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

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The long term series of studies on the morphological and physiological effects of ^{131}I on the thyroid have continued along several lines of investigation as described in the more recent progress reports. The observations continue on both man and animals. Previous reports have provided detailed descriptions of the work. In most of these areas we continue to assemble data as the opportunities and time provide. The clinical data include studies on the Marshallese whose primary effects were caused by radioiodines from the thermonuclear bomb fallout.

The areas of study which we have discussed in considerable detail in previous reports and continue to be investigated are as follows:

Variations in clinical responsiveness to ^{131}I therapy for Graves' disease.
The detailed collection of radiation effects continue on highly selected cooperative patients.

The appearance of significant amounts of mono (M_1) and diiodotyrosine (D_2) and a rise in iodide in the circulation following therapeutic doses of ^{131}I
Appearance of M_1 and D_2 is followed by thyroid failure within a very few months.

The failure of lightly radiated thyroid cells to hypertrophy when stimulated by giving goitrogenic substances.
In animals increase in cell height may occur through the action of TSH release but the gland does not increase in size (weight) because the capacity for cell multiplication is inhibited. The question is whether those cells that can and do multiply are the source of neoplasms and whether reduction in the capacity for replication of normal cells reduces the chance of neoplasms.

The actual buildup in the amount of DNA in the nucleus of radiated thyroid cells during the interphase but the failure of cells to divide.
The buildup we have shown may exceed two times the diploid value. The result is bizarre nuclear forms seen in many radiated thyroids in both animal and man.

We know from previous animal experiments and suspect from observations on clinical 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 is to determine these respective doses.

There is a dose level of ^{131}I in man that results in a latent failure of the thyroid long after all of the isotope is gone (years).
This seems to be attributable to intrinsic nuclear damage which does not destroy the cell nor its hormone production but does result in the intrinsic damage which is ultimately lethal when mitosis is attempted.

Following a modest dose of ^{131}I to rats and a latent period during which no cells can be shown to be synthesizing DNA from thymidine, a surge of many cells synthesizing DNA has been found.
Since the relative number of cells incorporating thymidine exceeds the controls, the surge of attempted proliferative activity may have a special meaning. The question is whether a relative physiological failure of thyroxine output acting through a TSH mechanism is responsible for such a surge.

In our laboratory we have a unique balance of detailed studies in clinical subjects treated with ^{131}I and simultaneous studies on the long term effects of ^{131}I on thyroids of rats, particularly the formation of neoplasms. In addition to the work on radiation effect carried out under this contract, the principal investigator has been an active participant in the Cooperative Thyrotoxicosis Follow-up Study of 19,000 patients treated with ^{131}I in 26 centers. The project was prematurely terminated but it is now known that the interval for the development of neoplasms is much longer than was provided for. The reopening of the Study is urgently needed. The principal investigator has been a participant in the studies of the Marshallese bomb casualties (whose primary insult was from radioiodines). He has been the surgeon operating the individuals and carrying out the laboratory studies of the tissue removed. He has been one of the consultants screening the southern Utah and Nevada children for goiter in the fallout area, a study concluded without positive findings pertaining to radiation, but yielding other scientific data on thyroid disease (the last manuscript has just been submitted for publication). These collateral studies have contributed greatly to the experimental objectives and designs for work carried out under this contract.

The laboratory and personnel that are supported mainly under this ERDA contract are a part of Cleveland Metropolitan General Hospital and Case Western Reserve University. This laboratory is responsible for all diagnostic and therapeutic use of ^{131}I in thyroid disease at Cleveland Metropolitan General Hospital. All costs for the routine work and many of the special radioiodine studies are carried by the hospital. The principal investigator is a surgeon and because of research interests, consults on all medical or surgical thyroid problems 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 precise preparation so that the most pertinent information concerning radiation effect can be obtained from the tissue removed.

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

Serial observations continue on selected patients whom we are 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 and the amount of radioactivity circulating for several weeks as well as the loss of the isotope in the urine for 3 days are followed after the treatment dose is given to get a better idea of the kinetics of the treatment dose of ^{131}I. This is ultimately related to the changes that take place in the clinical picture and the degree of recovery from hyperthyroidism. These observations are providing better information from which the actual rad dose delivered to the thyroid can be calculated.

Some patients develop 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 slowly to a euthyroid state. Other patients seem relatively uneffected by what was calculated to be an effective dose. Multiple doses may be required in some of these patients. In such cases, the serial studies have been repeated to see how the behavior of the ^{131}I the second time compares with the first, especially if there is some but not enough radiation effect. As has been pointed out in previous reports, some of these data collected in the past represent some of the most complete 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 physiological function of the gland. The only way to judge the possible outcome

of radioiodine therapy is to know precisely the rad dose that was received by the gland. The important question is whether there is a just adequate dose of ^{131}I which will bring a hyperthyroid patient to a euthyroid state without ultimately developing hypothyroidism. The other question is whether an inadequate dose of ^{131}I is more inclined to lead to neoplasm formation.

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 that required treatment. The procurement of tissue is providing an opportunity to study the effects of the radiation in various areas of the gland by autoradiography.

Thus, we continue to follow our radioiodine therapy cases diligently with repeated examinations to look for the development of masses and to reappraise thyroid function.

The Problem of Selecting the Right Dose of ^{131}I for the Treatment of Graves' Disease

An important variable in trying to anticipate and estimate the effectiveness of a dose of ^{131}I is the structural character of the particular gland that is to be irradiated. This is probably as important as the weight or size of the gland because all of the volume may not be attributable to thyroid cells - histologically the thyroids of Graves' disease may be quite variable. The gland may be extensively infiltrated with lymphocytes, in which case, it will be very firm and rubbery on examination 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 be involuted with a large part of its volume occupied by colloid. Such a gland is rather firm, but not as firm as when extensively infiltrated with lympocytes. This gland often feels rather pebbly. The very hyperplastic gland with few lymphocytes or colloid is composed of relatively more cells. Such a gland is quite vascular. The history is of short duration. This type of gland usually occurs in younger individuals. We believe these intrinsic characteristics of the gland of Graves' disease 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 characteristics can only be inferred from a knowledge of the pathology as it relates to the features of the physical examination. Unfortunately a biopsy of a large number of thyroids before ^{131}I therapy is given is not justifiable. 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 the personally reviews the histology on each gland that is operated should be able to correlate the findings with reasonable accuracy. Information on the probable true character of the gland, along with kinetic data on the behavior of the dose of ^{131}I in a given individual should help unravel the explanation as to why the dose of ^{131}I produces prompt hypothyroidism in some patients and is relatively ineffective in others. In a similar manner the surgeon who records his estimate of the weight of a thyroid in Graves' disease from his preoperative physical examination and then sees and weighs the amount of tissue he removes and from that estimates the amount of tissue that remains has performed a unique control on his preoperative estimate. With experience, he can refine his judgement of thyroid weights for those to be treated with ^{131}I.

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

The Development of Neoplasms of the Thyroid in Animals and Man

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, resulting in a secondary stimulus to the gland. The other concerns the intrinsic nuclear damage that results in impairment, abnormality, or even failure of cell division. In referring here to damage to the nucleus it is important to keep in mind that the effects often 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 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.

As is pointed out in two accompanying manuscripts, 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 examinations the first palpable nodule was not found for 9 years and neoplasms are still appearing at 22 years. The maximal dose estimated to the thyroid in the Marshallese was 1,100 rads in children and far less in the adults. Only two of 19 children exposed developed clinical hypothyroidism and that required a number of years (This raises a question as to whether the estimate that has been made was low). Both were irradiated at about 1 year of age. Of the 17 other children who were exposed under the age of 10, 15 did not display hypothyroidism but have developed neoplasms and been operated. To date 6 carcinomas have been removed from 30 individuals from several atolls 3 from an atoll with extremely low exposure. Since this is a population which seldom ever develops thyroid nodules, the relationship to the radiation which was primarily radioiodines is most impressive.

Our experience finding carcinomas in radioiodine treated patients in the National Cooperative Thyrotoxicosis Follow-up Study seems to be quite contrary. An unusually small number of carcinomas was found; in fact, considerably fewer than would have been expected, considering the large number of Graves' disease treated by surgery and found to have an occult carcinoma. Such incidental lesions might have been expected to grow if left in and ^{131}I treatment given. The difference between the dose received by the Marshallese thyroids and those of Graves' disease is considerable if individuals beyond 10 years of age are considered. Graves' disease patients probably receive 5,000 to 12,000 rads while the Marshallese received less than 1,000, probably nearer 500. Furthermore, the hyperplastic gland of Graves' disease is 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 others in rats seem to strongly suggest that it is the smaller doses of ^{131}I that produce circumstances under which neoplasms arise. Indeed the effects are sufficiently mild so that no microscopic architectural distortion is recognized in animals most inclined to develop neoplasms.

The Contribution of the Study of the Marshallese in our Laboratory

The Marshallese materials are of great importance to the study under this contract. The fresh tissue when removed here by us is studied promptly by methods in use under this contract. Although most of the neoplasms found in the Marshallese have been benign, it is important to note that a total of 6 carcinomas have been found. Two of these had metastasized to 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.

At the time of the last annual report we described a 21 year old Marshallese who we had just operated for multiple benign adenomas. He was 6 months in utero when his mother was exposed to fallout. The special studies of that thyroid tissue showed the bizarre nuclear forms recognized as evidence of radiation effect. At the time of preparation of this report, we have just operated and removed several benign but atypical adenomas from the thyroid of his mother who had developed masses in the last year.

The factor of long delay in the development of neoplasms is emphasized in both animals and man. Our experience has shown that the rats do not develop lesions unless they are radiated when very young and 1/2 to 2/3 of the life span passes. The first Marshallese lesion did not develop for 9 years. Many of the early lesions came from the atoll with the highest fallout (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, but in the most recent years, 8 individuals have been operated and 3 carcinomas found. These observations seem to emphasize the risk of the low dose range.

Further support for the concern for the low dose and long latent period is illustrated by the very considerable number of individuals coming to medical attention for neoplasms of the thyroid after irradiation to the head and neck. Relatively small doses to shrink lymphoid tissue and even smaller doses for acne seem implicated in our experience and that of others. We are screening a very considerable number of persons referred because of such known exposure.

The Part Played by TSH in Neoplasm Formation Following ^{131}I Radiation

Although each rat experiment has required almost 2 years lapse time, we have succeeded in obtaining neoplasms in rat thyroids using ^{131}I alone with increasing frequency. The yields have become (60%) sufficiently good so that the model may now be used to test inhibiting and stimulating effects on the induction and growth of such lesions.

For many years thyroid stimulating hormone (TSH) has theoretically been implicated in the development of thyroid tumors. First by the chronic application of an iodine deficient diet; then by the chronic use of goitrogenic substances and finally in recent years by radioiodine. Simply stated, the lack of iodine to make hormone, the block of the synthesis of hormone or the damage of the cellular mechanism for hormone production may lead to a deficiency of thyroid hormone and a compensatory increase

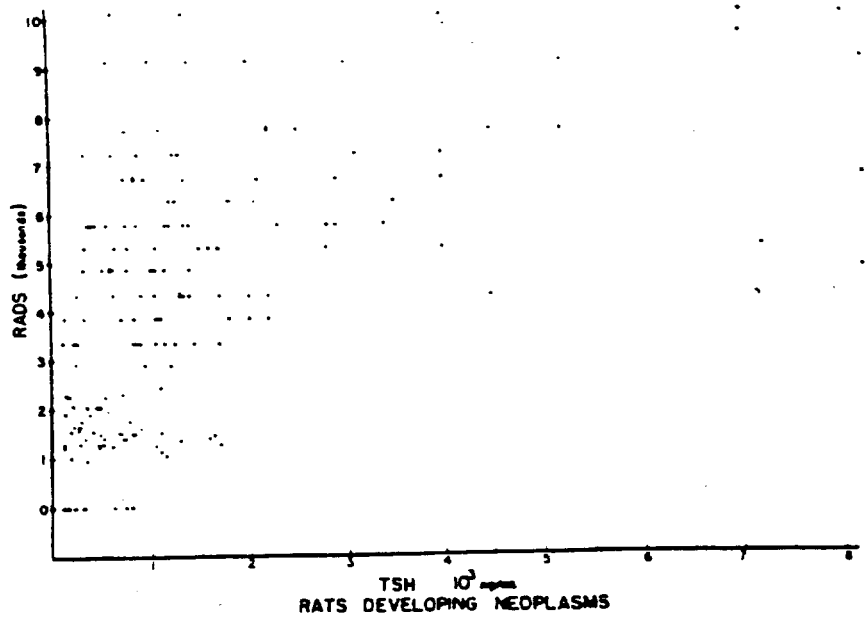
in output of TSH. The chronic stimulating effect of TSH on the thyroid does produce hyperplasia and glandular hypertrophy which ultimately leads to uncontrolled growth of cells in areas of the thyroid. In the past few years at least two groups of investigators (Lindsay and Furth) have suggested that there may be a double mechanism responsible for radiation induced neoplasms; i.e.: that radiation initiates the neoplasm and that TSH promotes its growth. The principal investigator first noted cellular hypertrophy (increase in cell heights) in some of Skanse's chicks that had been given ^{131}I (published in 1948). This has been observed by many investigators since and has been considered a manifestation of the stimulus from TSH, but assays for TSH have, until recently, not been sufficiently sensitive to detect very slight elevations of TSH. We have learned from our own investigations in animals and the publications of others that neoplasms are produced much more consistently by surprisingly small doses of ^{131}I rather than large ones. We also know that, if there is more than the most subtle evidence of histologic damage from ^{131}I, the incidence of neoplasms is much decreased. In studies published under this contract and former contracts, we have shown that radiation from ^{131}I caused intrinsic damage that might impair the capacity for cellular replication without seriously hampering hormone production. Thus, too much damage might cause a rise in TSH to significantly detectable levels but the degree of damage precludes 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 on neoplasm development.

Our collaborative studies with Dr. Robert Conard and Brookhaven National Laboratory on the Marshallese have prompted serious questioning of the TSH origin of neoplasms in these people. Nine years after the 1954 thermonuclear bomb accident, the first thyroid neoplasm appeared. When more Marshallese developed nodular goiters, the question was raised concerning the efficacy of the prophylactic administration of thyroid hormone (T_4) to inhibit the development of more neoplasms in these people. On the basis of a consensus of opinion, it was elected to give T_4. As near as we can tell, T_4 tablets have been taken rather well and T_4 serum levels (at first PBIs) have been kept at a respectable level in most individuals. More recently TSH levels in the serum have been determined. They have rarely been found elevated. In spite of these observations we continued to find new lesions on almost every annual examination of these exposed people. We have found a total of 6 carcinomas in 28 operations. Adenomatous change was found in all. The observations have prompted some skepticism as to whether TSH induced neoplasms and whether T_4 administration inhibited them. This has become the basis for some animal experiments in the past few years.

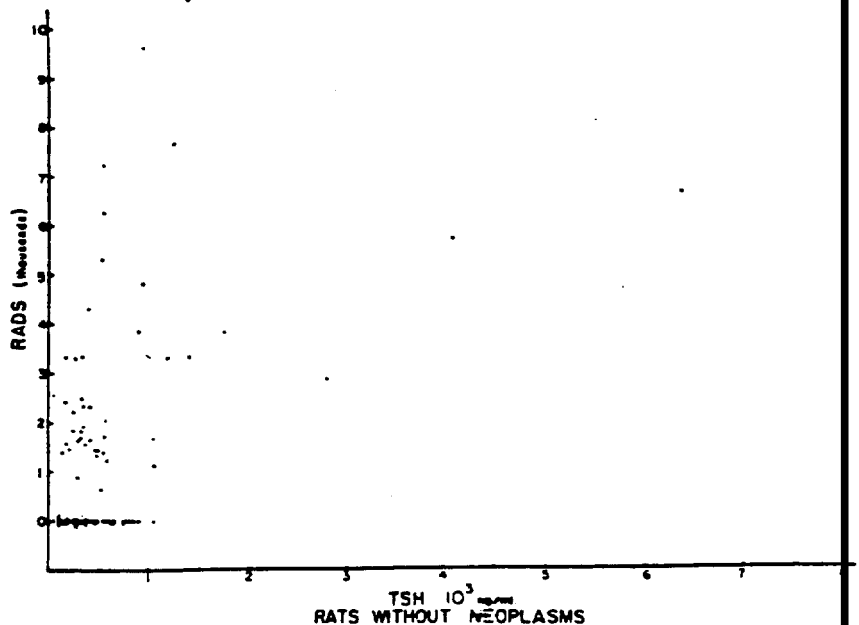
Radioimmunoassay for TSH and its Application to the Study of Neoplasm Induction

During the past year under this contract we have instituted and tested the radioimmunoassay (RIA) for rat TSH in our laboratory. This has been done according to the NIAMDD method with assistance of Dr. P. Reed Larsen (formerly of Pittsburgh and now Peter Brent Brigham Hospital, Boston), who has been a collaborator on the Marshallese work. During the last few months we feel our assay has become well established and is reliable. We are using the NIAMDD Rat TSH: I-1 (potency 35 I.U./mg) for iodination and Rat TSH RP I-2 for standard. Our range of normal values in several groups tested is from 40 to 800 ng/ml with a mean of 410 ng/ml. Experimental animals whose thyroids had been removed or destroyed with ^{131}I range as high as 3,500 ng/ml.

One year ago when more than 100 animals were being sacrificed from a series of radiated animals (at 22 months), animals were anesthetized and exsanginated by direct heart puncture. The blood was centrifuged and the serum frozen until the RIA was fully operational in our laboratory. Information was subsequently assembled regarding the presence of microscopic neoplasms in the thyroid, rad dose of ^{131}I originally re-



RATS DEVELOPING NEOPLASMS



RATS WITHOUT NEOPLASMS

ceived by each thyroid and other data. Preliminary graphs have just been prepared to relate the rad dose received by each animal to the level of TSH at the time of sacrifice. The data on animals that had developed neoplasms have been placed on one graph, those that had no neoplasms are on another (Illustration attached). It would appear that those animals with neoplasms had higher TSH levels. It should be emphasized that this is only a very preliminary consideration. Duplicate assays on these serum samples will be run to confirm these TSH levels. The relationship of the types of tumors to the TSH levels, an attempt to weed out the parafollicular cell (medullary) tumors, that presumably have no relationship to radiation, and an attempt to relate those lesions that appear malignant to TSH are some of the things that must be considered. (Other rat experiments dealing with the effects of T_4 replacement are in progress, see below.)

A new group of weanling rats has been given ^{131}I at a dose level that has yielded about 60% thyroid neoplasms in the past. The initial group numbered 450, including controls. The details of preparation and observations on these animals follow the plan described in the attached manuscripts. However, this group was prepared and irradiated with even more experimental data being acquired than in the past. All animals are individually and permanently identified as in the past 2 series that have extended over two years each. Rather than measure the accumulation and disappearance of thyroid ^{131}I on only 30 or 40 representative animals and from this calculate an effective half-life (which in the past served as an estimate for all), the actual data have been acquired individually on all animals. Although thyroid weights must still be estimated on the basis of 30 or 40 animals, these weights are quite uniform. With individual effective half lives known, the actual rad dose for each animal is much more precise. The animals of the present series are having several milliliters of blood drawn by direct heart puncture for TSH assays several times during the 2 year period. These animals are regrouped on the basis of the rad dose received by the thyroid rather than on the dose administered to the animal. One half of the radiated groups are receiving T_4 to test its inhibitory effect on neoplasm development. The effectiveness of the T_4 therapy is being checked by the TSH assay. Thus far the survival of the animals is excellent. The results of the first TSH assays on these animals have prompted us to increase the amount of T_4 supplement to be sure TSH was adequately suppressed. The experiment seems to hold promise but the length of time and labor involved constitutes considerable risk with respect to survival of the animals and the possibility of oversites in the experimental design. The mortality from cardiac puncture has become negligible.

Neoplasms of the Thyroid Following X-Irradiation in Clinical Subjects

With the recent increase in publicity concerning the development of carcinoma of the thyroid in adults who had received x-irradiation to the tonsils and thymus when they were infants and children, and skin of head and neck for acne as adolescents, there has been a large number of persons come to be examined from a wide area. If such persons have a proven history of exposure and have detectable lesions 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 any lesions found. The fact that our laboratory, supported in a major way by this contract, has techniques in everyday operation and personnel particularly interested and experienced in these activities makes it possible to take advantage of these opportunities. The number of individuals coming to our attention in the past year has increased noticably. Our pathologists under the direction of Dr. John D. Reid have been especially interested in our work and have given us special support in this clinical area. When

the probability of lesions of special interest is anticipated, a tracer of ^{131}I is given in advance and with autoradiographic techniques in operation, the necessary work on the tissues can begin as soon as the tissue is removed.

Although we have been interested in the primary malignant lesions found, the multiple minute lesions and the frequency of the "atypical" small lesions that have not yet declared their clinical or microscopic malignant potential have drawn our special attention. A very important clinical consideration here is whether such thyroids that are at risk (including the Marshallese) should be totally removed.

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. Of those that were living, sixty of these individuals were traced and examined for thyroid nodules. There were 12 found to have masses and 10 underwent thyroid exploration. Three carcinomas were found; the remaining were adenomas. Postmortem records here showed carcinoma of the thyroid in another patient not operated. One of the several that was considered an "atypical adenoma" has recurred in the last year and now on reoperation shows microscopic features of carcinoma. Two of the patients who originally were found to have masses have continued to refuse exploration or a recent reexamination by us. These observations have not been published because we have hoped to complete the observations on all of the group.

The Kinetics of Various Treatment Doses of ^{131}I (An Addendum)

We have described in previous reports our accumulation of data in 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. Serial measurements of iodinated compounds were carried out by quantitative chromatography. Through the years we collected detailed data on over 75 Graves' disease patients following the treatment doses. These quantitative data were given to Dr. A. B. Brill (Vanderbilt University) who with his associates had been studying this material in models devised by Berson. These analyses have then been related to the 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. We have had an opportunity to review and comment on two rough manuscripts prepared by Dr. Brill describing interesting results from these studies. Answers to further questions are being sought. The manuscripts are being revised. It is hoped that some definitions may be found for determining the appropriate dose of ^{131}I for given patients and for better predicting the outcome by making certain early critical measurements. Dr. Brill tells us the new drafts will soon be available for further review.

The details of some of the more recent experimental results are contained in two recently submitted manuscripts. Copies are attached. The reader is encouraged to review them because much of their content is not included in the progress report. Photocopies of the illustrations have been used but actual photographs to show detail can be provided. Reprints will be available.

The Importance of These Studies

If the risk of nuclear accidents or military action is real in the future, it seems that this area of investigation is vital to our population. Sound information on the effectiveness of prophylactic measures to thwart neoplasm development after accidental exposure to fallout which contains radioiodines could be very valuable.

The increasing use of ^{131}I as a therapeutic agent raises some uncertainty. Although it seems to be a tried, proven and safe therapeutic procedure, perhaps this judgement is based on an inadequate period of observation. Since the Thyrotoxicosis Follow-up Study was based on an average follow-up period of only 8 years, more study is needed. Most of the thyroid lesions produced by radiation are slow growing lesions so that the presence of a mass without apparent growth for some period is no assurance against malignancy. Reopening that study to follow up patients already studied for some years is important. However, tracing 45- individuals in that study who had no palpable masses at the time of treatment but possessed an unoperated palpable mass at the close of the study is most important.

From the purely practical standpoint, it is important to a considerable segment of the population who develop thyrotoxicosis to have available a simple method of therapy that does not ultimately lead to hypothyroidism and myxedema, as is now the case with ^{131}I therapy. The treatment is so simple from the patient's standpoint that he forgets he was so treated (or for the first several years proves he does not need thyroid supplement) and then much later develops hypothyroidism and becomes helpless without the problem being recognized. Many physicians who treat hyperthyroidism seldom see their patient again because they are well and there is a long lapse of time before they need further attention.