.2	7.17 ± 0.14
Hemoglobin (g/100 cc)	14.3 ± 0.1	13.8 ± 0.1	14 ± 0.1
Hematocrit (cc RBC/100 cc whole blood)	56 ± 0.7	46 ± 0.5	48 ± 0.5

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	No. rats autopsied	Body wt (g)	Thyroid analysis wt (mg)	%I^{131}	%I^{131}/g	Weight of organ (mg) 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.

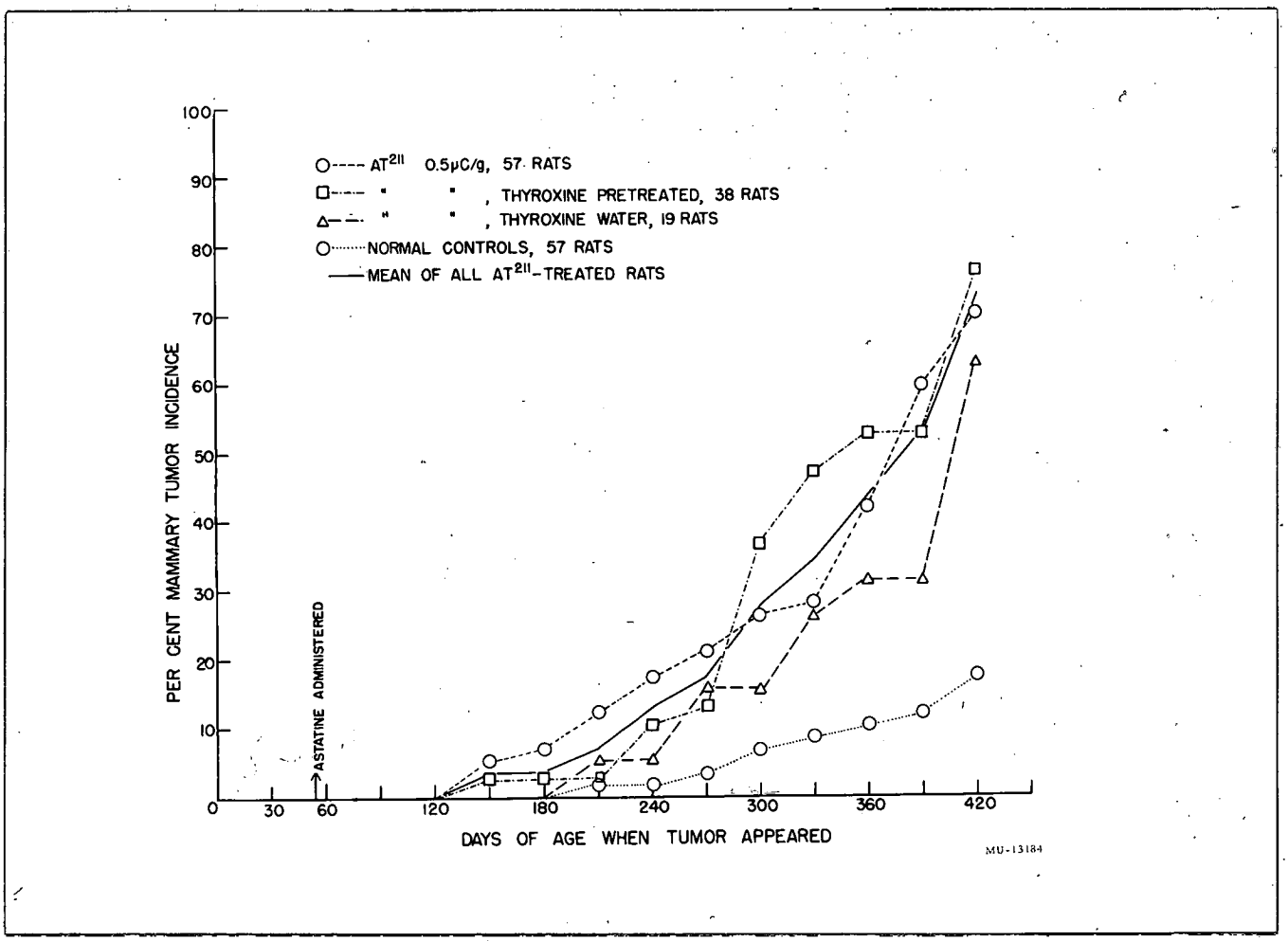
[b] Data available for 9 additional rats for a total of 55 individual measurements.

Table 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


100
90
80
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120
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240
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360
420
PER CENT MAMMARY TUMOR INCIDENCE
DAYS OF AGE WHEN TUMOR APPEARED
ASTATINE ADMINISTERED
AT211 0.5μC/g, 57 RATS
, THYROXINE PRETREATED, 38 RATS
, THYROXINE WATER, 19 RATS
NORMAL CONTROLS, 57 RATS
MEAN OF ALL AT211-TREATED RATS
MU-13184

Fig. 1