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A STUDY OF THE PHYSIOLOGICAL
FUNCTION AND HISTOLOGICAL CHANGES
OF THYROIDS IRRADIATED WITH
RADIOACTIVE IODINE

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1

This contract has supported a series of long term investigations on the morphologic and physiologic effects of radioiodine (^{131}I) on the thyroid. Earlier the attention was directed more toward the biochemical changes. In the last few years, more attention has been directed toward cellular changes, cellular replication, and neoplasm formation. A very detailed report was submitted three years ago describing all of the studies that had been carried out since the beginning of contracts on this subject in 1949. These studies will not be reviewed again here. However, the most notable work accomplished and published on radiation effects of ^{131}I under our AEC contracts may be listed as follows: The quantitative changes in iodinated amino acids and polypeptides in the circulation of man using quantitative chromatography following therapeutic doses of ^{131}I , particularly as it relates to the clinical variations in Graves' disease; the increase in thyroid cell height without significant cellular destruction following modest doses of ^{131}I in rats and chickens; the failure of the radiated thyroid to hypertrophy (when stimulated) but with the survival and synthesis of hormone by the radiated cells; the quantitative determination of DNA in radiated thyroid cells, showing the build up of chromatin but failure to complete the cell division, resulting in bizarre nuclear forms; the failure and degree of recovery of thyroid cells to synthesize DNA following non-lethal doses of ^{131}I ; the latent failure of mitosis long after all radiation was gone; the temporary retardation of mitotic capability followed by a surge of mitotic activity in radiated thyroids after certain modest doses of ^{131}I ; and a variety of successes and failures in attempts to produce thyroid tumors with ^{131}I with and without the accompaniment of goitrogenic agents.

In addition to the work on the biological effects on the thyroid in clinical subjects treated with ^{131}I and the changes produced in rat thyroids with ^{131}I , there are three other areas dealing with the effects of ^{131}I , but these are not directly supported by this contract. They have, however, contributed a great deal to this work supported by the AEC contract. The first collateral area indirectly related to this contract has been the National Thyrotoxicosis Follow-up Study based on 35,000 patients, more than half of whom had been treated with ^{131}I . The principal investigator has been serving as the Chairman of the Steering Committee which deals with the analysis of the data in that study. A publication concerning the benign and malignant lesions that have developed in these patients is appearing in the current issue (June 1974) of the Journal of Clinical Endocrinology and Metabolism. Another large publication is now in preparation dealing with the circumstances surrounding the development of hypothyroidism following ^{131}I therapy in the same large group of patients. Further mention of this work will be made as it relates to work under this contract.

A second collateral area related to the studies here is the survey and study of the Marshallese who were exposed to ^{radioiodines in} thermonuclear fallout in 1954 and who after the ninth year have been developing nodules in the thyroid. Three to 4 weeks have been spent each year or alternate years on the annual survey and thyroid examinations of these people. Almost annually new nodules have been found on the surveys and the patients have been brought back to the U.S. for thorough study of the radiation effects at Brookhaven National Laboratory and the removal of the thyroid nodules and special studies of the tissue here at this institution by the principal investigator. The maintenance of personnel and techniques for the study of radiation effects on thyroids of patients and rats under this present contract makes possible

the utilization of the Marshallese material. Observations on these people have been the basis for some of the experimental designs in rats.

The third area of collateral study is now coming to a close. A somewhat similar study dealing with possible lesions in the thyroids of children from ^{131}I in fallout areas of Southwest Utah and adjacent Nevada has also occupied considerable time in the past several years and has contributed to the work under this contract. The principal investigator has participated in the examinations and investigations of children who had been screened from the population and suspected of having lesions. Although the work has now come to a close and the evidence suggests there is no relationship between the radioiodines and the lesions found, some other important contributions have come out of this study. A total of four manuscripts have been submitted with Dr. Marvin Rallison of the University of Utah, School of Medicine. These deal with thyroiditis, the occurrence of nodules in a normal population and the course of adolescent goiter, all in school children.

Our laboratory is responsible for all diagnostic and therapeutic use of ^{131}I in thyroid disease at Cleveland Metropolitan General Hospital. Costs for the routine work are carried by the hospital. The principal investigator is a surgeon and because of research interests, consults on all thyroid problems--medical or surgical--in this hospital. This provides a unique opportunity to select from a large hospital population any patient for special study in whom a radiation effect is to be produced with ^{131}I . When an operation on the thyroid is necessary, this also provides an opportunity to procure thyroid tissue under special conditions and with exact preparation so that the most information concerning radiation effect can be obtained from the tissue removed.

The Effects of ^{131}I Radiation on the Function of the Thyroid in Clinical Subjects with Hyperthyroidism

In the past we made a large number of serial observations on each of over 200 patients who we were treating with large doses of ^{131}I for hyperthyroidism. The uptake of ^{131}I by the thyroid, its disappearance day by day from the gland for several weeks and the loss of the isotope in the urine were followed. In over 150 of these, the changing pattern of iodinated amino acids in the blood was followed by quantitative chromatography to observe the appearance and changing amounts of these compounds over periods of hours, days, and weeks as the radioactivity was taken up and then lost from the gland and from the circulation. The changing clinical picture and the degree of ultimate recovery from hyperthyroidism were correlated with the biochemical changes observed. This has provided information on the radiation effect. Some patients developed hypothyroidism very rapidly in spite of the fact that the dose of radiation per estimated gram of gland was thought to be appropriate to bring the patient to a euthyroid state slowly. Other patients seemed relatively unaffected by the calculated dose. Multiple doses were required in some of these patients. In such cases, the serial studies were repeated to see how the behavior of the ^{131}I the second time compared with the first. Unfortunately with the limitation of funds and personnel these detailed studies have been limited to only one or two patients a month.

These data already collected represent some of the most complete series of observations on individual patients available. In addition to enormous amounts of chemical data collected over several months on each patient, there is in progress

the long term follow-up of physiologic function of the gland. In a few cases, histologic samples of the gland have become available because surgery was indicated owing to the appearance of a mass or persistence of hyperthyroidism. This has provided an opportunity to study the effects of the radiation in various areas of the gland by autoradiography. Several publications have come from this work and have been listed in past reports.

The Kinetics of Therapeutic Doses of ^{131}I in Clinical Subjects

Up until recently, the study of the kinetics of the ^{131}I from our data has been at a standstill for want of persons capable of carrying out this work. Our hope that a computer analysis could be made of the kinetics of the ^{131}I in some of these patients is now being realized. This may furnish some insight into the reasons for success or failure of radioiodine therapy.

Many of our older cases on whom data were collected were included in the National Cooperative Thyrotoxicosis Follow-up Study. The kinetics of the ^{131}I in a few of the cases that had been submitted to that study from several laboratories were considered in a preliminary way. Although the need for information on the exact amount of radiation delivered was very important to the National Follow-up Study when considering the results of the therapy, the data were not analyzed because of the incompleteness of much of the data and for want of personnel who could deal with the problem. Dr. A. B. Brill, Vanderbilt University, helped set up the National Study and has been very interested in this problem. He has recently been in a position to start the study of the kinetics of ^{131}I from the data on some of the patients that we had submitted to the study. In the previous annual report we explained that some of the data on the changing pattern of radioiodine in the thyroid, blood and urine in selected patients had been sent to Dr. A. B. Brill of Vanderbilt University. When these data were studied in compartmentalized models, according to Berson's original model, and with modifications he suggested to better accommodate the therapy dose of ^{131}I , there were some interesting leads. We have had one joint meeting in Nashville and as a result the data on the quantitative changes in the individual iodinated compounds in the serum (rather than the serum compartment as a whole) have been supplied. Although there are variables that are not understood or even identified, Dr. Brill and his associates who are working with him seem very enthusiastic about the "fits" that seem to be evolving. The analyses at the present are being confined to data from the patients with uniformly diffuse thyroids of Graves' disease. Among the special cases with extensive data, we have selected some examples of patients who unexpectedly and rapidly went into hypothyroidism presumably because of extensive destruction of the gland. Others have been selected where the course of hyperthyroidism seemed completely unaffected in spite of a high uptake and satisfactory retention of the isotope in the gland. Another example of a special situation for study is a patient who had triiodothyronine (T_3) thyrotoxicosis and required four separate doses of ^{131}I at 3 to 4 month intervals. After each, serial chromatograms of serum at hours and days were made to quantitatively determine the changing amounts of circulating iodinated compounds. Here the large amount of circulating T_3 with protein binding characteristics different from thyroxine (T_4) presents a very different kinetic picture. One of the most important but unavailable variables that (by the nature of the ^{131}I therapy) cannot be directly obtained is the structural character of the particular gland which is being radiated. (We have felt that biopsy before ^{131}I therapy is not justified.) From a knowledge of the variable pathology that may exist in such thyroids, the question is whether the gland is extensively infiltrated with lymphocytes, whether

a large part of its volume is blood, whether it is involuted with a relatively large part of its volume occupied by colloid or whether it is very hyperplastic consisting mainly of cells. Whichever the case may be, the result of radiation undoubtedly will be different. These characteristics within the gland must have a major effect on the effectiveness of the radiation but such characteristics can only be inferred from a knowledge of the pathology as it relates to the features of the physical examination of the gland, i.e. firm, soft, spongy, pebbly, etc., but such information along with kinetic data may help unravel the explanation for the variable and sometimes unanticipated results of the radiation.

Basic Considerations of Neoplasm Formation in Animals and Man

We have been particularly interested in trying to observe the manner in which neoplasms of the thyroid evolve. As shown in our earlier experiments in rats and in studies by others, there is a considerable lapse of time between the administration of ^{131}I and the beginning of nodule or neoplasm formation. In our rats the interval is usually 14 to 18 months. The lengthy interval in man is very clearly shown by the observations in the Marshallese. Nine years elapsed following the ingestion of radioiodines in fallout before the first palpable nodules of the thyroid developed. The maximal dose of radioiodines to the thyroid of the Marshallese children was estimated to be about 1,400 rads. Two of the children (1 yr. old at the time of exposure) sustained enough damage so that severe hypothyroidism developed after some years but no neoplasms have been palpated in these boys so far. Of 17 other children who were exposed under the age of 10, there have been 15 who did not display evidence of hypothyroidism but have developed nodules.

It has been postulated that a chronic endogenous stimulus from the pituitary, resulting from some subtle degree of hypothyroidism, promotes the development of thyroid neoplasms; therefore, for the past 10 years all exposed Marshallese have been given thyroid and most of them took it. Although the thyroid neoplasms may have taken origin before the thyroid supplement was started, palpable masses have become evident with increasing frequency even while they were taking thyroid supplement. More will be said of this under proposals for continued work.

In the therapeutic use of ^{131}I for the treatment of hyperthyroidism it now seems clear from the National Thyrotoxicosis Follow-up Study that carcinoma of the thyroid is a very uncommon sequella of this treatment. In these cases the dose of radiation to the thyroid commonly ranges from 5,000 to 20,000 rads. It seems possible that the doses used in the treatment of thyrotoxicosis are sufficiently great so that the damage to replication (in spite of the survival of the cells) is such that the formation of new cells and perhaps neoplasms has been inhibited. From that study it also seems that neoplasms are more likely to be found when ^{131}I therapy was used in younger individuals. As will be explained later, it now seems that neoplasms are more likely to form after less damaging doses of ^{131}I .

At least two circumstances may have an important bearing on the development of neoplasms following ^{131}I . One concerns the damage to the cell which results in impairment to the manufacture and output of thyroid hormone and a secondary stimulus to the gland that may result from the deficiency. The other concerns the intrinsic nuclear damage that results in impairment, abnormality, or even failure of cell division. In referring to damage here it is important to keep in mind that the effects produced are not rapidly lethal to the cells, rather the cells survive for long periods with only mild impairment of function. If the development of benign or malignant neoplasms is dependent on chronic TSH stimulation then profound and

prolonged hypothyroidism should be an important promoting factor. On the other hand, if the cellular damage is so devastating as to cause severe hypothyroidism then that same damage may be so crippling as to hinder the development of a new growth which is dependent on cell division.

Experimental Production of Thyroid Neoplasms with ^{131}I in the Rat

In the most recently sacrificed large series of rats, there are some very interesting results that have come to light and lead to further points for investigation. Several important generalizations seem apparent in this series. We have been much more successful in producing neoplasms in the same strain of animals than at any time before. By using lower rad doses to the thyroid than formerly, neoplasms have developed in almost half of the animals. From our experience following and studying the Marshallese where doses of radioiodines to the thyroid were probably 1/5 to 1/10 that used therapeutically in man and where nodules have developed with surprising frequency, it seemed that lower doses in rats might be more productive of neoplasms. The neoplasms in rats began appearing with considerable frequency after doses as low as 2,000 rads. In previous experiments the dose of radiation received by the thyroid was based on the average uptake of representatives of what were thought to be identical members of a group of animals administered the same dose of ^{131}I . It has become apparent that there was considerable individual variation between the uptake and the rad dose received by the thyroid among these animals. In the current experiment, the actual uptake was measured in vivo in each rat at 24 hours to obtain the amount of ^{131}I in each gland. In addition the uptake was measured at 24 and 48 hours and almost daily for 19 days on 1/10 of the animals to get the effective half life of the ^{131}I in the glands. This required individual identification of the animals throughout the experiments. The observations proved the wide variation in the actual rad dose received and related much better to the degree of architectural distortion produced by the thyroid. Thus the animals in the most recent experiment were regrouped on the basis of the rad dose received rather than the dose of radioiodine given and an average uptake.

In this experiment doses ranging from several hundred rads up to approximately 10,000 rads were received by the thyroids. The thyroids of the majority of the animals received 1,000 to 6,000 rads. Some animals were sacrificed at 14 months and others at 18 and 22 months. At that time, the function of the thyroid was tested by ^{131}I uptake. For doses between 1,000 and 6,000 rads the uptake was not significantly different from the non-radiated animals. In alternate animals the ability of the thyroid cells to undergo mitosis and the ability of the gland to increase in weight was tested when the stimulus of thiouracil was introduced for 5 days. Although the thyroid weights of the radiated animals were much lower than those of the non-radiated animals, the thyroids of these radiated animals, when stimulated for 5 days, increased in weight markedly, but they did not reach the weight of the unstimulated non-radiated controls. The fact that the radiated thyroids responded by an increase in weight when a stimulus (thiouracil) was applied showed that the natural stimulus from any thyroid hormone deficiency would have come into play if the animals had been significantly deficient. This suggests that any functional damage to these glands, which were developing neoplasms, was minimal. Autoradiographs were made to show ^{131}I in the thyroid (and in neoplasms if present) and delayed autoradiographs were made to show the incorporation of ^3H thymidine incorporated into DNA in nuclei of thyroid cells if mitosis was occurring. The data on an increase in mitotic activity are not yet assembled.

The attached graph (Figure 1) shows the occurrence of neoplasms found in a total of 191 animals sacrificed at 14 months and later. Many of the neoplasms that were found were only microscopic in size at 14 months. One fourth to 1/3 of the animals whose thyroids received 2,000 to 6,000 rads were developing neoplasms at this time. A few tiny neoplasms were seen in thyroids which received somewhat lower doses of radiation. The development of neoplasms in the animals, however, increased in frequency later. At 18 and 22 months, almost 3/4 of the animals showed neoplasms. Some were grossly large and infiltrating the larynx and trachea (Figure 2). Although many non-radiated controls were sacrificed at the earlier periods no spontaneous neoplasms were found until the sacrifice at 22 months. The occurrence in the more heavily radiated animals (6,000 to 11,000 rads) was no greater than in those which received 2,000 to 6,000 rads (Unfortunately because of limitation of funds this series of rats did not include enough animals at the higher dose range, but the higher dose range used in the previous series had been considerable less productive of neoplasms). These observations are noteworthy for two reasons: 1) It now appears that we have found a dose range at which thyroid neoplasms develop quite readily. In our former experiments we were using considerably higher ^{131}I dose levels (more comparable to that used therapeutically in man) and finding fewer neoplasms. 2) The finding of fewer neoplasms at the earliest sacrifice in those exposed at the lowest dose levels and the development of the lesions in this group after more time elapsed may possibly be likened to the observations in the Marshallese (to be discussed elsewhere), where on the last three annual surveys (18th, 19th and 20th years) nodules were picked up for the first time in individuals on atolls lying somewhat further away (suspected lower dose) from the high concentration of fallout that had been found on Rongelap where the lesions began to appear at the 9th year. It seems that in the rats a longer interval was required for development of tumors after the lower doses, but they probably did not develop in significant numbers until a critical dose range (yet to be determined) was reached.

In our earlier experiments where larger doses of ^{131}I (10,000 to 100,000 rads) were given to the rats and fewer tumors formed, there was microscopic evidence of structural damage to the gland. Although thyroids were often not seriously altered in microscopic appearance, there was evidence of some architectural distortion. It was felt at that time that this was the desirable dose range for the best experimental design because it was more nearly the dose used therapeutically in man, and at least some architectural alterations has almost always been found in the human tissue we have obtained after ^{131}I therapy. Furthermore, it was postulated that to produce neoplasms there should be enough physiological impairment produced to bring into play the stimulus of TSH from the pituitary. It was the latter reasoning that prompted us in earlier experiments to use chronic thiouracil administration in some of the radiated animals to make sure that there was a stimulus from the pituitary just in case the damage to the thyroid had not been sufficient to reduce hormone production and provide an endogenous stimulus. We now have reason to question the part hormone deficiency may play in neoplasm formation but not necessarily in their growth.

The use of chronic administration of a goitrogen after radiation in earlier experiments has lead to uncertainty (as it has in the work of others) because it is not clear just how much the radiation effect contributed and how much the chronic stimulus of thiouracil contributed to the formation of neoplasms. As a consequence of the latter uncertainty we have used the antithyroid drug in the recent experiments for only 5 days to test other parameters (described elsewhere)

and otherwise eliminated the possibility of thiouracil as a cause of the neoplasms. The elimination of the goitrogen together with the lower dose of radiation has brought out several interesting facts; There has been little if any architectural distortion of follicles and little increase in connective tissue or inflammatory reaction from the smaller doses of ^{131}I that have proven to produce more neoplasms. The lobulated thyroids with involutionary areas and hyperplasia as seen with chronic thiouracil administration have been almost absent in the recent series of animals. Finally the recent material suggests that we have many more solid cellular tumors than papillary tumors in the present series. Thus it seems that the radiation effect is probably more prone to produce solid cellular tumors, which are often quite undifferentiated and infiltrating, and that factors which produce chronic hyperplasia are more likely to produce papillary neoplasms. The following table, although compiled from three different large series of animals representing a wide variety of ^{131}I dose levels, serves to illustrate the different conditions under which types of neoplasms have developed:

Treatment	Type of Neoplasm Produced		
	Solid Cellular	Follicular	Papillary
^{131}I Only	52	23	14
^{131}I + Chronic Thiouracil	14	16	19
Chronic Thiouracil; No ^{131}I	0	1	5
No Rx.	4	1	0

149 neoplasms in 323 animals. In some instances more than one neoplasm is registered in the same animal but in general only those of significant size could be classified.

The predominance of solid cellular undifferentiated neoplasms following ^{131}I radiation in the rats may relate to the observations in the 35,000 cases of treated thyrotoxicosis in the National Thyrotoxicosis Follow-up Study where all the undifferentiated lesions were found in those treated with ^{131}I while none of the lesions in those treated by primary thyroidectomy were anaplastic. There were no anaplastic lesions found among 47 malignant lesions removed in patients treated primarily by thyroidectomy while there were 5 anaplastic lesions in patients that had been treated with ^{131}I among 16 malignant lesions found.

Apropos of the observation that neoplasms did not develop as readily in rats in experiments where higher doses of radiation were used, it is interesting that a similar happening may have taken place among the Marshallese children. (There were 19 exposed under the age of 10). The two children who sustained the most physiological damage to their thyroids (ultimately juvenile myxedema) have not developed nodules, but 15 of the remaining 17 have developed nodules and undergone thyroidectomy. None were hypothyroid before operation. In earlier rat experiments we found that there was impairment to the capacity to undergo mitosis after rad doses which were still not sufficient to cause much architectural alteration. The damage in the Marshallese may have been sufficient to interfere with cellular replication and stop the normal replacement of thyroid cells (hypothyroidism became evident in the two children in 5 to 8 years), and in a similar manner may

have been sufficient to interfere with the development of neoplasms, which is also dependent on the capacity of the cell to replicate. More insight into this hypothesis may be gained by further studies comparing the capacity of rat thyroid cells to replicate after a 5 day stimulus following thyroid exposure to 2,000 to 3,000 rads on one hand and 7,000 to 10,000 rads on the other. The capacity for replication in each thyroid may then be related to the presence or absence of tumors that may have developed in these thyroids.

The Study of Manner in Which Neoplasms Develop in the Rat Thyroid Following ¹³¹I

One objective of our studies of thyroid neoplasms has been to identify the lesion at its earliest inception to see how such lesions originate. One of the questions was whether the neoplasm arises from cells within the wall of a well formed follicle or whether from clusters of epithelial cells lying as rests between the follicles. We feel that we have evidence that the lesions are arising as a proliferation of follicular cells in the wall of the follicle. In the most recent series of animals, where neoplasms have formed more readily, we have found numerous further examples of heavy labelling with ³H thymidine in the nuclei of cells filling the follicle. By comparison labelling in the normal follicles of the same gland is usually much less frequent. The earliest indication that a neoplasm is starting is the proliferation of cells in a follicle that is usually somewhat larger than others. It seems quite clear that these clusters of cells are proliferating from the wall of the follicle as seen in figure 3 where the proliferating cells have an abundance of nuclei bearing tritiated thymidine. Later the cells fill the follicle, as seen in figure 4, and distend it. The thymidine labelling in the cells in the follicles in figure 4 show these are multiplying cells and not desquamation of follicular cells. In reviewing serially the microscopic sections it is clear that these areas are arising as multicentric origins. These expanding follicles full of cells appear to become irregular and lobulated as seen in figure 5, or the cells appear to be breaking from the original follicle permitting the cells, with abundant nuclear labelling, to spread out among other adjacent follicles as seen in figure 6. As will be described later, similar solid cellular lesions have been found in Marshallese thyroids, see figure 7. In some instances the proliferating cells take on frond-like patterns in a giant follicle filled with colloid or just solid sheets of cells. An example of both types of lesion is seen in the two lobes of the same rat thyroid in figure 8. The neoplasms that are presumably rapidly growing (based on abundance of nuclei with the ³H thymidine label) are frequently necrotic in the center as seen in figure 9. The lesion illustrated has broken through the capsule of the thyroid and at that point of breakthrough the cells in mitoses are exceptionally abundant. Autoradiographs of the radioiodine in such tissues show no uptake of ¹³¹I in most all of these neoplasms, see figure 10.

The question may be raised whether these minute solid cellular lesions might be tangential cuts through the epithelioid bodies seen commonly in rat thyroids and thought to be rests arising from the ultimobranchial bodies. The solid cellular neoplasms may be distinguished from these keratinized bodies in two ways: 1.) The autoradiographs usually show the ³H in nuclei of a good many of the cells composing a neoplasm, indicating mitotic activity; while such labelling is essentially never seen in the ultimobranchial body rests that are buried within the thyroid. 2.) All thyroids are completely sectioned for microscopic preparations. Multiple representative sections through both lobes and the isthmus after serially sectioning the entire gland are mounted and studied, thus permitting a review of the entire gland.

Any mass of cells, whether an ultimobranchial body rest or a neoplasm, thus usually appears in more than one section. The ultimobranchial body rest is easily recognized by the keratinized center.

Studies on the Radiation Effects in the Marshallese

The principal investigator's participation in the annual surveys of the Marshallese people who were accidentally radiated by fallout primarily consisting of radioiodines from the thermonuclear shot at Bikini in 1954 has been referred to above. The principal investigator like some others, was consulted when the first nodules were palpated and removed nine years after the exposure. Aside from beta burns to the skin and temporary suppression of hematological elements (the first case of leukemia occurred just a little over a year ago), the only serious lasting effect was the latent damage to the thyroids in these people. The estimated dose to the thyroids on Rongelap ranged up to 330 rads in adults and up to 1,400 rads in children. The next nearest atoll was Ailingnae where the dose was estimated to be 132 in adults and 480 rads in children. The next and most remote atoll was Uterik where the estimate was 55 to 110 rads. A month was spent on one of the surveys in 1973 when five more individuals were discovered to have nodules. Three more cases were found later in 1973 making eight in one year. The observations made while screening the subjects on the atolls and the special studies on these people and their thyroid tissues have been extremely valuable to this project. These observations on the Marshallese have run parallel to and been the basis for some of the long term studies on radiated rat thyroids in this laboratory.

There have been discrete nodules removed from 19 of 82 exposed persons (53 still living) from Rongelap (3 were malignant) and 6 of 157 lightly exposed from Uterik (1 was malignant). Of the three new patients we have operated within the last month, one was an individual exposed at the 6th month of intrauterine life. This may be a very important case since the human thyroid does not take up iodine until the 13th or 14th week and then does so avidly after that time. Since this boy's thyroid should have been taking up radioiodines when his mother was exposed, the finding of 3 discrete adenomas in his gland may have been significant. There are two others known to have been exposed in utero. Although we have conducted experiments on pregnant rats, we have not used an experimental design comparable to this Marshallese observation. We think the observation should be explored - see proposal for continuation of work.

All of this Marshallese material is of great importance to the study under this contract. Although most of the neoplasms found in the Marshallese have been benign it is important to note that several carcinomas have been found. One of these had metastasized to many of the regional cervical lymph nodes. The radiated Marshallese thyroids often have many minute solid cellular or mixed papillary and follicular lesions in addition to a much larger lesion which had drawn primary attention. Many of the tiny lesions are occupying an area no larger than a dozen normal follicles just as is seen in the radiated rat thyroids.

The problem of drawing a distinction between the benign and the malignant nature of these tiny lesions on the basis of the microscopic anatomy in the Marshallese is similar to that in the rat. Although there has been some difference of opinion among some pathologists who reviewed the microscopic sections of these tiny neoplasms, they must abide by the accepted criteria upon which the

malignant nature of a lesion is based even if some bear the pleomorphic features of a lesion that might spread. Those larger lesions diagnosed as malignant have been uniformly considered so by all who reviewed the tissue. In all probability the minute lesions have not yet declared their malignant potential at this seemingly early stage of their development. In addition to the minute discrete lesions, there are often found follicles which are filled with a cluster of epithelial cells also similar to those seen in the radiated rat thyroid. An example of a microadenoma in a Marshallese thyroid is seen in figure 8. Unfortunately tritiated thymidine studies cannot be made on this human material to show mitotic activity as is done in the rat.

Radiation Effect on the Thyroid of Children in the Utah Study

The principal investigator's participation in the studies of the population of children exposed to radioiodines in fallout in southwest Utah has been mentioned above. A variety of pathological conditions came to light. Numerous children were recommended for further study at the clinical research unit of the University Hospital. When surgical exploration seemed indicated, tissues removed were studied by our laboratory for evidence of radiation effects comparable to those seen in the Marshallese and in the experimental rats. Children who were residing in this area at the time of fallout have sustained no detectable change in the thyroid that can be attributed to radiation. It is not clear just how much exposure to radioiodines in the fallout these individuals received.

Rallison, M.L., B. M. Dobyns, F. R. Keating, J. E. Rall, F. H. Tyler Thyroid Disease in Children: A Survey of Subjects Potentially Exposed to Fallout Radiation. Am. J. of Med. 56:457-463 April, 1974

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Attempted Experiments for Evidence of Genetic Effects

The question of a genetic effect in the offspring of radiated animals has been raised. An experiment which attempts to answer the question of genetic effects is difficult to design because any genetic effect transmitted to the fetus from the mother may have a superimposed hypothyroid milieu which may in itself affect a change in the fetus or its thyroid. Several observations may be made on the radiated mother rat from the standpoint of her reproductive capacity as well as the status of the fetus which she carries.

Two long term experiments were carried out using 19 female rats in the first experiment and 26 in the second with 5 serving as controls in each group. The animals were given 10, 20, 50, or 100 microcuries of ^{131}I . The thyroids took up approximately 2, 4, 10, and 25 microcuries respectively. After 4 weeks had passed

and all ^{131}I had disappeared from the thyroids, the animals were introduced to males. After two weeks, the males were removed. The pregnant animals were placed in individual cages and delivered litters which when weaned were identified as to litter and mother and isolated as to sex in cages for offspring. These young offspring were allowed to grow to a minimum of 200 grams at which time they were sacrificed. Shortly after the first litters were weaned all females were again bred and the same procedure followed for a maximum of five possible pregnancies. If a female remained barren after one mating, another breeding was not attempted until all other females were rebred at the same time.

Before sacrifice, the mother rats were given tritiated thymidine intraperitoneally and 1.0 uc. of ^{131}I to label those cells undergoing mitosis and to demonstrate thyroid function by autoradiography. The offspring were given only a tracer dose of ^{131}I to measure thyroid function in vitro. When the animals were killed, the weight of the thyroid and the uptake of ^{131}I in it were determined. Thyroid weights of both the mothers and offspring were expressed as milligrams of thyroid weight per 100 grams of body weight. The body weight was recorded as a measure of growth. The histological preparations of the mother's thyroids were prepared in serial fashion to permit a search not only for neoplasms but for evidence of architectural distortion produced by the radiation.

The experiments extended over a period of two years and three months. The thyroid weights and the ultimate ^{131}I uptakes of the radiated mother rats were significantly lower in proportion to the rad dose received by the thyroid. The body weights of these animals were comparable to non-radiated controls. There were a few barren breedings in both controls and the radiated rats. The doses of ^{131}I given and the degree of damage sustained by the thyroids of the mother rats were not sufficient to reduce the chance of pregnancy nor the size of the litters. The thyroid weights of the offspring, even the most heavily radiated mothers, were not significantly different from the offspring of the non-radiated mother controls. Histologic study of the thyroids of the offspring did not seem worthwhile. Neoplasms were found in the thyroids of some of the radiated mothers. The experiments although preliminary in nature revealed no evidence of genetic effect on the thyroid of the offspring nor reflected a hormone deficiency in the milieu within the mother.

Behavior of Some Rapidly Growing Thyroid Neoplasms Produced by ^{131}I

Some of the thyroid neoplasms in the rats were very rapidly growing lesions. In an effort to conserve radiated animals in some experiments until thyroid neoplasms were present, some animals were anesthetized and the thyroid exposed. If there was no suggestion of nodularity developing, the animal was permitted to survive longer in hope of finding a neoplasm. In a few instances when the animal was sacrificed only a few weeks later, a large neoplasm was found, indicating very rapid growth.

The abundant nuclear labelling in these anaplastic neoplasms with tritiated thymidine is very much in contrast to the labelling in the papillary lesions which often become quite large but show only an occasional nucleus labelled. The phenomenon of intense labelling of nuclei may be found in some very minute lesions, suggesting that such lesions display the apparent rapid rate of growth from their inception. Some of the small solid cellular neoplasms have only infrequent labelling and appear to be well contained within a capsule. In contrast, others (illustrated in figure 6) even though very small, have no capsular limitation and show a large number of nuclei bearing the tritiated thymidine label.

Some of the anaplastic lesions were found to be extensively necrotic in the center as some highly malignant lesions are inclined to outgrow their blood supply. Figure 9 shows not only a lesion with central necrosis but also shows near the capsule an abundance of nuclei which had taken up tritiated thymidine showing that a high proportion of the cells were undergoing mitosis within the preceding four hours before sacrifice. The illustration also shows how at the point where the cells had breached and had spread beyond the capsule, the rate of mitotic activity was even faster.

Since by trial and error we have not only found a range of rad dose of ^{131}I that produces neoplasms readily but also by sampling at different intervals have found about when such neoplasms might be expected to begin to form, we have a unique way to study the development and the behavior of neoplasms.

The Life Span of the Normal and Radiated Thyroid Cell

On several occasions we have proposed ways of determining the life span of the normal thyroid cell and then judge the life span of the cell crippled by ^{131}I . Partly because of time and funds but more importantly because the proposed experimental designs that we have contrived may not really answer the question, no substantial experiments have been carried out in this area. All of the experiments in the areas described in the report require such prolonged periods of time and expense that we must be reasonably satisfied with the approach before the experiment is undertaken. Many of the experiments reported under this contract have contributed ideas regarding the probable life span of the normal thyroid cell which is quite long, perhaps almost the life of the individual as suggested by Doniach; but just how long is yet to be determined.

The Importance of These Investigations

The importance of these studies is obvious because of the possibility of accidental fallout which might deliver sizable doses of radioiodines to the population. The theory that supplemental thyroid hormone is a deterrent to the development of thyroid neoplasms has never been adequately tested although the theory in the minds of some has been accepted. The reasons for uncertainty about the effectiveness have been stated.

In the clinical use of radioiodine there seems to be no very great fear of those doses now widely used therapeutically. The National Study tends to confirm this. However, it would seem from animal experiments that there must be in man a dose level that most likely could produce neoplasms, especially in younger individuals. This is suggested not only by the rat experiments but by the Marshalllese observations as well. The level that might produce neoplasms is probably something less than those levels used therapeutically but more than the lowest levels used for clinical testing. An attempt to identify those individuals in the National Study who had received inadequate therapy and thus possibly be more subject to neoplasms was considered but such patients were not permitted to go on untreated. Further treatment invalidated observation on this point.

Unfortunately the interval of time required to develop neoplasms in the thyroid is so long that it is difficult to make valid observations in man. It is almost impossible for one human investigator to collect sufficient comprehensive data and survive to interpret the outcome on his own fellow man. Without much doubt the National Study was terminated prematurely; furthermore, many nodules had appeared in patients who had been treated with radioiodine but the masses were being watched rather than removed. Obviously if these lesions had been the anaplastic type, they would have declared themselves promptly by a lethal outcome. On the other hand, the great majority of thyroid neoplasms are very slow growing and may exist for years before causing symptoms. A longer period of clinical observation would have been very valuable.

The latent development of hypothyroidism long after radioiodine treatment of hyperthyroidism was predictable and has become a serious problem. The National Follow-up Study which went to great extremes to find all patients, showed clearly how hypothyroidism had come on so insidiously and so long after radioiodine therapy that not even the patient remembered the warning of ultimate hypothyroidism that had been given him at the time of treatment. For the average overworked physician, hypothyroidism is a diagnosis that is readily overlooked in elderly people. When a large number of these patients were found, they were out of the active stream of society and their problem not recognized - often considered premature senility.

It is most important to study the radiation effects on the thyroid, hoping to just control the hyperthyroidism without producing another disease - hypothyroidism.

Figure 1

The occurrence of thyroid neoplasms in rats given various doses of ^{131}I when weanlings. The animals were sacrificed at 14, 18 and 22 months. The total heights of the columns represent the total number of animals sacrificed. The blackened portions represent the number whose thyroids contained one or more neoplasms. It is seen that only two non-radiated animals developed neoplasms and these were not found until 22 months. As time passed after 14 months more neoplasms were found. At 18 and 22 months, almost $3/4$ of the animals harbored a neoplasm.

Figure 2 A large carcinoma in each lobe of a rat thyroid. The lesion on one side had infiltrated the trachea. The dose of ^{131}I that had been delivered to this thyroid was rather high--10,800 rads.

Figure 3 A probable illustration of a thyroid neoplasm in a rat previously given ^{131}I . An example of thyroid epithelium proliferating in the wall of a follicle. Many of the cells forming the cluster have nuclei which have taken up tritiated thymidine, a precursor of DNA that is only taken up immediately prior to cell division. It appears that this represents a clone of cells multiplying more rapidly than cells in other follicles.

Figure 4 A follicle containing a cluster of thyroid epithelium. The nuclei of most all of the cells within the follicle contain the tritium label. This shows that they are proliferating cells and not just desquamation.

Figure 5 A minute solid cellular neoplasm of the thyroid from a rat 22 months after 3943 rads to the thyroid. This is an autoradiograph to demonstrate the location of tritiated thymidine in nuclei. There are many nuclei with blackened areas on the superimposed film suggesting rapid multiplication of cells. The extra nodular tissue shows only rare labeling of nuclei in comparison to the tiny neoplasm. There is little or no architectural distortion caused by radiation. Mag. xl20

Figure 6 A solid cellular neoplasm in a rat thyroid which received 2366 rads. Sacrificed at 18 months. This is an autoradiograph showing the location of cells undergoing mitosis. There is an enormous number of cells undergoing mitosis in this neoplasm. The lesion is somewhat larger than that in Figure 5. Its margins are ill defined showing lack of encapsulation and proliferation out among normal thyroid follicles whose cells show minimal (normal) mitosis. Mag. x120

Figure 7 An atypical thyroid adenoma apparently arising in a follicle in the thyroid of the Marshallese patient. Many such lesions may be found scattered throughout both lobes and are only coincidental to a larger lesion which had drawn attention and prompted surgical exploration. Mag. x85

Figure 8 Two lobes of a rat thyroid which received 2366 rads. The animal was sacrificed at 22 months. The solid cellular lesion can be seen breaking through its capsule into the surrounding normal tissue. The superimposed autoradiograph shows many of the nuclei of cells composing this lesion contain tritiated thymidine (not seen at this magnification) implying rapid cell division. The papillary lesion distending a follicle in the other lobe has few labelled nuclei in it and is probably slow growing.

Figure 9 A large solid cellular neoplasm in a rat thyroid which received 5914 rads 22 months before sacrifice. This is an autoradiograph of a lesion which is presumably growing very rapidly. The entire center of the lesion is necrotic apparently from outgrowing its blood supply. The black dots superimposed over the nuclei of cells at the advancing edge of the tumor are evident. At the top the malignant cells have penetrated the capsule of the thyroid and are spreading into adjacent fat. The mitotic activity at this point is such that about 1/3 of the cells were in the process of mitosis within the preceding 4 hours. (Tritiated thymidine was given 4 hours before sacrifice) Mag. x50

Figure 10 A microscopic section of a solid cellular neoplasm in a rat thyroid with a superimposed autoradiograph to show the distribution of ^{131}I uptake. Black dots over the neoplasm identify mitosis in this lesion. The thyroid received 4731 rads 18 months before sacrifice. This neoplasm takes up no ^{131}I but many of the nuclei of the neoplasm took up tritiated thymidine.