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

MASTER

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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

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

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

Our laboratory represents one of 19 centers included in the Public alth follow-up study of I^{131} treated patients. Our detailed data on proximately 175 of these patients, studied under this contract, have oven to be the most thorough in this national study. The kinetics of the 31 in these treatment patients, along with the collateral observations, e now serving as the basis for attempting to define the patterns of behvior of I^{131}. It is the hope of those in charge of the national project at, having established kinetic patterns from the data on our patients, en fragments of data from other less completely studied patients from other nters can be analyzed and the missing data estimated for those patients the use of computors. The reasons for the variations in the therapeutic tcome may thus be learned in a large number of patients now being followed. responsible investigator of this contract has been appointed Chairman of Steering Committee concerned with the analysis of the clinical data that e now been assembled from the 19 centers. It should be admitted that h of the data that have been acquired under this grant are not fully lerstood by us, but with the collaboration of others more knowledgeable the study of kinetics and the use of patients from other centers, more uld be learned. We continue to carry out a detailed study on selected ients when: 1) an appropriate patient is to be treated, 2) when he is ilable for intensive study, and 3) when the personnel working under the tract have a sufficient block of consecutive days to complete the study that patient.

. Synthesis in Radiated and Stimulated Rat Thyroids

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

A manuscrppt entitled "The Acute and Long Term Effects of Various Doses Radioiodine on the Thyroid of the Rat as Demonstrated by Mitotic Activity ng Tritiated Thymidine"(4) was appended as a separate manuscript with our prehensive report one year ago. That manuscript was sent to Endocrinology publication. Some relatively minor editorial suggestions were made by editor. With the lapse of time, and reconsideration of the manuscript, seemed the paper could be improved upon, not only by making some suggested or revisions, but by redesigning the presentation with some change in hasis. In that study tritiated thymidine was used in rats to show radiographically 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 μc) 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 μc 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 ceived none. Each animal was given tritiated thymidine four hours before crifice, so that contact radioautographs might be made to determine which d how many cells possessed nuclei that were preparing for mitosis. In me instances the rats were also given minute trace doses of I^{131} to test e function of the thyroid before they were sacrificed. The radioautographs r tritiated thymidine were not prepared until this I^{131} had completely cayed. Some animals were killed early in the course of the experiments to ther additional data on the supramaximal surge of DNA formation that had en observed in previous experiments two months after the I^{131} was given. dy weights, thyroid weights and thyroid function, as measured by I^{131} upke, as well as gross changes in thyroids, are all being determined at the me of sacrifice.

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

clear Changes in Human Radiated Thyroid Tissue

Over the years there have been opportunities to procure by surgical ans, samples of thyroid tissue from patients previously treated with I^{131}. ving firmly established the method of Feulgen staining and quantitative crospectrophotometry on animal thyroids in this laboratory(3), the rigid thodology for procuring and processing the tissue was set into operation each ne human material was to become available. Thus, the quantitative measurent of DNA in individual nuclei was undertaken in human tissue as we had ne in the past on animals. Over a period of almost 10 years, thyroid ssues from 13 I^{131} treated patients have been obtained for this study. addition, 4 tissues from thyroids previously subjected to x-ray radiation d 4 controls were obtained. Ten of the 13 I^{131} patients were subjected surgery because of masses which had developed in the thyroid; one patient s operated on for persistence of hyperthyroidism; in two instances tissue s obtained at prompt post mortem examination. Recently the final steps in e preparation and staining of these radiated thyroids and tissues from ntrol thyroids were concluded. During the last year the quantitative asurement of DNA in individual nuclei was completed in these human tissues. review of alternate sections stained with the customary hematoxylin and sin method, it was found that somewhat fewer of these radiated tissues splayed bizarre nuclear forms than was observed in our previous radiated nan thyroids described many years ago(2). However, four of 13 I^{131} treated tients showed an abundance of the bizarre nuclear forms in extranodular ssue. One of the most obvious had been given propylthiouracil before e operation because of continued hyperthyroidism. The use of this drug, ich acted as a stimulus to the patient's thyroid, may have behaved in a shion comparable to our animal experiments where an abundance of bizarre clear forms developed when a similar stimulus was applied. In this case he natural stimulus of the disease to produce hyperplasia had obviously ersisted at the time the tissue was obtained. In the other cases following 131 treatment, it is ultimately difficult to know whether a given patient s in a euthyroid state because the driving force that caused Graves' isease has abated or whether the force is still there, but the thyroid is o damaged that hyperthyroidism is not possible. Quantitative measurement f DNA in individual nuclei using Feulgen staining and microspectrophotometry howed considerable variation in DNA content and nuclear volume in some, ut not all of the radiated tissues. Measurements indicated that the amount f DNA in some cells was greater than 2 times the diploid value. This is s was observed in the stimulated thyroids of animals which had previously een given I^{131} and is interpreted as a build up in DNA, but thwarted cell ivision.

Some of the adenomas which developed in these radiated human glands ere also similarly studied for DNA content of individual nuclei. Considerable variation was found in nuclear volume and DNA content in these tumors. n 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 iven. It would be particularly interesting to know which tumors arose rom radiated cells which bore a potential for bizarre nuclear forms and hich were tumors whose cells were themselves subjected to the radiation ecause the tumor was already present. Certainly the former must be true in me cases. One follicular adenocarcinoma was encountered in a patient who d been treated with I^{131}, but unfortunately, the special preparations on is neoplasm were not adequate for our studies. The bizarre nuclear forms re present, but not abundant in the extranodular tissue of this thyroid. 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} ich for a time after the radiation is given, neither completely destroys e function of the thyroid cell, nor (as shown in animals) interferes with e capacity of those cells to multiply and make a larger gland. After longer lapse of time and long after the dose of I^{131} is dissipated, a fect develops in the ability of the radiated cell to divide, although A may build up. Clinical observations in the human show that although is subtle damage may be caused to the thyroid cell, it continues to survive d make thyroid hormone maintaining the individual in a euthyroid state. perficially, it may appear that an ideal euthyroid state is achieved in is clinical subject. In fact, the euthyroid state persists for a good many ars. However, we now are beginning to observe at 12, 15 and more years ter I^{131} therapy that these human glands, which appeared to have adequate pacity to manufacture hormone, ultimately begin to fail and the individual gins to suffer from hypothyroidism. This has become apparent from our ng term study of these patients. It is thus a reasonable assumption from e animal experiments that the expected normal replacement of thyroid cells not taking place.

romosome Abnormalities in Circulating Leucocytes of Patients Treated with 31

Several years ago we solicited the assistance of Professor Neil cintyre of this University in the study of chromosomal anomalies in circulating white cells in a patient treated with large doses of I^{131}. In omprehensive review of a year ago, we described our observations ng the very high incidence of chromosomal anomalies in this patient ad been studied in great detail. We published these observations(5) e first American publication of its kind several years ago. In those es we found that a high incidence of anomalies 6½ years after the last total of 475 millicuries of I^{131}. With the very extensive experience romosomal preparation and interpretation by Dr. Macintyre and his atory personnel, it seemed appropriate to carry these observations er and look for anomalies in individuals who had received doses of 15 millicuries of I^{131} as treatment for hyperthyroidism. In the mean- observations have been reported by others who used one or two ob- tions on each patient rather than a series to prove unquestionably a change had taken place and to observe a sequence of changes. It een our policy to make multiple cultures from a series of 8 to 12 es of blood following a treatment dose of I^{131}.

Two years ago we completed studies on a total of 6 patients given moderate therapeutic doses of I^{131} for hyperthyroidism, but un- nately, the full series of cultures was not always complete on each nt. Some cultures failed and in two instances, the large number of al control observations were not fully acceptable. From the meager on these patients, it appeared that there was a slight rise in lies. Continuation of the work was limited at that time because of age of personnel on our own staff and on Dr. Macintyre's staff. g the last year we have resumed these studies with the participation graduate student who is addressing himself to this problem. We have r in the past year assembled information on an additional 8 patients. xperimental design has been as follows: Samples of blood for culture btained before any I^{131} is given. Sufficient blood is obtained so multiple cultures will be available to clearly establish an incidence malies before the radiation is given. Subsequently, samples of are obtained for culture at 1, 4, 10, and 24 hours, and 2, 3, 7, and ys. The large battery of observations on the kinetics of the I^{131} e patient are also carried out (as they have been described earlier in eport for estimating whole body radiation). Because all series of some counts are done as complete unknowns, the results of most of observations are not yet identified, because study of slides is still ogress. In at least two instances, where the observations on the s is complete, it appears that chromosomal anomalies are being pro- at a just significant level. It will require a considerably larger r of patients and probably several years to obtain enough data to get ar answer to this issue. In the more recent studies, non-radioactive reatment) serum is obtained from the patient and stored to be used to e the serum (in cell cultures) that bears I^{131} when the blood is drawn. ing the serum in the culture eliminates any radiation effect that occur while the culture is being incubated. The fact that the nt's own blood bearing some of the therapeutic dose was used in our periments was an objection to our original experimental design. It be argued that under the former conditions some radiation might be ined by the cells during culture rather than be sustained only the cells were withdrawn from the patient. The background of de- l studies to determine the radiation exposure in these treated patients for the chromosome studies is adding to our total number of patients d in great detail for purposes of ultimate relationship to effects on yroid.

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 fore 1950 received x-ray radiation to the neck which presumably included e thyroid area. All of these individuals were given x-ray therapy for berculous cervical lymphadenitis. Most of them were children or young ults when treated. Of almost 200 patients so treated, 66 have been aced and brought back for our personal examination of the thyroid. elve of these were found to have at least one discrete mass nn the thyroid. ght have submitted to surgical removal of the mass. There were two rcinomas, two Hurthle cell tumors (one with capsular invasion) and four th follicular adenomas. Of 60 additional individuals known to have died, e records of post mortem examinations are available on 46. Two had sions of the thyroid; one of these was a carcinoma. Most of the patients o died did so within 5 years after the radiation therapy had been given. ur patients who have very discrete firm masses, but refuse operation, ise considerable concern to us. A final effort will be made once more induce these patients to submit to surgery. The study should be cometed soon.

ferences

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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. Dobyns, 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. Dobyns, M. D., Ph.D. (Submitted to Journal of Clinical Endocrinology and Metabolism)