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

Final Report
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FINAL REPORT

This has been a broad and long term study of the morphological and physiological effects of radiation from radioiodine (^{131}I) on the thyroid of both man and rats. Numerous lines of investigation have been in progress since the first of a series of these contracts which began in 1951. Earlier more time was devoted to changes observed in man treated with ^{131}I for hyperthyroidism. With the work primarily centered in the department of surgery and being in charge of radioiodine therapy, there was a unique opportunity not only to study in great detail the precise behavior of a treatment dose of ^{131}I, but to obtain tissues under controlled conditions (when a thyroid operation was otherwise indicated) and thus relate the findings to extensive observations that had been made with respect to radiation long before. More than 500 patients with hyperthyroidism have been studied in great detail (to be described) and followed for many years with the hope that with the initial information stored on each of these cases the ultimate long term results over many years might be more meaningful. These human studies were embarked upon because of the bizarre nuclear changes we discovered in the late 1940's and the then unpredictable outcome of the radiation effect that resulted in hypothyroidism.

Simultaneously, experiments on the effects of ^{131}I radiation in rat thyroids were designed and carried out on many groups of several hundred rats each, trying to find the amount of radiation which would lead to the bizarre nuclear changes which might also lead to the formation of neoplasms. Although years were spent dealing with varying doses which for a long time were far too large; in later years it gradually became evident that thyroid neoplasms were more readily produced when the dose was so small that no obvious histologic changes were produced. Furthermore, it was necessary to use very young animals and observe the animals for at least one half of their normal life span. Each animal experiment required approximately three years for pos

irradiation observations and the many physiological and histological studies to be made on tissue to appraise the results. With experience, it was discovered that the neoplas not only appeared frequently but resulted with a high degree of predictability, if the actual uptake and retention of ^{131}I was known and the long period of post radiation observed. This predictability has provided a means for watching the development of neoplasms from their inception to large destructive growths in the radiated thyroid without the use of thyroid stimulating substances.

The various techniques of radioautography, developed or modified in our laboratory, using ^{131}I in the humans at the time of an operation and ^{131}I and tritiated thymidine (^{3}H-TdR) in the rats have provided important information on the physiological function and mitotic activity as a neoplasm developed. Many of these observations and the techniques used to study tissues lead to the studies on the Marshallese who we have operated. The detailed biochemical studies on individuals treated with radioiodine also lead to important contributions to kenetic data on some patients in the National Thyrotoxicosis Follow-Up Study with which we have a major part.

These studies have lead to the collaboration with numerous other groups with similar or collateral interests: Dr. A.B. Brill (formerly Vanderbilt, now of Brookhaven National Laboratory) for analysis of 9 compartment iodine kinetics; Dr. P. Reed Larson (Harvard) for refinement of our rat TSH assay; Dr. Hunter Heath (Mayo Clinic) for rat calcitonin assays; Dr. Robert Conard (Brookhaven National Laboratory) in the study of Marshallese; Dr. E.D. Williams (Cardiff) for identification of C-cell neopla

Objectives

The objectives of the research in clinical subjects were:

1. To obtain precise detailed data on the amount and behavior of ^{131}I radiation delivered to the thyroid for reference to the subsequent observations on that thyroi

2. To obtain sequential biochemical and kinetic data on the behavior of therapeutic ^{131}I for 3 or 4 weeks in selected cases of hyperthyroidism.

3. With the above extensive data (not commonly available with routine treatment) in hand, to follow the clinical results in order to determine the causes for failure of therapy, for overtreatment and for the development of nodularity; and, in addition, to find out what pretreatment characteristics will alter the retention and therapeutic behavior of the dose to determine the need for adjustments of the therapeutic dose upward or downward.

4. To study in detail (functionally as well as morphologically) any irradiated thyroid tissue that becomes available from whatever the source may be: ^{131}I therapy, x-ray therapy to the head or neck, or exposure to fallout in the case of the Marshall islanders.

The objectives of the animal research were concerned primarily with the production of neoplasms with ^{131}I in rats:

1. To develop a model for readily producing thyroid tumors solely with ^{131}I.

2. To use the model to study the circumstances that lead to neoplasms in the irradiated gland.

3. To study the development of neoplasms from their earliest inception and to see how different histologic patterns evolve. This may be seen in rat thyroids of certain age destined to develop neoplasms at a given time after irradiation.

4. To determine whether a hypothyroid state and/or TSH play a part in the induction of such neoplasms by using radioimmunoassay for TSH.

5. To see whether the predictable development of neoplasms may be inhibited.

6. To appraise the nature of the failure to produce neoplasms after somewhat larger doses than the "critical" dose which produces such neoplasms but causes no obvious evidence of histologic damage.

Results

Radiation Effects of Therapeutic Doses of ^{131}I in the Thyroid in Clinical Subjects with Hyperthyroidism

Serial observations have been carried out on selected patients whom we treated with therapeutic doses of ^{131}I for hyperthyroidism. The patients have been followed and studied for many years thereafter. The important question was whether there is a minimal dose of ^{131}I which will bring a hyperthyroid patient to a euthyroid state without ultimately developing hypothyroidism. The other question was whether an inadequate dose of ^{131}I is more inclined to lead to neoplasm formation.

Extensive pretreatment data was collected on each patient selected for this special study. The uptake of ^{131}I by the thyroid, its disappearance day by day from the gland, and the amount and the changing chemical form of radioactivity in the circulation for several weeks, as well as the loss of isotope through the urine for 3 days, have been followed after the treatment dose. The results obtained were ultimately related to the changes that took place in the clinical picture and the degree of recovery from hyperthyroidism. These observations provided information from which the actual rad dose delivered to the thyroid could be determined.

The therapeutic objective in the use of ^{131}I was to bring the patient down to a euthyroid state and no lower. However, some patients developed hypothyroidism very rapidly in spite of the fact that the dose of ^{131}I per estimated gram of gland was thought to be appropriate to bring the patient slowly to the euthyroid state. Other patients were relatively unaffected by what was calculated to be a fully effective dose. The observer accuracy of the estimated weight of the glands was constantly checked by the same observer who was operating similar surgical cases and saw the glands. Multiple doses were required in approximately one-fourth of these patients. In such cases, the serial studies following a subsequent treatment were repeated to see how the behavior of the ^{131}I the second time compared with the first. When

repeated doses were required, the uptake was usually progressively less and the retention of the isotope more sustained. As has been pointed out in previous reports, some of the data collected in the past represents some of the most complete observations on individual patients available. These became a part of the National Thyrotoxicosis Follow-Up Study. In addition to enormous amounts of chemical data collected over several months on each of these patients, there has been continuous long-term follow-up of physiological function of the radiated gland. A knowledge of the precise rad dose received by the gland is the only way that the outcome of radioiodine therapy can be evaluated. Unfortunately, the dose that was administered to the patient is no measure of the amount of radiation received without detailed study

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. The procurement of tissue has provided an opportunity to study by autoradiography the effects of the radiation in various areas of the gland. Thus, we have continued to follow our radioiodine therapy cases diligently with repeated examinations to look for the development of masses and to reappraise thyroid function.

Some of the significant biochemical, radiological, and clinical correlations that have been found are:

If the serial quantitative chromatograms on the blood following a therapeutic dose of ^{131}I show radioiodinated mono- and diiodotyrosine after a few days, a precipitous decline in radioactivity over the thyroid usually occurs and this is followed by extensive destruction of the gland. Chromatographically insoluble iodinated polypeptides also appear in the blood. This indicates gross destruction of the gland with the release of colloid material into the circulation.

If the radioactivity in the gland followed a reasonably straight line decay, representing normal cycling of iodine back to the thyroid, but subsequently (after 7 to 14 days) followed an altered and more rapid decline, hypothyroidism was predictable at a reasonably early date. Simultaneous with the change in the thyroidal disappearance curve, there was a rise in radioactive iodide in the serum and in the urine, showing that the trapping mechanism for iodide in the thyroid had been damaged. Loss of this mechanism appears to be the first physiological evidence of radiation damage. The free iodide which becomes available from the deiodination of thyroxine (T_4) is not returning to the gland.

If the amount of free circulating radioactive iodide is less than the amount of radioactive thyroxine 4 or 5 hours after a treatment dose has been given, the disappearance from the thyroid will be rapid, the circulating radioactive T_4 will be sustained high, the dose of radiation to the thyroid will be less than anticipated and the treatment will not be fully effective. This indicated a very rapid synthesis and turnover and was associated with the more clinically toxic patient. Inadequate therapy was most unfortunate.

If radioactive triiodothyronine (T_3) was found to be high in the circulation a few hours after treatment, it will rapidly find its way into the urine and thus not be available to recycle through the thyroid. The result has been less than the expected therapeutic effect. If the desired dose is calculated in the usual way, three or four treatments will be required in high T_3 producing patients.

The ideal treatment of hyperthyroidism with ^{131}I should be to bring the patient to a euthyroid level and no lower. Throughout these studies over 30 years, it is apparent that almost all patients treated with ^{131}I will ultimately become hypothyroi Although some physicians give a "standard dose" of ^{131}I and wait to see if it has cor rected the disease, it is more desirable to strive for the euthyroid state. As a res of these studies, it is clear that the dose required among individuals is quite varia

The problem of selecting the right dose has been approached. It is well known that the histological structure of glands that cause hyperthyroidism is quite variable. That variability appears to have considerable bearing on the effectiveness of the size of the dose of ^{131}I required. Although we have not resorted to biopsy before treatment is given, much has been gained from reviewing the histologic features of all glan thoroughly studied and characterized before surgery and then by using those clinical features (hardness, softness, lobulation, nodularity, bruit, etc.) to estimate the structural character of the particular gland that is to be irradiated. This has appeared to be as important as the weight or size of the gland because much of the volume may not be composed of thyroid cells. With the limited range of the beta ray within the thyroid gland, ionization may take place in structures distributed among thyroid cells. Thus, the ionizing effect may take place at sites other than the thyroid cell. The gland may be extensively infiltrated with lymphocytes, in which case, it will be more firm and rubbery on examination. In contrast a considerable part of its volume may be blood and as a result it will be very soft, compressible and have a bruit. The gland may have become lobulated and thus involuted with a considerable part of its volume occupied by colloid with only spotty areas of hyperplasia. We kno from the radioautographs that the ^{131}I is then taken up in a very mottled pattern. Such a gland is lobulated and rather firm, but not as firm as when infiltrated with lymphocytes. This gland often feels rather pebbly. The very hyperplastic gland with few if any lymphocytes or colloid is composed of relatively more cells. From our observations, we believe these intrinsic characteristics of the gland of Grave's disea play a major part in the effectiveness of the radiation. The greater the space occupied by lymphocytes, colloid, or connective tissue the more ionization is spent in these structures rather than in the cells that produce hormone. Such characteristic can only be inferred from a knowledge of the pathology as it relates to the features of the physical examination. However, any physician or surgeon who devotes a great

deal of his time to examining thyroids, judges and records the character of the gland at physical examination and then personally reviews the histology on each gland that is operated should be able to correlate the physical findings with the histology with reasonable accuracy. We believe that we have shown in these studies why the dose of ^{131}I produces prompthypothryoidism in some patients and is relatively ineffective in others. The calculated dose to be taken up by the gland may be modified to fit the circumstances. Similarly the physician who records his preoperative estimate of the thyroid weight and then sees and weighs the amount of tissue removed, has performed a unique control of his ability to estimate accurately the weight of the thyroids. With experience he can refine his judgement of weight for those to be treated with ^{131}I.

Our data to be collected on the clinical features of the gland in patients before treatment with ^{131}I have been assembled on index cards so that many of these considerations have been explored in the light of the thoroughly studied clinical result. If a more precise estimate of the dose of ^{131}I that is needed can be achieved, then perhaps there can be more correct doses given, fewer early developments of hypothyroidism and fewer instances of repeated treatments being required.

The Kinetics of Various Treatment Doses of ^{131}I

The collection of data on the changing pattern of ^{131}I iodinated amino acids and free iodide in the serum and urine and the disappearance of ^{131}I from the thyroid following treatment doses for hyperthyroidism has been mentioned. Serial measurements of iodinated compounds were carried out by quantitative chromatography almost daily for a week and weekly for several weeks. There are over 175 Graves' disease patients so studied. The quantitative data on blood and urine along with estimated weight of the gland, the size of the dose given, the size of the dose lodged in the gland and finally the features of the clinical response that followed were given to Dr. A.B. Brill (formerly of Vanderbilt University) for analysis. He and his associates have been studying and analyzing this material in compartmentalized models originally

devised by Berson. Rates of flow of ^{131}I among compartments reflect the physiological state of hyperthyroidism and the development of a radiation effect. The results are in draft form in the hands of Dr. Brill. Two manuscripts have been prepared describing the results from these analyses. Publication has been delayed by Dr. Brill's recent move to Brookhaven National Laboratory.

The Development of Neoplasms of the Thyroid Following Irradiation

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 synthesis and output of thyroid hormone, resulting in a secondary stimulus to the gland from the pituitary (TSH). The other concerns the intrinsic nuclear damage that results in the production of abnormalities within the cell, or failure of cell division. In referring here to damage to the cells, it is important to keep in mind that the effects produced are often 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 prolonged mild 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 would be so crippling as to hinder the development of a new growth which is dependent on cell division.

Our studies have shown that neoplasm formation in the rats requires an interval of 14 to 18 months from the time of the irradiation. The lengthy interval in man is clearly shown in the Marshallese where, in spite of thorough annual physical examina the first palpable nodule was not found for 9 years and neoplasms are still appearing at 26 years. (See twenty-six year report). Many of the experimental ideas for our laboratory have come from our experience in operating the thyroid neoplasms of the Marshallese. The maximal estimated dose to the thyroid in the Marshallese was 1,150

rads in children and 550 in the adults. Only 2 of 19 children exposed under the age of 10 developed frank clinical hypothyroidism and that required a number of years to become evident. This raised a question whether the estimate of the dose in the Marshallese was too low. Both children were irradiated at about 1 year of age. Neither of these has developed nodules. Presumably, cellular damage was sufficiently great to preclude thyroid cell replication. Of the other 17 children who were exposed, 15 did not display clinical hypothyroidism but have developed neoplasms and been operated.

Our experience finding carcinomas in radioiodine treated patients in the National Cooperative Thyrotoxicosis Therapy Follow-Up Study seems to be quite contrary to the Marshallese experience. An unusually small number of carcinomas was found; in fact, considerably fewer than would have been expected, considering the relatively larger number of occult malignant lesions found in Graves' disease treated by surgery. Such incidental lesions found at surgery might have been expected to grow if unknowingly left in place and ^{131}I treatment had been given. The difference between the doses received by the Marshallese thyroids and those of Graves' disease is considerable. Graves' disease patients usually receive 5,000 to 14,000 rads while the adult Marshallese received an estimate of 550. Furthermore, the hyperplastic gland of Graves' disease would be expected to be far more sensitive to the radiation than the normal glands of the Marshallese.

Our animal experiments and the most recent studies of other investigators using rats seem to have suggested strongly that it is the smaller doses of ^{131}I that produc circumstances under which neoplasms arise. Indeed the effects are sufficiently mild so that no microscopic architectural distortion is recognized in the thyroids most inclined to develop neoplasms.

In our studies we have shown that there was a failure of the moderately irradia rat thyroid to hypertrophy when stimulated by giving an antithyroid substance. We h also shown in such thyroids that there is a marked reduction in the capacity of such

irradiated cells to take up tritiated thymidine and thus prepare for cell division under the stimulus. This explains the failure of the thyroid to enlarge when the stimulus is applied. In other earlier experiments under these contracts we showed by quantitative histochemical measurements of DNA that the nuclei of an occasional cell contained much more than two times the diploid value, at which time the cell should have divided. We pointed out many years ago when we obtained the first biopsies of thyroid treated with ^{130}I and ^{131}I that the large bizarre nuclear forms with excessive chromatin resembled neoplastic cells that might have malignant potential. (This was the primary basis for our original AEC Contract). The cummulative evidence increasingly supports the idea that under such conditions those altered cells, which can and do synthesize DNA and occasionally complete the process of mitosis are the source of neoplasms. It does appear that with heavier irradiation the number of cells retaining a capacity for mitosis is reduced and thus the chance of a neoplasm developing is les

We know from previous animal experiments, and suspect from observations on clini subjects, that there is a difference between the effect of a relatively large dose of ^{131}I which causes both impairment to the synthesis of thyroxine and intrinsic nuclear damage (that precludes subsequent cell division) and the small dose which damages the nucleus but does not seriously impair thyroxine production. The problem has been to determine these respective doses in the experimental animal.

There is a dose level of ^{131}I to the thyroid in man that results in a latent failure of the thyroid hormone production after all of the isotope is gone and years have passed. (See report by Larson, et al including this author). As we pointed out more than 20 years ago from the work under the earlier AEC contracts, the failure is attributable to intrinsic nuclear damage which does not destroy the cell nor its hormone production but does result in ultimate death of the cell when mitosis is attempted.

Several months following a modest dose of ^{131}I to rats and a latent period during which few cells can be stimulated to synthesize DNA, a supra-maximal surge by many cells synthesizing DNA was produced by stimulating such a gland. This occurred before many of the cells had succumbed and the gland had begun to shrink in size.

It was these and other related observations that led to further experiments in which progressively smaller and smaller doses of ^{131}I were used in large groups of rats. With each succeeding experiment which required 2 to 3 years to complete, more neoplasms developed. The need for longer periods of observation became apparent. The neoplasms first began to appear at 14 to 16 months. When there was reasonable expectation that neoplasms were soon to appear, many animals were sacrificed and autoradiographs were prepared by giving a trace amount of ^{131}I just before sacrifice to locate sites of function and tritiated thymidine (^{3}H-TdR) to locate the nuclei synthesizing DNA. With longer periods of waiting, neoplasms in all stages of development were found and studied. Three types of lesions commonly seen in these animals were papillary, follicular and solid cellular. At least some of the lesions that develop spontaneously in elderly rats were parafollicular cell (medullary) type as described by Lindsay and by Williams. The remainder of the solid cellular lesions were rapidly growing as implied from the abundant labelling of the nuclei by ^{3}H-TdR.

The details of the development of the lesions have been described in a manuscript which was submitted for publication but publication has been delayed for reasons to be described later. When large numbers of animals were sacrificed at the time neoplasms could be expected to be developing and thereafter for months, the sequence of the development of neoplasms could be studied. The multicentricity of lesions in the same animal and the lesions found in multiple animals sacrificed at intervals permitted an interpretation of the development of the lesions. Papillary lesions arose from follicular epithelium. Occasional follicles several times the diameter of the surrounding normal follicles appeared. This was the first sign of a localized abnormality develop

Such follicles often contained darker staining colloid. Autoradiographs showed that such follicles have essentially no uptake of ^{131}I. The cells comprising the wall of such a follicle are thus functionally different from others nearby that are functioning Such giant follicles may be found containing a cluster of cells arising from one local in the wall rather than the normal single layer of cells lining the rest of the follicl Some of the masses of cells may be forming papillary tufts protruding into the follicle These cells have a discernably higher incidence of nuclei bearing the ^{3}H-TdR label than cells in other parts of the wall of that follicle or in other follicles nearby. The follicle appears to enlarge to accomodate the expanding papillary lesion within. Ultimately the cells of the parent follicle are replaced by connective tissue forming a capsule as the total mass comes to occupy more of a lobe. Although there may be quite a number of the enlarged non-functioning follicles in a single irradiated thyroid and a few of the lesions described above appear in a single thyroid, but they are in different stages of development in a given animal. This is the evolution of papillary neoplasms.

The follicular neoplasms appear to evolve much as the papillary lesions. Tiny follicles develop among the papillary structures. The parent follicle continues to expand until the structure appears to be an encapsulated follicular adenoma. Multiple lesions are again seen in a single thyroid, where as solid cellular lesions are most often single but may be accompanied by papillary or mixed papillary and follicular lesions in the same thyroid. The application of autoradiography using ^{131}I and ^{3}H-TdR separately prepared from adjacent sections of tissue, with such observations on very large numbers of rats, have made the described sequences of development rather obvious The material acquired for these observations was based on four experiments composed of several hundred animals each and lasting 16 to 22 months.

The solid cellular lesions are more inclined to arise in follicles which are similar to the normals in size. A site of cellular proliferation occurs in the follicular wall. As many as 50% of the cells forming a tiny mass of perhaps a dozen cells are labelled with the ^{3}H-TdR label showing that within the 4 hours before sacrifice many of the cells were picking up tritiated thymidine in preparation for mitosis. Before the relatively small follicle is filled with cells, some of the cells bearing the ^{3}H-TdR label are found outside of the basement membrane of the follicle. These apparently rapidly proliferating cells appear to be spreading to form a solid cellular mass. Some of these lesions are partly encapsulated but remnants of what appear to be partial encapsulation are seen in the tumor mass. Spread of the neoplasm beyond the true thyroid capsule ultimately may be found. Examples may be found where the ^{3}H-TdR label is moderate, but at the point where the lesion has breeched a thyroid capsule and spread into surrounding fat, the ^{3}H-TdR labelling in the advancin margin of cells in greatly intensified.

Rats, particularly the Long-Evans strain as used by Lindsay & Chaikoff and associates, are prone to develop spontaneous neoplasms composed of interfollicular or C-cells as animals age. The neoplasms form solid masses of cells which in the rat may be confused with the solid lesions arising from the follicular cells. Some rats also show considerable hyperplasia of C-cells which may be a precursor of C-cell neoplasms. In our early experience we could not distinguish between the two types of lesions and considered them all "solid cellular". We intially assumed (rightly or wrongly) that C-cell tumors did not represent a significant number of the neoplasms in our irradiated rats because the solid cellular lesions were very uncommon among controls in our particular strain of Sprague-Dawley rats. The failure to be able to identify which solid cellular lesions were of C-cell origin and which were of follicular cell origin not only left our analysis of the incidence of the two types in question but also weakened our hypothesis that most of the solid lesions were of follicular

cell origin and that C-cell neoplasms were not readily induced by radiation. The solid lesions were very infrequent in controls of the same age.

Radioimmunoassays for rat calcitonin in serum collected at the time of sacrific from animals which had been found to have solid cellular tumors, did not clarify the issue because the titre was quite variable from animal to animal and the frequency of interfollicular cell hyperplasia (found in rats) varied from animal to animal, many of which did not have a demonstrable solid cellular tumor. We are grateful to Dr. Hunter Heath of the Mayo Clinic for carrying out sample assays of rat calcitonin for us. Dr. E.D. Williams of the University of Wales, Cardiff, being an author on the medullary carcinoma of man and the interfollicular cell lesions of rats, was called upon for assistance in the identification of the C-cell lesions. He has point out to us by direct personal assistance the characteristic features of these lesions while reviewing many microscopic examples. The features are rather easily recognized.

Since the interfollicular cells normally lie between the follicles, their proliferation, as in hyperplasia of such cells, tends to spread the follicles apart. A neoplasm arising from such cells not only expands the interfollicular space in a local area, but appears to completely surround an occasional normal follicle. The expanding neoplasm becomes lobulated with thin connective tissue margins and septae around the clusters of proliferating cells, a feature which Williams has appropriatel called "packeting". Normal follicles often are trapped in these growing lobules. Fortunately our procedure of double labeling with ^{131}I and ^{3}H-TdR with radioautograph has not only shown increased mitotic activity in such spreading lesions, but has alsc distinguished a trapped normal follicle (because of ^{131}I uptake in it) from the tiny follicles sometimes seen in the undifferentiated solid cellular lesions with an occasional primative follicle which does not pick up iodine.

The uncertainty of distinguishing C-cell neoplasms from other solid cellular lesions prompted us to hold up publication describing the high incidence of neoplasms following low doses of ^{131}I. This was the first of a series of 3 manuscripts submitt

for publication and submitted (in manuscript form) with a former annual report. Since it now appears that we can distinguish C-cell neoplasms from other undifferentiated solid cellular lesions in irradiated animals, several thousand microscopic preparations with radioautographs are being re-reviewed. Thus, the previous manuscripts will soon be modified to account for the two types of solid cellular tumors and resubmitted.

The Contribution of the Marshallese To The Radiation Study In Our Laboratory

As has been mentioned above, the study of the Marshallese has contributed a great deal to the study of radiation effects on the thyroid. The Marshallese were exposed to radioiodines in the thermonuclear fallout in 1954. They are continuing to develop masses in their thyroids more than 25 years after exposure. We have now performed thyroidectomies on 45 out of a little over 330 that were known to be exposed. The tissues are of great importance to the study under this contract. The fresh tissue when removed in this institution by us has been studied promptly by methods established and maintained in use under this contract. Autoradiographs for ^{131}I have been prepared on all tissues removed.

This investigator has participated in nine of the annual survey examinations of thyroids of the exposed persons in the Islands. During the course of the present contra (since 1975) 18 exposed individuals have developed masses and have been brought back to Brookhaven National Laboratory for clinical study and then to us for thyroid exploration. All exposed thyroids operated since 1975 have contained adenomas and six have had carcinomas. To date we have removed 9 carcinomas and 2 adenomatous nodules with papillary carcinoma from the 45 exposed individuals of all ages from several atolls. Three of the carcinomas had metastasized to regional cervical lymph nodes. Four carcinomas came from an atoll with extremely low exposure. Since this is a population which seldom seems to develop thyroid nodules, the relationship to the radiation which was primarily radioiodine is most impressive.

The irradiated Marshallese thyroids often have many minute solid cellular or mixed papillary and follicular lesions in addition to the much larger lesion which had drawn primary attention on physical examination. 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.

An exposed Marshallese woman on whom we did a total thyroidectomy in 1969 for carcinoma has developed a pituitary tumor, as evidenced by erosion of the sella tursica. This may be the first human example of the Furth mice in which the thyroids were destroyed and a pituitary tumor rich in TSH subsequently developed. In this clincal case, the woman had presumably been taking the T_4 supplement as supplied. The final important observation among the Marshallese has been the development of lesions in 2 young men who at the time of exposure were in utero. As near as can be determined, the fetuses were sufficiently developed so that the thyroid should have taken up radioiodine. The thyroids in these cases showed multiple adenomas. We have recently operated one of the mothers for what proved to be multiple "atypical" adenoma

The long delay in the development of neoplasms following irradiation is emphasiz in the Marshallese as well as in the animals. Our experience has shown that the rats do not develop lesions readily unless they are irradiated when very young and 1/2 to 2/3 of the life span has lapsed. The first Marshallese lesions did not develop for 9 years. Many of the first lesions found came from the atoll with the greatest fall-out (Rongelap). It was quite some years later that lesions began appearing in people who were on the next nearest atoll (Alingnae) where the dose had been somewhat less. While lesions were appearing on the nearer atolls, the low dose received on an atoll much further away (Uterik) seemed to have produced no lesions. However, in the most recent years, 8 individuals have been operated from this most remote atoll. Four of the carinomas found have come from this atoll. These observations emphasize even more the risk of the low dose range and the long latent period to produce neoplasms.

Endogenous Thyroid Stimulating Hormone (TSH) in Neoplasm Formation Following ^{131}I Radiation

In one of the more recent rat experiments using small doses of ^{131}I and periods of observation of almost 2 years, we have succeeded in obtaining neoplasms in almost 75% of the rat thyroids. The yield has become sufficiently high in this model so that it has been possible to test the factors which inhibit or stimulate the induction and growth of such lesions.

TSH has been theoretically implicated in the development of thyroid neoplasms. Observations that tumors were produced by the use of chronic iodine deficient diet or by the chronic use of goitrogenic substances supported the belief that the damage caus by radioiodines might operate in a similar fashion. Just as the lack of iodine to mak thyroid hormone, or the block of the synthesis of hormone a deficiency of thyroid hormone with a compensatory increase in the output of TSH, so radiation damage to the cellular mechanism for hormone production may work in a similar way. Lindsay, Furth and Doniach have independently suggested that there may be a double mechanism responsible for the radiation induced neoplasms, i.e., that radiation might initiate the neoplasm and TSH promote its growth. The principal investigator first noted cellu hypertrophy (increase in cell height) in the thyroid of some of Skanse's chicks that had been given ^{131}I (1948). This has been observed by many investigators and has been considered a manifestation of the stimulus from TSH, but assays for TSH, until the las few years, have not been sufficiently sensitive to detect reliably the very slight elevations of TSH unless there was major damage to the gland. We have learned that ne plasms develop much more consistently following surprisingly small doses of ^{131}I rathe than large ones. We also have learned that when there is histologic evidence of damag from ^{131}I, the incidence of neoplasms is much decreased. In studies published under our former AEC contracts, we have shown that radiation from ^{131}I caused intrinsic dama that might impair the capacity for cellular replication without seriously hampering hormone production. Thus, if damage is sufficient to cause a rise in TSH to significa

detectable levels, the degree of damage may then preclude neoplasm development. The need for a very sensitive TSH assay was obvious in an experimental design to test the subtle effects that TSH might have in irradiated animals that should be developing neoplasms some of which were given supplemental T_4.

Some of our observations on the Marshallese patients have prompted us to question the part played by TSH in the origin of neoplasms, because when the first Marshallese began to develop nodules, they were given prophylactic thyroid hormone (T_4) to inhibit the development of more neoplasms. It has subsequently been shown that many of these people became at least mildly hypothyroid after many years if they were not given T_4 supplement (Larsen, et al, including this principle investigation, in press). As near as we can tell, most of the Marshallese have taken the T_4 tablets rather well and when taken, the T_4 levels (at first PBI's) have been at a respectable level in most individuals. In spite of the administration of T_4 for more than 15 years, we have continue to find new lesions at almost every annual examination of these exposed people. The observations have prompted some skepticism as to whether TSH induced neoplasms or whether T_4 adminstration had been as successfully followed as was intended. Using the rat model in which neoplasms were expected to develop, we have used a very sensitive rat TSH assay on serum collected at the time animals were sacrificed. (Experimental data still incom-

Radioimmunoassay for TSH and its Application To The Study of Neoplasm Induction

We have modified and tested a radioimmunoassay (RIA) for rat TSH in our laboratory under this contract. This has been done according to the method of Kieffer, et al, 1974, using NIAMD materials, and with advise of Dr. P. Reed Larsen who has been a collaborator on the Marshallese work. Our assay has become well established in our laboratory and is reliable. The NIAMD Rat TSH: I-2 (potency 35 I.U./mg) was used for iodination and RAT TSH RP I-2 for standard. Our range of normal values on a large number of male control animals was 40 to 800 ng/ml with a mean of 412 SD $\pm$ 263 ng/ml

Female control values are significantly lower (confirmed by others). Experimental male animals whose thyroids had been meticulously removed or deliberately destroyed with ^{131}I ranged as high as 3,500 ng/mg.

A series of about 300 surviving radiated male rats of one experiment were sacrificed 14 to 16 months after a range of small doses of ^{131}I. As per routine, all animals were individually identified and all data was collected including the rad dose of ^{131}I originally received by the thyroid. The animals were anesthetized and exsanguinated by direct heart puncture. The serum was frozen until the IRA was fully operational in our laboratory. Information was subsequently assembled regarding the presence of microscopic neoplasms in each thyroid. The results of TSH assays among those animals that had developed neoplasms and those that had no neoplasms are the basis for a report in manuscript form. The levels of TSH in general were higher among animals which developed neoplasms. The incidence of neoplasms in this series was 79.7%. An elevation in serum TSH was often found in rats whose thyroids had been irradiated with ^{131}I and also had become the site of one or more growing neoplasms. There were, however, numerous instances of neoplasms having developed without accompanying elevation of TSH. From this study it may be concluded that both elevation in TSH and neoplasm formation results from irradiation but may not have a cause and effect relationship. TSH elevation was not often found until the rad dose received by the thyroid was 3,000 rads or more. Thyroid neoplasms were, however, readily produced by doses between 1,500 and 2,000 rads. As a preliminary conclusion, it may be said that ^{131}I radiation in the thyroid may result in a rise in TSH and may also initiate changes that result in neoplasm formation but it cannot be assumed that the rise in TSH resulting from irradiation initiates neoplasms. On the other hand, once a neoplasm is initiated, TSH may be a growth promoting factor.

Another group of 450 female rats were given small doses of ^{131}I. The details of preparation and observations on these animals followed the plan previously used.

All animals were individually and permanently identified. Rather than measure the accumulation and disappearance of thyroidal ^{131}I on 30 or 40 representative animals and from this calculate an effective half-life (which in the past served as an estimate for all if the sample was relatively uniform), the actual data have been acquired individually on all animals. Although the initial thyroid weights at the time of irradiation must be estimated on the basis of 30 or 40 animals initially sacrificed these weights were quite uniform. With individual effective half-lives known, the actual rad dose for each animal is much more precisely known. In this current series several milliliters of blood were drawn by direct heart puncture for TSH assays several times during the 18 month period to see when the TSH rose following irradiation, if it did occur. T_4 was administered to some of these animals.

There were several new and further objectives in this experiment:

1. To sample the TSH levels in animals several times during an 18 month interval between irradiation and sacrifice. If thyroid function fails, it probably will occur after a long interval, considering the rad dose used (approximately the same dose rate in the thyroid was achieved in this experiment as in the past). If it does fail, how does the date of failure relate to the development of a neoplasm?

2. To see if there is an inhibitory effect of thyroxine (T_4) administration on the development of neoplasms. T_4 was given to half of the animals in this group. TSH assays served to test the completeness of the TSH suppression by T_4. The dose of T_4 was adjusted accordingly.

3. To appraise changes in the pituitary by weighing the gland and assaying its TSH content and relating the results to the presence of thyroid tumors, rad dose received by the thyroid and the effects of T_4 suppression.

4. Since neoplasms of the breast occasionally occur spontaneously in Sprague-Dawley rats and since their occurrence had seemed high in some irradiated rats in some past experiments when females were used, another objective was to record the occurrence of breast tumors and to remove each breastlesion to preserve the life of the animal.

The relationship of these observations to those on the thyroid is being considered. At least one breast tumor has been removed from each of over 100 animals. Preliminary observations suggest that radiation was associated with an increased occurrence of breast tumors. The data from this series of observations are not complete.

Neoplasms of the Thyroid Following X-radiation in Clinical Subjects

With the increase in publicity concerning the development of carcinoma of the thyroid in adults who had received x-radiation to the tonsils or thymus when they were infants or children and to the skin of head and neck for acne as adolescents, there have been a large number of persons examined by us from a wide area. If such persons have a proven history of exposure and have a palpable lesion we have had the opportunity to explore the thyroid. This has provided an opportunity to anticipate the findings and make plans for special study of the lesion when removed. The fact that our laboratory, supported in part by this contract, has had the technique in everyday use and personnel particularly interested and experienced in these activities make it possible to take advantage of these opportunities. When the probability of lesions of special interest was anticipated, a tracer of ^{131}I was given in advance, and with autoradiographic techniques immediately available, the necessary work on tissue was begun as soon as the tissue was removed.

Although in clinical practice the interest and concern in the malignant lesions draws primary consideration, the multiple minute lesions and the great frequency of the "atypical" lesions among previously irradiated persons has drawn our special attention. These tiny lesions show cellular pleomorphism, nuclear irregularity and occasional mitotic figures suggesting malignant characteristics but often from the standpoint of microscopic anatomy have not yet declared their malignant potential by showing capsular or blood vessel invasion. A very important clinical consideration here as well

as in the Marshallese is whether thyroids being operated for the gross lesions should be subjected to total thyroidectomy. Very often the malignant lesion found in an irradiated gland is not the lesion that prompted exploration.

Between 1956 and 1960 when the risk of carcinoma of the thyroid following radiation to the head and neck became an issue, we had available the records of 200 individuals who had received x-radiation between 1939 and 1949 for tuberculous cervical lymphadenitis in this hospital. Of those that were living, 60 were traced and examined for thyroid nodules. There were 12 found to have masses and 10 thyroids were explored. Three carcinomas were found; the remaining were adenomas. Post-mortem records of one additional patient in the group showed carcinoma. One additional lesion of the several removed and considered to have been an "atypical adenoma" has recently recurred and now shows microscopic features of carcinoma.

PARTICIPATION IN OTHER WORK IN THIS LABORATORY CONCERNING RADIATION EFFECTS

In recent years, the principal investigator has participated in several other projects concerned with radiation effects on the thyroid. Many of the observations and analyses of findings have contributed to the investigation under this contract.

1. The opportunity to study the Marshallese patients has been described above. This is a very unique situation where a low but a critically oncogenic dose of radioiodines reached the thyroid of normal persons. These were normal thyroids in contras to hyperplastic thyroids of Graves' disease treated with ^{131}I. The cells of the latt are more sensitive to radiation than the former. This difference is illustrated by t far greater dose of ^{131}I necessary to cause therapeutic reduction in function of the normal thyroid of the cardiac patient than the amount used to produce a similar effec in the hyperplastic gland of Graves' disease. Thus, it may be assumed that rad for r ceived, the radiation effect was far less in the Marshallese where neoplasms were for than in Graves's disease where neoplasms are rarely produced. The similarity between

the results in the non-hyperplastic thyroid of the rat and the Marshallese is striking.

2. The survey of 5,000 school children for thyroid lesions in the southern Utah area, where there was exposure to small amounts of radioiodines in fallout, was concluded some years ago. This investigator was one of three who at 6 month intervals examined each child considered to have a thyroid abnormality by local screening physicians. Although some nodules were found in this formerly endemic goiter area, no carcinomas were found that could be attributed to fallout. Several interesting series of collateral observations (which were not directly related to radiation effect) on the course of adolescent goiter and the course of thyroiditis in children came to light during the survey. Several publications by Ralison, et al, including this investigator have resulted. It now appears that the interval of time after exposure was relatively short in view of some of the above observations.

3. The analysis of data in the National Cooperative Thyrotoxicosis Therapy Follow-Up Study, for which the principal investigator was Chairman of the Steering Committee, came to a standstill for lack of funds several years ago. The relatively few (several hundred) thyrotoxicosis treatment cases this laboratory contributed to the 35,000 cases in that study were the most thoroughly studied cases from the standpoint of ^{131}I kinetics. The data submitted from our cases had been developed as a result of work under this contract (Mentioned elsewhere in this report is a collaborative analysis of those data by Dr. A.B. Brill). No significant number of carcinomas came to light in the National Cooperative Thyrotoxicosis Therapy Follow-Up Study, as we reported in 1974. There were, however, 494 individuals in that Study who had developed a mass in the thyroid that had not been present when ^{131}I was given. These masses that had developed had not yet been removed when the study closed.

4. Several years ago the principal investigator participated in a Committee of the National Academy of Sciences studying the use of radiation in the treatment of benign diseases. The use of radioiodine was one.

The participation in the various activities described above has contributed in a variety of ways toward the laboratory work supported by this contract. The observations supported in this laboratory have in turn contributed to those projects. Scientific inquiry and experimental design take their origin from experiences such as these.