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RESEARCH PROJECT AT(30-1)-1243 H.C. $300 ; MN 65

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

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JUL 19 1967

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ANNUAL REPORT - June 1, 1966 to June 1, 1967

This project has represented a broad study of both physiological and morphological changes produced by ^{131}I in the thyroid of both animals and man. Early in the history of the project much time was devoted to gathering kinetic data on patients treated with ^{131}I and observing the associated changes in the physiology of the gland. Gradually more time has been devoted to morphological changes in these human thyroids and to animal thyroids with special emphasis on the fate of the cell, its potential for mitosis in normal replication and neoplastic change. Much of what has been learned and objectives in experimental design have been related to two other important national studies of the Division of Radiological Health with which the principle investigator has been associated. One is the study of nodular goiters in children in the atomic bomb fallout area of Utah. The other is the cooperative radioiodine therapy follow-up study of which the responsible investigator is the chairman of the Steering Committee.

A very detailed report covering all activities of this project from the beginning was submitted two years ago. Because of this some of the review will be brief.

The Study on Radiation Effect on Thyroid Function in Clinical Subjects Given ^{131}I

Early in this project enormous amounts of time were devoted to collecting very detailed data on selected patients who were given ^{131}I therapy for hyperthyroidism. Only selected patients could be used because of the inordinate amount of time required for a single patient. The clinical effects of ^{131}I therapy have been ultimately 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. There are many studies that have been done, and are being continued, on each of the selected patients. This has been discussed in detail in the previous reports, especially in the lengthy review submitted two years ago. The series of observations on each patient are therefore merely listed here. We have felt strongly that only subsequent interpretation of the effects of radiation can only be evaluated on the basic of very detailed kinetic data and the changing pattern of iodinated compounds in the blood rather than the superficial observations commonly made in the course of clinical follow-up of the usual patient given ^{131}I therapy.

Besides 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 ^{131}I and the clinical judgement of the severity of the hyperthyroidism, a series of special observations were made on these selected patients. It is these special studies that have been 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 ^{131}I from the thyroid over a period of up to three weeks. 2) Sampling of the total radioactivity per ml 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 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. The cases represent a wide range of response. The ultimate value of the data is not fully known because the long term effects of ^{131}I are still coming to light.

Our laboratory represents one of 19 centers included in the Radioiodine Therapy Follow-Up Study of the Radiological Division of the United States Public Health Service. (We received a small grant from this source solely for the purpose of tracing and keeping contact with all of these special patients as well as others treated in our center without special study). The detailed data described above on approximately 185 of these patients, studied under this Atomic Energy Contract, have proven to be the most thorough in this national follow-up study. The kinetics of the ^{131}I in these treatment patients, along with the collateral observations, are now serving as the basis for attempting to define the patterns of behavior of ^{131}I. A special committee headed by Dr. Mones Berman has been set up (May 1967) to study this material. It is hoped that after the committee has established kinetic patterns from these detailed data that fragments of data from other less completely studied patients 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. Additional and more meaningful information should come from the data assembled under the Atomic Energy Commission contract as we follow the outcome of the radiation in the patients and have the collaboration of others who are more knowledgeable in the study of kinetics and who are using our data. 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 of blood and urine of that patient.

DNA Synthesis in Radiated and Stimulated Thyroids

Gradually, as the project has progressed, emphasis has shifted somewhat more from the purely physiologic toward the morphologic changes caused by

^{131}I radiation. The large bizarre nuclear forms originally found and described at the beginning of this project have received increasing attention. The production of the odd nuclear forms in animals after only small doses of ^{131}I followed by a stimulus of thiouracil has been reported from this project. The finding of excessively large amounts of DNA as demonstrated by Feulgen staining and quantitative microspectrophotometry in nuclei of thyroid cells of animals was reviewed in detail in the complete review submitted two years ago.

A manuscript entitled "The Acute and Long Term Effects of Various Doses of Radioiodine on the Thyroid of the Rat as Demonstrated by Mitotic Activity using Tritiated Thymidine" when submitted to Endocrinology received some relatively minor editorial suggestions which, with the lapse of time, reconsideration and additional data prompted redesigning the entire presentation. This was retitled "Desoxyribonucleic Acid (DNA) in the Radiated and Stimulated Thyroid". It was resubmitted on January 24, 1957. It has been accepted and is in press. Copies of this manuscript are attached. Its contents may be briefly reviewed as follows:

Tritiated thymidine was used in rats to show radioautographically 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 ^{131}I 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 after the ^{131}I, but before the stimulus was applied. This response occurred long after the ^{131}I was gone, but while the gland as a whole still had a capacity to enlarge under the antithyroid stimulus.

It had been learned from the earlier experiments that after giving a dose of ^{131}I 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 that soon after an intermediate (5-20 μc) dose of ^{131}I (25 to 35% uptake) 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 ^{131}I 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 among those glands which had received lesser doses of ^{131}I and still had a capacity to enlarge that developed more of the abnormal nuclear forms. It was these nuclear forms that had excessive DNA.

An initial iodine deficient diet had been given to induce a good uptake of ^{131}I. This part of the experiment showed how thyroid cells when iodine deficient displayed a high degree of DNA synthesis in preparation for cell division. When the iodine deficiency was corrected, DNA synthesis promptly declined. More attention in the revised manuscript was given to the observations on DNA synthesis under these conditions of iodine deficiency.

Nuclear 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 ^{131}I. Having firmly established the method of Feulgen staining and quantitative

microspectrophotometry on animal thyroids in this laboratory, the rigid methodology for procuring and processing the tissue was set into operation each time human thyroid 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 in animals. Over a period of almost 10 years, thyroid tissues from 12 ^{131}I treated patients were obtained for this study. All had had Graves' disease. In addition, 4 tissues from thyroids previously subjected to x-ray radiation and 2 controls were obtained. Ten of the 12 ^{131}I patients were subjected to surgery because of masses which had developed in the thyroid; one patient was operated on for persistence of hyperthyroidism and was receiving an antithyroid drug at the time the tissue was obtained; in two instances tissue was obtained at prompt post mortem examination. All tissues had been processed and stored in paraffin blocks so that simultaneous staining could be accomplished on all tissue at the same time. Recently the final steps in the preparation and staining of these radiated thyroids and tissues from control thyroids were concluded. During the past 1½ years the quantitative measurement of DNA in individual nuclei was done on these human tissues.

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 ^{131}I and is interpreted as a build up in DNA, but thwarted cell division.

On 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. However, four of 12 ^{131}I treated patients showed an abundance of the bizarre nuclear forms in extranodular tissue. One of the most obvious was a patient who had not been cured of hyperthyroidism and had been given propylthiouracil before the operation. This drug 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 the natural stimulus of the disease to produce hyperplasia had obviously persisted at the time the tissue was obtained. In the other cases following ^{131}I treatment, it is 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.

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 DNA content and nuclear volume in these tumors. In the final analysis, it is not entirely clear which adenomas arose following ^{131}I and which were present, but not detected, at the time ^{131}I was given. It would be particularly interesting to know which tumors arose from radiated cells that 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 ^{131}I, but unfortunately, the special preparations on this neoplasm were not adequate for our studies. The bizarre nuclear

forms were present, but not abundant in the extranodular tissue of this thyroid. A copy of the manuscript describing these findings and entitled "DNA Content Associated with Nuclear Changes in ^{131}I Radiated Human Thyroids" was submitted to the Journal of Clinical Endocrinology and Metabolism and is attached to this report.

Our experimental results in animals suggest that there is a dose range of ^{131}I which for a time after the radiation is given, neither completely destroys the function of the thyroid cell, nor 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 ^{131}I is dissipated, a defect develops in the ability of the radiated cell to divide, although DNA may build up. Clinical observations in the human show that although the 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 such a 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 ^{131}I 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 and explains the ultimate failure of the thyroid.

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

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

We have in progress two rather extensive experiments on the frequency of individual cells forming DNA at various intervals of time during which neoplasms may be expected to be developing. The purpose is to watch the development of changes in DNA formation in a population of radiated thyroid cells. A rate of mitotic activity should be manifested by uptake of tritiated thymidine.

After a brief period of iodine deficient diet to insure a high uptake of ^{131}I, a large series of approximately 100 rats were injected with either 5, 10, or 50 μc of ^{131}I. Others received none. Following the ^{131}I and a brief respite, chronic administration of thiouracil in the drinking water was begun in some rats at each radiation dose level. This series of rats were pubescent and weighed 120 to 140 grams when ^{131}I was given. Another large series of rats which were somewhat younger and weighed from 80 to 100 g grams were prepared with similar doses some months following the above series. Representatives of the various groups were killed soon after ^{131}I was given to determine the actual uptake of ^{131}I in the average gland. Rats representing the various experimental groups were subdivided following ^{131}I so that in addition to those which received thiouracil chronically, others received it acutely shortly 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 ^{131}I to test the function of the thyroid before they were sacrificed. The radioautographs for tritiated thymidine were not prepared until the ^{131}I had completely decayed. Some animals were killed early in the course of the experiments to gather additional data on the supramaximal surge of DNA formation that had been observed in previous experiments two months after the ^{131}I was given. Body weights, thyroid weights and thyroid function, as measured by ^{131}I uptake, as well as gross changes in thyroids, are all being determined at the time of sacrifice.

The longest of these experiments have been in progress for about 1½ years. Intervals thus far selected for sacrifice have been 3½ months, 9 months, 1 year and 1½ years. They will be sacrificed at 2 years and 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. As the neoplasms begin to develop, it is anticipated that clusters of cells which represent incipient tumors may display different proclivity for synthesizing DNA. At the time of the last progress report no gross tumors had appeared in animals that had been sacrificed. At the end of one year 5 sacrificed animals showed some irregularities in the thyroid. Radioautographs were not as good as they should have been for interpretation of tritium in nuclear DNA. New radioautographs have just been completed and are not particularly revealing, probably because the nodular areas are not sufficiently distinct. Tissues obtained at 18 months show 4 examples of more irregularity in each series. Radioautographs are in preparation on these tissues. It is interesting that the irregularities are developing in the gland a little more frequently in the animals given ^{131}I alone than in ^{131}I plus chronic administration of antithyroid drug. It now seems apparent that there should be a third series of animals that are much younger than either of these two series when ^{131}I is given, i.e., weanling rats.

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

Several years ago we solicited the assistance of Professor Neil Macintyre of this University in the study of chromosomal anomalies in circulating white cells in a patient treated with large doses of ^{131}I. In our comprehensive review two years ago, we described our observations showing the very high incidence of chromosomal anomalies in a patient to whom we had given several very large doses of ^{131}I and had studied in great detail. These observations were published several years ago as the first American publication of its kind. In those studies we found that a high incidence of anomalies 6½ years after the last of a total of 475 millicuries of ^{131}I. With the very extensive experience in chromosomal preparation and interpretation by Dr. Macintyre and his associates, it seemed appropriate to carry these observations futher and look for anomalies in individuals who had received doses of 8 to 15 millicuries of ^{131}I as treatment for hyperthyroidism. In the meantime observations have been reported by others who have used one or two observations on each of several patients rather than a series of observations on each patient 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 during a two week period following a treatment dose of ^{131}I.

Two years ago we completed studies on a total of 6 patients given these moderate therapeutic doses of ^{131}I 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 initial control observations were not fully acceptable. From the meager data on these patients, it appeared that there was a slight rise in the incidence of chromosomal 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 past 1½ years we have resumed these studies with the participation of a graduate student who is addressing himself to this problem. We have so far assembled information on 14 additional patients. The experimental design has been as follows: Samples of blood for culture are obtained before any ^{131}I was given. Sufficient blood was obtained so that multiple cultures would be available to clearly establish an incidence of anomalies before the radiation was given. Subsequently, samples of blood have been obtained for culture at 1, 4, 10, and 24 hours, and 2, 3, 7, and 14 days. Multiple cultures are prepared from each of 11 to 18 samples of blood from each patient. The usual large battery of observations on the kinetics of the ^{131}I as described in the first part of this report were also being carried out. All series of chromosome counts are done as complete unknowns. At the time of reporting one year ago the data on 8 patients studied thoroughly suggested that chromosomal anomalies were being produced at a just significant level in patients given 5 to 8 millicuries of ^{131}I. It was recognized that a considerably larger number of patients would be required to obtain enough data to get a clear answer to this issue.

The new cases added to the study in the past year have not shown a very significant rise in the number of chromosomal anomalies following ^{131}I except in one very significant case. This particular case received a somewhat higher dose of ^{131}I than did all of the others. Although all of the kinetics are not fully analyzed, this patient received 19.7 Mc. Attention is now being directed toward patients who happen to be receiving doses in this range.

There has arisen some concern that cells if incubated in their own serum (which contained ^{131}I) might sustain significant further radiation effect during that incubation. It could be argued that under the former conditions some radiation might be sustained by the cells during culture rather than sustained solely before the cells were withdrawn from the patient. It seems that replacing the serum in the culture eliminates any radiation effect that might occur while the culture is being incubated. In the more recent studies, non-radioactive (pretreatment) serum has been obtained from the patient and stored and later used to replace the serum (in the cell cultures) that bears ^{131}I when the blood is drawn. Special studies to compare the effects of ^{131}I in the cultures have been tested. By comparing the results with and without the radioactive serum it seems clear that the presence of the small amount of ^{131}I has no detectable influence on the number of anomalies found.

X-Ray Radiation Effect on the Thyroid

As part of our studies on radiation effect on the thyroid we have had in progress a follow-up study of a selected group of patients who before 1950 received x-ray radiation to the neck and presumably to the thyroid area. All of these individuals had been [illegible]

cervical lymphadenitis. Most of them were children or young adults when treated. Of almost 200 patients so treated, 67 have been traced 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. One year ago eight had been submitted to surgical removal of the mass. There were two carcinomas, two Hurthle cell tumors (one with capsular invasion) and four with follicular adenomas. Three patients who had been examined and found to have discrete firm masses in the thyroid refused surgical exploration at the time of reporting one year ago. One of these has been explored and carcinoma was found. Of 60 additional individuals known to have died, the records of post mortem examination 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. Two patients who have very discrete firm masses, but refuse operation, raise considerable concern to us. A final effort will be made once more to induce these patients to submit to surgery. Publication of this series of cases has been held up in the hope that all of the lesions about which we are concerned can be removed. The finding of 3 carcinomas and and two possible carcinomas in nine thyroids explored from among 66 patients called back for observations lends support to the belief that radiation had some relationship to the development of these lesions.

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