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TITLE OF INVESTIGATION: A Study of the Physiological Function and Histological Changes in Thyroids Irradiated with Radioactive Iodine

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The work carried on under this project represents a broad study of physiological and morphological changes in the thyroid of man and animals following the administration of radioactive iodine (I^{131}).

A very detailed report covering activities from the beginning of the project was submitted one year ago.

Radiation Effect on the Function of the Thyroid in Clinical Subjects

During the early years of the project more time was devoted to the physiologic effect of the radiation than to the morphologic aspects of it. Selected patients were studied in very great detail so that the clinical effects of I^{131} therapy could ultimately be measured against a large background of data on each individual patient. It was hoped that in this way, it might be possible to find explanations for the great variation in the response of different patients to this therapy. The many studies that have been done, and are being continued, on each of the selected patients were discussed in detail in the previous reports, especially in the lengthy review submitted one year ago. The series of observations are therefore merely listed here.

In addition to the usual thorough clinical work-up of the patient including: hematologic studies, estimation of the character and weight of the thyroid, systematic recording of all features of ophthalmopathy, the PBI, the uptake of I^{131} and the clinical judgment of the severity of the hyperthyroidism, a series of special observations were made on these selected patients. It is these special studies that are supported by this grant. They include the following: 1) The uptake of the treatment dose by the thyroid and repeated (almost daily) observations thereafter to determine the pattern of disappearance of the I^{131} from the thyroid over a period of up to three weeks. 2) Sampling of the total radioactivity per milliliter of blood over the same period of time. 3) Serial quantitative chromatograms to show the amounts of various iodinated compounds in the blood (5 to 8 samples per patient) to reflect the changing pattern of these compounds following the administration of the treatment dose. 4) Similar observations on the urine with attention to the daily total loss of radioactivity from the body. These serial observations not only initially reflect the abnormalities of the disease process and variations among patients before a substantial

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radiation effect occurs, but they also reflect changes that are induced by the radiation.

The data have been gradually accumulated and subsequent evaluation of the course of the disease has been considered in the light of these findings. These studies go far beyond the usual routine observations commonly made on patients treated with I^{131} . The ultimate value of the data is not fully known because the long term effects of I^{131} are only recently coming to light.

Our laboratory represents one of 19 centers included in the Public Health follow-up study of I^{131} treated patients. Our detailed data on approximately 175 of these patients, studied under this contract, have proven to be the most thorough in this national study. The kinetics of the I^{131} in these treatment patients, along with the collateral observations, are now serving as the basis for attempting to define the patterns of behavior of I^{131} . It is the hope of those in charge of the national project that, having established kinetic patterns from the data on our patients, the fragments of data from other less completely studied patients from other centers can be analyzed and the missing data estimated for those patients by the use of computers. The reasons for the variations in the therapeutic outcome may thus be learned in a large number of patients now being followed. The responsible investigator of this contract has been appointed Chairman of the Steering Committee concerned with the analysis of the clinical data that have now been assembled from the 19 centers. It should be admitted that much of the data that have been acquired under this grant are not fully understood by us, but with the collaboration of others more knowledgeable in the study of kinetics and the use of patients from other centers, more could be learned. We continue to carry out a detailed study on selected patients when: 1) an appropriate patient is to be treated, 2) when he is available for intensive study, and 3) when the personnel working under the contract have a sufficient block of consecutive days to complete the study on that patient.

Synthesis in Radiated and Stimulated Rat Thyroids

Gradually, as the project has progressed, emphasis has shifted somewhat from the purely physiologic toward the morphologic changes caused by I^{131} radiation. The large bizarre nuclear forms originally found and described (1,2) at the beginning of this project have received increasing attention. The production of the odd nuclear forms in animals after only small doses of I^{131} followed by a stimulus of thiouracil has been reported (1,2) from this project. The finding of excessively large amounts of DNA as demonstrated (3) by Feulgen staining and quantitative microspectrometry in nuclei of thyroid cells of animals was reviewed in detail in a complete review submitted last year.

A manuscript entitled "The Acute and Long Term Effects of Various Doses of Radiiodine on the Thyroid of the Rat as Demonstrated by Mitotic Activity Using Tritiated Thymidine" (4) was appended as a separate manuscript with our comprehensive report one year ago. That manuscript was sent to Endocrinology for publication. Some relatively minor editorial suggestions were made by the editor. With the lapse of time, and reconsideration of the manuscript, it seemed the paper could be improved upon, not only by making some suggested corrections or revisions, but by redesigning the presentation with some change in emphasis. In that study tritiated thymidine was used in rats to show radio-graphically which cells were forming DNA in preparation for mitosis.

When an antithyroid drug was added to the drinking water two months after a small dose of I^{131} was given, there was a much greater rise in the incidence of cells forming DNA than when such a stimulus was applied to non-radiated controls. This propensity to over-respond with DNA formation on stimulation occurred even though there was no obvious microscopic change in the cells before the stimulus was applied. This response occurred long after the I^{131} was gone, but while the gland as a whole still had a capacity to enlarge under the antithyroid stimulus.

We had known from our earlier experiments that after giving a dose of I^{131} which was insufficient to produce recognizable microscopic changes in the thyroid, a latent effect was produced that later resulted in large abnormal nuclear forms when the stimulus of thiouracil was applied. We also had known from earlier experiments⁽³⁾ that soon after an intermediate (5-20 μ c) dose of I^{131} had been given, the thyroid could be induced to hypertrophy, as does the normal gland when an antithyroid drug is administered. However, several months later and long after all the I^{131} had disappeared from the gland, this ability of the gland to hypertrophy was gradually lost. It seemed in the more recent experiments that it was just those glands which still have a capacity to enlarge that develop the abnormal nuclear forms. It was these nuclear forms that had excessive DNA.

More attention in the manuscript has been placed on the rapid decline of DNA synthesis when the initial iodine deficient diet was discontinued. By chance this part of the experiment showed how thyroid cells when placed under the influence of iodine deficiency displayed a high degree of DNA synthesis in preparation for cell division. When the iodine deficiency was corrected, DNA synthesis promptly declined. The manuscript has been considerably revised and will be resubmitted very soon to Endocrinology. It has been re-titled "DNA Synthesis in the Radiated and Stimulated Thyroid".

A Study of Nuclear Changes at the Time of Neoplasm Formation Following I^{131}

Since we know that neoplasms sometimes develop in rat thyroids following small doses of I^{131} , and since the frequency of the occurrence of these tumors is enhanced by giving thiouracil, it seemed appropriate to use the tritiated thymidine technique to observe the behavior of thyroid nuclei as tumors are beginning to develop.

We now have in progress two rather extensive experiments on the development of changes in DNA formation at the time neoplasms are beginning. We are interested here in the preparation for mitotic activity as manifested by uptake of tritiated thymidine.

After a brief period of iodine deficient diet to insure a high uptake of I^{131} , a large series of approximately 100 rats were injected with either 5, 10, or 50 μ c of I^{131} . Others received none. Following the I^{131} and a brief respite, chronic administration of thiouracil in the drinking water of some of the rats was begun. This series of rats were pubescent and weighed 120 to 140 grams when I^{131} was given. Another large series of rats which were somewhat younger and weighed from 80 to 100 grams were prepared some months following the above series. Representatives of the various groups were killed soon after I^{131} was given to determine the actual uptake of I^{131} in the average gland. Rats representing the various experimental groups were subdivided following I^{131} so that some received thiouracil

ronically, others received it acutely before sacrifice, and still others received none. Each animal was given tritiated thymidine four hours before sacrifice, so that contact radioautographs might be made to determine which and how many cells possessed nuclei that were preparing for mitosis. In some instances the rats were also given minute trace doses of I^{131} to test the function of the thyroid before they were sacrificed. The radioautographs for tritiated thymidine were not prepared until this I^{131} had completely decayed. Some animals were killed early in the course of the experiments to obtain additional data on the supramaximal surge of DNA formation that had been observed in previous experiments two months after the I^{131} was given. Body weights, thyroid weights and thyroid function, as measured by I^{131} uptake, as well as gross changes in thyroids, are all being determined at the time of sacrifice.

These experiments have been in progress for about one year. Intervals thus far selected for sacrifice have been 3½ and 9 months. Animals will be sacrificed at more frequent intervals as the expected time for the occurrence of neoplasms arrives. They will be sacrificed at intervals for 2 to 2½ years. It is hoped that the intervals elected for sacrifice will give radioautographs at the time when the first signs of the development of neoplasms occur just as we have seen in experiments under this contract in years gone by. As the neoplasms begin to develop, evidence for differences in rates of synthesis of DNA in different parts of the thyroid will be sought. At the time of the most recent sacrifice of animals, no gross evidence of tumors had yet appeared.

Clear Changes in Human Radiated Thyroid Tissue

Over the years there have been opportunities to procure by surgical means, samples of thyroid tissue from patients previously treated with I^{131} . Having firmly established the method of Feulgen staining and quantitative microspectrophotometry on animal thyroids in this laboratory (3), the rigid methodology for procuring and processing the tissue was set into operation each time human material was to become available. Thus, the quantitative measurement of DNA in individual nuclei was undertaken in human tissue as we had done in the past on animals. Over a period of almost 10 years, thyroid tissues from 13 I^{131} treated patients have been obtained for this study. In addition, 4 tissues from thyroids previously subjected to x-ray radiation and 4 controls were obtained. Ten of the 13 I^{131} patients were subjected to surgery because of masses which had developed in the thyroid; one patient was operated on for persistence of hyperthyroidism; in two instances tissue was obtained at prompt post mortem examination. Recently the final steps in the preparation and staining of these radiated thyroids and tissues from control thyroids were concluded. During the last year the quantitative measurement of DNA in individual nuclei was completed in these human tissues. In a review of alternate sections stained with the customary hematoxylin and eosin method, it was found that somewhat fewer of these radiated tissues displayed bizarre nuclear forms than was observed in our previous radiated human thyroids described many years ago (2). However, four of 13 I^{131} treated patients showed an abundance of the bizarre nuclear forms in extranodular tissue. One of the most obvious had been given propylthiouracil before the operation because of continued hyperthyroidism. The use of this drug, which acted as a stimulus to the patient's thyroid, may have behaved in a fashion comparable to our animal experiments where an abundance of bizarre nuclear forms developed when a similar stimulus was applied. In this case

he natural stimulus of the disease to produce hyperplasia had obviously persisted at the time the tissue was obtained. In the other cases following I^{131} treatment, it is ultimately difficult to know whether a given patient is in a euthyroid state because the driving force that caused Graves' disease has abated or whether the force is still there, but the thyroid is so damaged that hyperthyroidism is not possible. Quantitative measurement of DNA in individual nuclei using Feulgen staining and microspectrophotometry showed considerable variation in DNA content and nuclear volume in some, but not all of the radiated tissues. Measurements indicated that the amount of DNA in some cells was greater than 2 times the diploid value. This is as was observed in the stimulated thyroids of animals which had previously been given I^{131} and is interpreted as a build up in DNA, but thwarted cell division.

Some of the adenomas which developed in these radiated human glands were also similarly studied for DNA content of individual nuclei. Considerable variation was found in nuclear volume and DNA content in these tumors. In the final analysis, it is not entirely clear which adenomas arose following I^{131} and which were present, but not detected, at the time I^{131} was given. It would be particularly interesting to know which tumors arose from radiated cells which bore a potential for bizarre nuclear forms and which were tumors whose cells were themselves subjected to the radiation because the tumor was already present. Certainly the former must be true in some cases. One follicular adenocarcinoma was encountered in a patient who had been treated with I^{131} , but unfortunately, the special preparations on this neoplasm were not adequate for our studies. The bizarre nuclear forms are present, but not abundant in the extranodular tissue of this thyroid. A manuscript describing the bizarre nuclear forms and their excessive and regular amounts of DNA in human tissue is in preparation.

Our experimental results suggest that there is a dose range of I^{131} which for a time after the radiation is given, neither completely destroys the function of the thyroid cell, nor (as shown in animals) interferes with the capacity of those cells to multiply and make a larger gland. After a longer lapse of time and long after the dose of I^{131} is dissipated, a defect develops in the ability of the radiated cell to divide, although a may build up. Clinical observations in the human show that although a subtle damage may be caused to the thyroid cell, it continues to survive and make thyroid hormone maintaining the individual in a euthyroid state. Superficially, it may appear that an ideal euthyroid state is achieved in this clinical subject. In fact, the euthyroid state persists for a good many years. However, we now are beginning to observe at 12, 15 and more years after I^{131} therapy that these human glands, which appeared to have adequate capacity to manufacture hormone, ultimately begin to fail and the individual begins to suffer from hypothyroidism. This has become apparent from our long term study of these patients. It is thus a reasonable assumption from the animal experiments that the expected normal replacement of thyroid cells is not taking place.

Chromosome Abnormalities in Circulating Leucocytes of Patients Treated with I^{131}

Several years ago we solicited the assistance of Professor Neil McIntyre of this University in the study of chromosomal anomalies in circulating white cells in a patient treated with large doses of I^{131} . In

comprehensive review of a year ago, we described our observations on the very high incidence of chromosomal anomalies in this patient and how it had been studied in great detail. We published these observations (5) as the first American publication of its kind several years ago. In those studies we found that a high incidence of anomalies 6½ years after the last total of 475 millicuries of I¹³¹. With the very extensive experience in chromosomal preparation and interpretation by Dr. Macintyre and his laboratory personnel, it seemed appropriate to carry these observations further and look for anomalies in individuals who had received doses of 15 millicuries of I¹³¹ as treatment for hyperthyroidism. In the mean time, observations have been reported by others who used one or two observations on each patient rather than a series to prove unquestionably that a change had taken place and to observe a sequence of changes. It has been our policy to make multiple cultures from a series of 8 to 12 samples of blood following a treatment dose of I¹³¹.

Two years ago we completed studies on a total of 6 patients given moderate therapeutic doses of I¹³¹ for hyperthyroidism, but unfortunately, the full series of cultures was not always complete on each patient. Some cultures failed and in two instances, the large number of normal control observations were not fully acceptable. From the meager observations on these patients, it appeared that there was a slight rise in anomalies. Continuation of the work was limited at that time because of shortage of personnel on our own staff and on Dr. Macintyre's staff. During the last year we have resumed these studies with the participation of a graduate student who is addressing himself to this problem. We have recently in the past year assembled information on an additional 8 patients. The experimental design has been as follows: Samples of blood for culture are obtained before any I¹³¹ is given. Sufficient blood is obtained so that multiple cultures will be available to clearly establish an incidence of anomalies before the radiation is given. Subsequently, samples of blood are obtained for culture at 1, 4, 10, and 24 hours, and 2, 3, 7, and 14 days. The large battery of observations on the kinetics of the I¹³¹ in a patient are also carried out (as they have been described earlier in the report for estimating whole body radiation). Because all series of chromosome counts are done as complete unknowns, the results of most of the observations are not yet identified, because study of slides is still in progress. In at least two instances, where the observations on the kinetics are complete, it appears that chromosomal anomalies are being produced at a just significant level. It will require a considerably larger number of patients and probably several years to obtain enough data to get a clear answer to this issue. In the more recent studies, non-radioactive (treatment) serum is obtained from the patient and stored to be used to replace the serum (in cell cultures) that bears I¹³¹ when the blood is drawn. Replacing the serum in the culture eliminates any radiation effect that might occur while the culture is being incubated. The fact that the patient's own blood bearing some of the therapeutic dose was used in our experiments was an objection to our original experimental design. It can be argued that under the former conditions some radiation might be absorbed by the cells during culture rather than be sustained only while the cells were withdrawn from the patient. The background of detailed studies to determine the radiation exposure in these treated patients for the chromosome studies is adding to our total number of patients and is described in great detail for purposes of ultimate relationship to effects on the thyroid.

Ray Radiation Effect on the Thyroid

As part of our interest in radiation effects on the thyroid we have most completed a follow-up study of a selected group of patients who before 1950 received x-ray radiation to the neck which presumably included the thyroid area. All of these individuals were given x-ray therapy for tuberculous cervical lymphadenitis. Most of them were children or young adults when treated. Of almost 200 patients so treated, 66 have been examined and brought back for our personal examination of the thyroid. Twelve of these were found to have at least one discrete mass in the thyroid. Eight have submitted to surgical removal of the mass. There were two carcinomas, two Hurthle cell tumors (one with capsular invasion) and four follicular adenomas. Of 60 additional individuals known to have died, the records of post mortem examinations are available on 46. Two had lesions of the thyroid; one of these was a carcinoma. Most of the patients who died did so within 5 years after the radiation therapy had been given. Our patients who have very discrete firm masses, but refuse operation, give considerable concern to us. A final effort will be made once more to induce these patients to submit to surgery. The study should be completed soon.

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RECENT MANUSCRIPTS SUBMITTED

Discussion of Paper entitled "Definitive Treatment of 570 Cases of Hyperthyroidism by Either I^{131} or Surgery", by Dr. H. T. Caswell, et al. at the meeting of the American Surgical Association, Boca Raton, Florida, March 24, 1966 (In press, Annals of Surgery)

"Desoxyribonucleic Acid Synthesis in the Radiated and Stimulated Thyroid", by Brown M. Jobyns, M. D., Ph.D., Ann E. Rudd, B.S., and Mary Ann Sanders, B.A. (Submitted to the ~~Journal of Endocrinology~~)

"Desoxyribonucleic Acid Content Associated with Nuclear Changes in Radiated Human Thyroids", by Leon R. Robison III, B.A. and Brown M. Jobyns, M. D., Ph.D. (Submitted to Journal of Clinical Endocrinology and Metabolism)