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TITLE OF INVESTIGATION

A Study of the Physiological Function and Histological Changes in Thyroids Irradiated with Radioactive Iodine

PRINCIPAL INVESTIGATOR

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Observations have continued in a variety of areas in both man and animals, as previously outlined and reported in great detail one year ago.

Effects of ^{131}I Irradiation on the Thyroid in Clinical Subjects with Hyperthyroidism

We continue to collect very detailed data on selected patients treated with ^{131}I for hyperthyroidism so that the clinical effects of the ^{131}I can ultimately be related to the behavior of ^{131}I that was observed in that individual. This is done in an effort to get a better understanding of why the effective dose of ^{131}I per estimated gram of thyroid tissue is so variable from individual to individual. Inspite of therapeutic doses, which are calculated to be just sufficient to bring the hyperthyroidism under control, we still observe far too high an incidence of hypothyroidism, not weeks or months, but years after the radiation is delivered. Since the responsible investigator is in charge of all ^{131}I therapy in this hospital, there is an opportunity to study selected patients in great detail. The following observations are made: 1) Careful pretreatment characterization of the gland and the patient, not only with respect to the uptake of a tracer of ^{131}I, but a variety of observations on factors that may explain differences among patients. 2) Determination of the amount of the treatment dose taken up by the thyroid, followed by almost daily measurements over the gland thereafter to determine the disappearance curve of the isotope from the gland. 3) Multiple observations on the concentration of ^{131}I in the serum as it relates to the thyroid at given times. 4) Determination of the extractable and non-extractable fractions in the blood (butanol) and in some cases the serial quantitative determination of iodinated compounds in the serum as the radiation effect takes place. 5) Similar observations are made on the total urinary excretion of ^{131}I

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for several days and in some cases determination of the compounds appearing there.

The principal investigator is Chairman of the Steering Committee of the National Co-operative Thyrotoxicosis Therapy Follow-up of the Radiological Health Center of the United States Public Health Service. Much time is being spent analyzing the data on radioiodine therapy from 19 centers participating in this large study. The patients that have been studied in our laboratory represent a relatively small fraction of those included in this large study of over 38,000 patients, half of which were treated with ^{131}I. The total data on our patients have proven to be the most complete set of data from various laboratories in this large group of centers. This consists of a disappearance curve of the therapeutic dose from the thyroid, the changes in the total amount of radioactivity in the serum, the changes in various iodinated compounds in the serum at intervals of hours, days and weeks after therapy, and the excretion of iodide in the urine. It has been thought that such data would be the basis from which to devise and test models that would reflect the nature and extent of the radiation effect, not only on the thyroid, but perhaps other tissues. It has been hoped that the application of such models, as that of Berman, might prove useful in explaining why some patients responded well to therapy and others did not. It has also been hoped that such models might be used to "plug in" more fragmentary data from less completely studied cases and thus estimate the blanks in the kinetics of these other patients.. Our need for mathematical assistance in the National Study has been longstanding. The demands on the personnel for analysis of these kinetic data in the National Study have been so heavy and the turnover so frequent among the persons concerned that the usefulness of the data is still not known. Although this study is revealing many interesting features on the comparative results of ^{131}I and thyroidectomy, it is apparent that the fragmentary nature of the kinetic data on individual patients in this retrospective study is such that the estimate of the individual doses of radiation to the gland is very difficult to ascertain. The data from our laboratory based on the above sequence of observations appears to be the most extensive. These data are now in the hands of Dr. A. Bertrand Brill of Vanderbilt University for analysis by the methods of Berman. The more recent data from our laboratory have been collected in a prospective manner and are much more appropriately obtained than formerly; unfortunately, however, the retrospectively studied patients are those with the long term follow-up and are now, many years later, the most important.

Any information that will lead to more precise application of the proper therapeutic dose of ^{131}I and the avoidance of long latent hypothyroidism is important. The observations being made are not experimental in nature, but merely prolonged and in great detail so that the outcome of therapy for that patient is much better understood and is of value if the individual requires more than one dose of ^{131}I.

A Study of Neoplasms as they Develop in Irradiated Thyroid Tissue

We have been concerned with, and published observations on, the bizarre nuclear forms occasionally encountered in human thyroids previously treated with ^{131}I. We have also been concerned with the occasional neoplasm which develops in rat thyroids that had been given small doses of ^{131}I and subsequently stimulated with antithyroid drugs. In the same rat thyroids, bizarre nuclear forms are seen. The method of using tritiated thymidine to identify by autoradiography those nuclei that are undergoing mitosis has been used in our laboratory for quite some years. As described in previous reports, we have been using this labeling technique to attempt to identify the onset of neoplasms as small aggregations of cells in irradiated rat thyroids. This is based on the assumption that any cluster of cells destined to represent a neoplasm will display a different rate of incorporation of tritiated thymidine in the nuclei.

In some recent experiments, a small trace dose of ^{131}I in addition to the tritiated thymidine has been given to the animals four hours before sacrifice. Autoradiographs prepared from microscopic sections promptly after the removal of the thyroid serve to identify any local areas in the gland where ^{131}I utilization is different from the rest of the gland. Because of the short half life of ^{131}I, this isotope disappears from the microscopic sections after two or three months. Now autoradiographs then prepared from other microscopic sections, adjacent to those used to demonstrate ^{131}I, will show only the location of the tritiated thymidine. Subsequent comparison of the two autoradiographs from microscopic sections, which are almost identical, reveals two types of information about the same cluster of cells which appear in both preparations.

We have several large groups of rats whose thyroids have been irradiated with various non-destructive doses of ^{131}I, ranging from 0 to 40 microcuries of ^{131}I. Some of these are under chronic stimulation with an antithyroid drug to see whether families of cells develop with different degrees of mitotic activity. Others are stimulated acutely for a few days before sacrifice. Still others remain unstimulated, except for the intrinsic stimulus which the animal may receive as a result of diminution in hormone output by the radiated thyroid. The rats of this strain (Charles River strain, since 1947) very rarely develop tumors spontaneously. Although the incidence of neoplasms induced by ^{131}I and goitrogens is relatively low, when lesions have been found their presence is more significant.

Rats weighing 100-130 grams were first used with ultimate poor survival, owing to an epidemic of pulmonary infection; however, the expected bizarre nuclear forms and some giant nuclei, which had previously been shown to contain excessive amounts of chromatin, did appear. Many of the large nuclear forms took up the labeled thymidine.

More recently, weanlings were prepared by giving doses of ^{131}I from 2.5 microcurie to 40 microcurie doses. Each rat has been identified and its individual uptake of the dose determined so that the individual amount of radiation can be more precisely known. Thus far, autoradiographs of rat thyroids radiated ten months or less before sacrifice have been reviewed. As we have previously observed, hypertrophy of the gland may be produced by goitrogens for quite some time after the ^{131}I is given, but after a considerable lag time (long after the ^{131}I is gone) ability of the gland to hypertrophy is impaired. Failure of the cells to undergo mitosis under stimulation is prompt following ^{131}I, as shown by failure of labeling after ^{131}I, but there is recovery of the capacity for mitotic activity after varying intervals. It now appears on preliminary survey of the autoradiographs from the current weanling rats that the supramonimal surge of mitotic activity after several months of recovery, as we had previously described in an earlier series, is confirmed. It is apparently at this time that hypertrophy of the gland can be produced even though it cannot be produced later.

Because evidence in both man and animals suggests neoplasms can be more easily produced in the young, still another series of animals only three days old have been prepared by giving various doses of ^{131}I. The first series of autoradiographs have been prepared, but there are many more to be made over the life span of this group of rats.

We have not reached the point in the sequence of observations on either of the last two series of animals when it will be economical to surgically expose the thyroids under anesthesia so that only those which have become nodular can be sacrificed. This is not to say that sacrifice will be avoided in all of those

without thyroid nodularity because the beginning of nodularity, which should be heralded by subtle variations in differentiation and dedifferentiation, is not evident from gross inspection of the gland.

Our clinical, as well as the animal experiments, seem to suggest that there is a narrow margin between the amount of ^{131}I which will promote abnormal cell division (and perhaps promote neoplasm formation) and the dose of ^{131}I which will prevent cell division (and perhaps inhibit the development of a neoplasm) even though the cell lives on and can continue to make hormone for a long time.

Anomalies of Chromosomes in Circulating Leukocytes in Man following Therapeutic Doses of ^{131}I

The first published observations in this country on chromosomal anomalies in circulating leukocytes following large doses of ^{131}I for carcinoma of the thyroid were made under this contract. These observations were made following 167 millicuries of ^{131}I for carcinoma of the thyroid. Not only was there an acute dramatic rise in the incidence of anomalies (amounting to almost one half of the circulating leukocytes cultured at the height of the radiation effect), but the preliminary observations on that patient before the 167 millicuries were given showed a higher than normal initial incidence of anomalies. This was apparently attributable to residual effects from massive doses of ^{131}I that we had given six years before. Since that time, we have attempted similar observations to detect chromosomal anomalies in patients treated with the usual therapeutic doses (five to fifteen millicuries) of ^{131}I for Graves' disease. Some publications have appeared describing changes caused by doses of ^{131}I of this range, but from our gradually accumulated experience under this contract, there is some question of the validity of conclusions based on simple "before and after" observations. We have felt that it was necessary to make observations on a large series of samples of blood, with multiple cultures from each, and a large number of preparations from each culture, along with extensive pretreatment control observations to make such observations valid. For quite some time, we have been cautious about concluding that there were demonstrable changes attributable to these common place doses of ^{131}I used for therapy. It now seems safe to say from our observations that for doses between 10 and 15 millicuries changes can be shown to occur. The maximal occurrence of serious chromosomal abnormalities, such as dicentrics, ring forms, etc., reach a peak of 3.5% in 24 hours. This is approximately a two-fold increase over pretreatment counts. The abnormalities referred to here are those most likely attributable to radiation and do not include chromatids or breaks in one leg of a chromosome, which may be artifacts and to which (along with non-modal counts) we formerly attributed more significance. David Satcher, a M.D., Ph.D. candidate, has been collaborating on this aspect of the work with the assistance of Dr. Neil Macintyre with whom we published the first work. The significant feature of the experimental plan is to show that there is a surge of anomalies in a large series of observations on the same patient, and to relate this to the blood and thyroid ^{131}I levels, as well as to the sequential changes in the amount of ^{131}I in certain organic compounds (which remain in the circulation), and to the ^{131}I iodide (which is cleared from the circulation by the kidney. Although no new cases have been studied in the past year, the enormous number of preparations have been under study and the significance of the changes being analyzed.

Observations on the Marshallese as they relate to Studies under this Contract

The responsible investigator has recently spent five weeks in the Marshall Islands participating in the annual review of the Marshallese exposed to the fallout from the thermonuclear devise detonated on Bikini in March 1954. The

observations on the nodularity developing in these exposed human thyroids seems to be not unlike that produced experimentally in animals under this contract. Five additional cases of nodularity in the Rongelap people turned up in the past year.

During the course of this recent survey, the exposed people that had been on Rongelap and Uterik, as well as many unexposed Marshallese, were given complete physical examinations. This included children born after exposure to the fallout. The occurrence on Rongelap of rather significantly enlarged thyroids in many of the adolescent and preadolescent ages was most interesting. These individuals were born of women who had been exposed, but these children had been conceived some years after that exposure. The significance of these goiters is difficult to evaluate at this time, because adequate data on the occurrence of adolescent enlargement in the unexposed population is not available, but it was surprising to see so many significant adolescent goiters on Rongelap in a strain of people who are notoriously free of goiter and seemingly free of adolescent enlargement in this iodine abundant environment. It should be emphasized that these adolescent goiters in individuals born of exposed mothers, although not exposed themselves, may have been influenced in utero by subtle thyroid deficiencies which were lingering in the mothers after all radioiodine from the fallout was gone. Under such an influence, the thyroid of the offspring may have been influenced in its early development. Such a postulation seems much more likely than that of a genetic defect. Control observations must be more fully scrutinized and expanded to validate the above observations, but, in the meantime, some animal experiments are getting under way in our laboratory under this contract and will be considered later.

As a recent participant in the Marshallese observations, the principal investigator has been particularly interested in the cellular changes in the thyroids of those Marshallese who have been subjected to thyroidectomy for nodular goiter. Aside from the neoplasms that have been removed from these individuals, the possibility of more minute and subtle changes in these thyroids were of interest because of the cellular changes we have studied in patients treated with ^{131}I and in animals studied under this contract. With this in mind, all microscopic preparations previously prepared from the Marshallese thyroids from Rongelap were mixed with nodular goiter preparations from the United States. With the identity of the slides unknown, the principal investigator reviewed the sections in an attempt to identify those tissues which had come from the Marshallese and had presumably received appreciable doses of radioiodine, and those which had not (there being approximately, but not exactly, one half from each source). All but one of the Marshallese thyroids were identified as having radiation changes. This judgement was based on nuclear changes as they had been observed during previous work under this contract. Only one of the non-radiated "nodular goiters" from the United States was classified as having received radiation. Although these judgements probably could not be repeated with the same degree of accuracy, because there was reasonable doubt in some cases. The majority were identified with reasonable certainty. The type of nodule observed was not a basis for judgement here. It was suspected that there is some difference in the criteria for judgement rendered here and criteria used by some pathologists.

The principal investigator has also been involved in the annual examination of the children in the Utah-Nevada fallout area (exposed in 1954) where some endemic goiter is present. A week was spent there again this year in the annual thyroid examinations of children that had been screened from the population by the United States Public Health Service screening teams. Discrete thyroid lesions,

which prompted concern, have been removed at the University in Salt Lake City. The application of the same criteria of judgement of radiation effect in thyroids excised from this population has revealed only an occasional thyroid tissue which hints at a radiation effect, contrary to the judgement of some others who reviewed the microscopic preparations of these cases.

The Study of Morphologic Changes in Human Thyroids Previously Treated with ^{131}I

In the past year, unfortunately, there has been no patients previously treated with ^{131}I for hyperthyroidism and studied extensively in our personal series that has had reason for thyroid surgery. Opportunities to obtain such tissues for study occur in a random fashion and only when a mass develops which causes concern.

The Relationship of Bizarre Nuclear Forms to the Persistence of LATS in Patients Treated for Graves' Disease and Previously Studied

Although we partially remove or partially destroy the excessively functioning thyroid in Graves' disease to correct hyperthyroidism in man, the unknown driving force that makes the thyroid overactive probably continues for a time. This stimulus in its effect may be comparable in some respects to the stimulus which is created by giving antithyroid drugs to normal rats. We know that antithyroid drug treatment to rats previously treated with ^{131}I makes the bizarre nuclear forms much more evident. The reason why we find bizarre nuclear forms in some ^{131}I treated human thyroids and not in others may be because of the continued stimulus to hyperplasia in some of the patients. Although we must admit we are not convinced that the long acting thyroid stimulator (LATS) is the mechanism which drives the thyroid in Graves' disease, it is demonstrable in at least half of these patients before treatment. We have the LATS assay method firmly established in our laboratory. Although, as stated above, we had no opportunity to obtain radiated human thyroid tissue during the past year and to carry out our usual studies with special attention to the occurrence of bizarre forms in such patients, when the opportunity does arise we expect to see whether the presence of LATS in the serum has any relationship to the bizarre nuclear forms found in some human thyroids which have not been too seriously damaged from ^{131}I.