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PROGRESS REPORT

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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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The investigations supported by this contract and dealing with the effects of ^{131}I on the thyroid continue in a variety of directions and range from clinical research in man to a variety of experimental designs in rats. Primary efforts are directed toward study of the long term effects of the radiation on the physiological function of thyroid cells, the nature of their survival, and the ultimate behavior of the cells with special attention toward the development of nodules and neoplasms. The ideas and experimental designs have not only grown out of the long term clinical follow-up of our own patients (more than 20 years), but from participation by the principal investigator (along with others) in several other major projects dealing with ^{131}I effects. These are:

1.) Chairman of the Steering Committee for analysis and study of almost 38,000 patients in the Cooperative Thyrotoxicosis Therapy Follow-up Study of the National Center for Radiological Health of the USPHS.

2.) Participant (with Dr. Robert A. Conard, Brookhaven National Laboratory) in the surveys of the Marshallese natives exposed to radioiodines from the first thermonuclear explosion.

3.) Participant in the surveys of goiter in children in the Utah-Nevada fallout (1954) area and in special studies performed on selected individuals in that population.

EFFECTS OF ^{131}I RADIATION ON THE THYROID OF CLINICAL SUBJECTS WITH HYPERTHYROIDISM

The description of this work continued as previously described. (The following two paragraphs are reproduced from the previous annual report with only slight revision because the basic outline for the collection of these data remain basically the same.)

Very detailed data continue to be collected on selected patients treated with ^{131}I for hyperthyroidism so that the ultimate clinical effects can later be related to the behavior of ^{131}I that was observed in that individual shortly after the treatment was given. This is done in an effort to get a better understanding of why the effect of a given dose of ^{131}I per estimated gram of thyroid tissue is so variable among individuals. In spite 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 and the excretion in the urine as they relate 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 for several days and in some cases determination of the compounds appearing there.

As Chairman of the Steering Committee for the Cooperative Thyrotoxicosis Therapy Follow-up Study of the National Center for Radiological Health of the USPHS, the principal investigator has devoted much time to analyzing the data on the results of radioiodine therapy from 19 centers participating in this large study. Although the patients studied in our laboratory represent a relatively small fraction of the total included in this large study of over 38,000 patients, half of which were treated with ^{131}I, the data on many of our individual patients has proven to be the most complete among the various laboratories. Our data 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 iodine in the urine. It has been thought that such data could be the basis from which to devise and test models that would reflect the nature and the extent of the radiation effect on the thyroid. It has been hoped by the Steering Committee that it might be possible to utilize the detailed data from individual patients (acquired under this contract) in kinetic models, such as those of Berman, to discover some

explanations for the success of ^{131}I therapy in some patients and the failure in others or for the overly destructive effect on the thyroids in still others. It has also been hoped that such models might be used to "plug in" more fragmentary data from less completely studied patients and thus estimate the blanks in the kinetics of other patients. Our need for mathematical assistance in the national study has been longstanding. The demands on the personnel for analysis of other data in the national study have been so heavy and the turnover of personnel so frequent that little more than a superficial review could be done with the studies on kinetics. Although this national study is revealing many interesting features on the comparative results of ^{131}I therapy and thyroidectomy, it is apparent from the superficial review that the kinetic data are so fragmentary on most of the patients in that retrospective study that the estimate of the individual doses of radiation to the glands will be impossible. On the other hand, the data from our laboratory, based on the above sequence of observations and collected in a prospective manner under this contract appear to be useful. These data are now in the hands of Dr. A. Bertrand Brill of Vanderbilt University for analysis by the methods of Berman.

During the past year, Dr. Brill has carried out some analyses on the data which we furnished him. He feels quite encouraged about gaining meaningful interpretations on the kinetics, using modifications of the models devised by Berman(which were devised for study of normal iodine kinetics with tracers rather than therapeutic doses). Dr. Brill is particularly interested in our cases on which we have serial quantitative determinations of changing iodinated compounds in the blood following treatment. Dr. Brill has submitted an application for a grant to support work with these data. It is hoped that the support will be available. He was instrumental in the initiation of the national study and has remained deeply interested in the problem.

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 currently on selected patients with hyperthyroidism are not experimental in nature, but merely prolonged and in great detail so that the outcome of therapy for that patient is understood much better and is of value if the individual requires more than one dose of ^{131}I or if the therapy is too destructive.

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 which had previously been 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. 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 be a neoplasm will display a different rate of incorporation of tritiated thymidine in its nuclei than will cells of the surrounding tissue.

A small trace dose of ^{131}I in addition to the tritiated thymidine has been given to the animals four hours before sacrifice. Autoradiographs promptly prepared from microscopic sections 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 the half life of ^{131}I is much shorter than the ^{3}H in tritiated thymidine, the ^{131}I disappears from the microscopic sections more rapidly and is gone after two or three months. New autoradiographs then prepared from other microscopic sections, adjacent to those used to demonstrate ^{131}I show only the location of the ^{3}H-thymidine. Subsequent comparisons of the two autoradiographs from microscopic sections, which are almost identical, reveal 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 doses of ^{131}I which cause relatively little or no obvious destruction of thyroid tissue, these doses ranging from 0 to 40 microcuries of ^{131}I with varying degrees of uptake depending on the experimental design. Each animal was individually identified and its uptake determined for future reference. Some of these animals were given an antithyroid drug chronically following the ^{131}I to produce hyperplasia. Others are stimulated acutely only for a few days before sacrifice to see how much mitotic activity could be produced. Still others remained unstimulated, except for the intrinsic stimulus which the animal may have received as a result of diminution in hormone output by its own radiated thyroid. The rats of this strain (Charles River strain, since 1947) very rarely develop tumors spontaneously. These animals have been observed up to two years.

Although most animals in the current series have been sacrificed and many autoradiographs are still in preparation, some general tentative observations may be described. Even though neoplasms are induced by goitrogens alone, they are produced sooner and more readily if relatively small amounts of ^{131}I have first been given.

It has been suspected that the age of the animal at the time ^{131}I is given is important in the frequency and speed with which neoplasms are produced. In previous experiments with juvenile rats approaching sexual maturation, the occurrence of neoplasms following ^{131}I treatment and chronic administration of antithyroid substances was fairly infrequent. In fact, the yield of rats with neoplasms with which to work was discouragingly low. A large series of weanling rats 65 to 85 gms has yielded a somewhat better occurence of neoplasms. These, like previous animals, were given Remington diet to enhance the uptake and make hyperplasic thyroids. In these animals, the uptake of the dose was 25 to 40%.

We are now well along in still another series in which very young rats were used by acquiring pregnant females and injecting the litters with ^{131}I after we were reasonably sure of the healthy survivors (15 days; 30 to 40 gm; no Remington diet) In this series, the uptake was about 10%. Animals were sacrificed in hours (to determine average thyroid weights and to measure actual uptake to check invivo counting), days, weeks and months up to almost two years. The dose among several hundred animals in these series ranged from 500 rads to 40,000 rads with a few considerably higher. These ranges have been explored in order to find the level at which neoplasms were most readily produced. Obvicusly doses which caused too much destruction left cells incapable of replacing themselves. Some other lighter doses were too small to cause any discernible untoward effects. In these experiments the higher doses of about 20,000 rads or more have produced considerable

damage, inhibiting the hypertrophy that results from thiouracil and leaving cells which survive but are incapable of replication. It now appears that for hyperplastic glands with doses of 2.5 to 5 uc per animal (yielding tissue doses of 10,000 to 20,000 rads) and for normal glands with doses of 10 to 20 uc (yielding the same amount of radiation) result in the greatest probability of neoplasm formation. This radiation causes little change in thyroid weight for the first six to twelve months provided no rapid growth of a neoplasm occurs. However, after about five months the expected increase in thyroid weight, when an antithyroid drug is given, cannot be produced as in the non-radiated controls.

Following a tissue dose of about 8000 rads animals whose thyroids are not chronically stimulated display thyroid weights which are slightly below those of non-radiated controls. Furthermore, their response to an antithyroid drug stimulus is greatly reduced. A full response is usually not displayed where doses over 2000 rads has been delivered. It should be emphasized that these are very preliminary conclusions and may be modified when additional material now in preparation is available and further analysis is made.

By sacrificing animals and testing the mitotic activity that is occurring under chronic stimulation or that may be produced by acute five day stimulation with antithyroid drugs, it has been possible to judge the temporary inhibition to mitosis resulting from the initial radiation insult. Depending on the dose there is recovery with the passage of time. The initial temporary reduction in mitotic response may be significant, but brief for doses as low as 2000 rads. After a short recovery period and after all ^{131}I is gone, there may be only partial recovery of capacity for mitotic activity at somewhat higher doses. At the same time, chronic stimulation with antithyroid drugs seem to produce appreciable degrees of hypertrophy of the gland. After long periods following ^{131}I, some degree of mitotic activity may be induced by acute administration of the drug, but in many cases the gland cannot be made to increase in weight.

The technique for acutely challenging a crippled thyroid by giving an antithyroid drug is a unique and roughly quantitative means of testing capacity for mitotic activity.

As time passes following the dose of ^{131}I, some recovery inability of the cells to undergo mitotic activity occurs. After 12 to 15 months, such thyroids show some areas of variability with respect to follicle size and cell size. When then challenged with the antithyroid drug, the labelling of nuclei is not uniformly displayed. Although there may be no discretely encapsulated groups of cells which are clearly a neoplasm, the tendency for cell division seems more prevalent in some areas than in others. The non-uniform distribution of labelling with tritiated thymidine presumably relates to the nodularity that evolves when these glands are stimulated to hypertrophy. Ultimately if such glands are chronically challenged in most cases they are found to contain lesions whose histologic appearance, degree of encapsulation and discretely different tendency for mitotic activity are quite evident.

A variety of thyroid neoplasms have now been observed in these animals. Most of the lesions are papillary in pattern. Many show single layers of epithelium in very simple configurations. Others show papillary structure with active proliferative activity that trends into solid cellular patterns composed of pleomorphic forms. Some thyroid lobes are completely replaced by what appears to be a benign lesion. Other lesions which appear malignant are found invading the thyroid capsule and the fat nearby. In some cases, the trachea has been invaded and malignant cells are seen within the trachea as well as musculature of the neck. Autoradiographs with ^{131}I, when satisfactory, have so far shown less ^{131}I in such neoplasms than in the normal thyroid tissue or none at all. There appears to be no significant difference in

histological patterns of those neoplasms produced by goitrogens along, or by ^{131}I alone, or by a combination of the two. Labelling of nuclei with ^{3}H-thymidine is usually more abundant in neoplasms than in the extranodular tissue. Increased frequency of labelling is more often seen in areas where normal follicles have given way to minute microfollicles or solid cellular areas between follicles. The preliminary impression is gained that these areas represent the origin of "nodules" or actual neoplasms.

As part of a background study of this investigation, it has seemed important to obtain information on the capacity of normal thyroids at different ages to respond with mitotic activity when challenged with the acute administration of antithyroid drug. It is also important to determine the frequency of mitosis in normal animals on a standard iodine intake. Although the data are not nearly complete or ready for presentation, it is now clear from preliminary review of the autoradiographs that in the very young growing rat, labelling of nuclei is abundant and that, as the age of the animal progresses, fewer and fewer cells are found in spontaneous mitosis. In very old animals it is rare to find even an occasional labelled nucleus indicating mitosis. In a similar way, the ability of a thyroid to respond to the acute challenge of antithyroid drug is very vigorous in the young animal. In the animals later in the life span, the response in the form of labelled nuclei is still clearly evident, but far less vigorous. This information is being assembled from control animals in these studies.

Efforts have been made to acquire information on time required for mitosis to take place in the normal thyroid and the degree to which the steps of mitosis may be affected by radiation. Data which we have previously published under this contract show that following radiation, DNA builds up in nuclei in preparation for division, but that actual division of the cell may be thwarted. The result is a very large nucleus which contains double or several times the amount of DNA customarily found in the normal nucleus, as if the cell had attempted to go through mitosis several times. In pilot experiments with autoradiographs, it appeared that following a single injection of ^{3}H-thymidine all labelling of nuclei was completed by four hours. Subsequent work of others has suggested that labelling is complete in one hour. In recent preliminary experiments, we have injected a large group of identical young rats with ^{3}H-thymidine and sacrificed them at 15 minute intervals in an effort to demonstrate when the rising incidence of labelled nuclei reaches a plateau. The plateau should indicate that no more labelled cells are still dividing. A plateau of labelling of nuclei was found, but it occurred later than expected. Data on paired nuclei was difficult to interpret. Although only those nuclei which are preparing for division take up ^{3}H-thymidine and the autoradiographs are beautifully imprinted from the isotope localized in individual nuclei, the geometry and range of the beta ray in this preparation poses problems in quantitation. This experiment is to be repeated soon with simultaneous observations on the ^{3}H-thymidine in the blood, its disappearance from the blood and the appearance of its metabolic degradation products. Information gained may be useful in determining the effect of radiation on the duration of the process of mitosis in radiated thyroids.

OBSERVATIONS ON THE DEVELOPMENT OF THYROID NODULES IN POPULATIONS EXPOSED RADIOIODINE FALLOUT AS THEY RELATE TO STUDIES UNDER THIS CONTRACT (MARSHALLESE)--(UTAH-NEVADA)

The Marshallese Study

In February and March of 1969, the responsible investigator spent five weeks in the Marshall Islands participating in the annual review of thyroids of the

thyroids was first observed nine years after exposure among these people. By 1967 fourteen of sixty-eight of the most heavily exposed had developed nodules which were of sufficient concern to require exploration. One carcinoma had been found. As a result of the survey early in 1969, five more individuals in this population were found to have developed thyroid masses which we felt should be explored. Four were from the more heavily exposed group on Rongelap atoll, i.e. several hundred to fourteen hundred rads estimated dose to the thyroid; and one on Utirik atoll who had probably received 30 rads. These were brought back to the U.S. for special study at Brookhaven National Laboratory. Because of our interest in and laboratory studies of radiated thyroids, the patients were brought to our institution in September, 1969 for surgical exploration. Three of the five proved to have lesions of the thyroid which were malignant (two Rongelap; one Utirik). One of these had extensive regional metastases. A fourth patient possessed several nodules, one of which was very small and has prompted serious consideration that this might also be malignant. Autoradiographs were immediately prepared in addition to a variety of observations commonly made on tissues from our animals (not ^{3}H-thymidine). Several of these thyroids had many minute solid cellular hyperplastic lesions, none of which took up significant amounts of radioiodine. Many of the lesions were papillary in structure and suggested papillary lesions of multicentric origin. All of these thyroids (except the woman from Utirik) showed the large bizarre nuclear forms repeatedly observed under this contract in animals and humans that had received ^{131}I.

Mention was made in the past annual report of a preliminary review of the Marshallese thyroid tissues for evidence of radiation effect based primarily on the bizarre nuclear forms and other subtle changes as observed in rats and human thyroid tissue. This study has just been completed in a formal manner.

To test the validity of these criteria as evidence of radiation effect, Dr. Robert Conard of Brookhaven National Laboratory assembled multiple slides of each thyroid of the Marshallese, of some thyroids of Japanese atomic bomb casualties, of thyroids of the Utah-Nevada children who may have been exposed (mentioned elsewhere in this report - from Dr. Marvin Rallison) and of miscellaneous unexposed nodular thyroids collected from elsewhere in Utah. More than 100 individual slides, completely disguised as to identity, were submitted to this writer for review and appraisal as to evidence of radiation effect in them. All slides were graded as to positive or negative evidence of radiation effect, and where positive, the evidence was further graded on a basis of +, ++, or +++; + being the slightest evidence and +++ being pronounced evidence. After individual slides were graded and reported to Dr. Conard, multiple slides that had come from the same thyroid were grouped together (still as unknowns) and reappraised. Although in most cases the ratings on several slides from the same individual were similar, it was appropriate to affix a single rating to each thyroid. In no case was the rating changed more than one degree to arrive at a common rating for a given thyroid. The results show that all of the Marshallese except one were identified as having received radiation exposure. Among these was a Marshallese woman who had been exposed and several years later had died of other causes on Majuro atoll where a post mortem provided the tissue which was free of gross nodularity. Several of the unexposed thyroids and a few of the Utah-Nevada group were graded as doubtful or +. Because of the correlation of the observations, Dr. Conard subsequently obtained the individual theoretical records of rad dose delivered to the thyroid (based on body burden determined some days after the removal of the Marshallese people from Rongelap). The degree of change attributed to radiation effect in the thyroids proved to correlate in surprisingly precise fashion with the degree of exposure previously calculated. Most of the Japanese tissues were graded only + or 0. Where as some observers have placed considerable weight of evidence for radiation effect upon the presence of lymphocytes,

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The writer returned to the Marshall Islands with the survey team again in March, 970. At least two more palpable suspicious nodules were found, one in a previously xplored individual who now has a 4 cm firm mass which is probably not compensatory ypertrophy. Plans for these two people have not yet become firm at this time.

he Utah-Nevada Study

The principal investigator has also participated in the annual examination of he children in the Utah-Nevada fallout area (exposed in 1954) where some endemic oiter is present. A week has been spent in this area each of the past five years in he annual thyroid examinations of children that had been screened from a very large opulation in the fallout area by a United States Public Health Service screening eam. Thyroid abnormalities which prompted concern were referred to a panel of three, f which the writer has been one. Those patients with thyroids judged to contain iscrete masses have been sent to the University of Utah Medical Center in Salt Lake ity for study. In some instances, the masses have been removed. The application of he same criteria of judgment of radiation effect in thyroids excised from this opulation has revealed only an occasional thyroid tissue which showed hints of a adiation effect. Reports are in preparation by Dr. Marvin Rallison. Another anuscript is ready for publication by Dr. Edward Weiss and others.

THE POSSIBILITY OF SPONTANEOUS GOITER ARISING IN THE OFFSPRING OF A PREVIOUSLY RADIATED ^{131}I MOTHER

During the survey of the Marshallese people on Rongelap early in 1969, both he exposed and unexposed population were subjected to complete examinations. This ncluded children born after exposure to the fallout. The occurrence on Rongelap f some rather significantly enlarged thyroids in some of the adolescent and pre-dolescent ages was most interesting. Some of these individuals were born of women ho had been exposed, but these children had been conceived some years after that xposure. It was surprising to see these adolescent goiters on Rongelap in a strain f people who have been regarded as free of goiter in this iodine abundant environment. he significance of these goiters was difficult to evaluate because adequate data on he occurrence of adolescent enlargement in the unexposed Marshall Island population as not available. It was suspected that some of these adolescents, although not xposed themselves, but born of previously exposed mothers, might have been influenced n utero by subtle thyroid deficiencies which were lingering in the mothers after all adioiodine from the fallout was gone. Under such an influence, the thyroid of the ffspring might have been affected in its early development. A subsequent review of he records of maternity of these children showed that not all had been born of xposed mothers.

With these uncertain observations in mind, a laboratory experiment using rats was designed to test the above hypothesis. A group of 16 young female rats identical to those used in other radiation experiments were given one of four dose levels of ^{131}I which were known to cause relatively mild degrees of thyroid damage. The rats were bred to normal males after all ^{131}I had disappeared from the gland. Litters were identified with mothers and body weights of litter mates recorded. They were weaned when approximately 40 gms, fed a diet with adequate but not excess iodine and sacrificed at about 250 gms of body weight. The thyroids were individually dissected out and weighed. These were compared with rats from litters born of non-irradiated female controls. Repeated litters (3 or 4) were obtained from the same females over a period of one year or until their reproductive capacities waned. The data showed that offspring from the females of the first three radiation dose levels displayed increasing thyroid weights but the offspring from the fourth and most heavily irradiated females had thyroid weight which more nearly resembled normal controls than the other. Unfortunately, the number of animals used in these experiments was small and the most heavily radiated mothers were not readily impregnated and had small litters. In spite of the heaviest dose of radiation, these few offspring did not fit the pattern seemingly established by the lower levels of the series. These experiments are again repeated.

The question of the adolescent goiters in children of exposed and unexposed Marshallese on Rongelap was re-explored more thoroughly in the most recent survey. With the previous incomplete observations in mind, the presence of goiter in adolescents born after the fallout exposure was reviewed: in children of exposed and unexposed population of Rongelap, in a street survey of all people on Utirik (who had received very low level exposure) and on a population of almost 200 on Likiep where there was no fallout. These data on the presence of diffuse thyroid enlargement and history of parents' exposure are now being analyzed. It is clear that goiter is very uncommon among the Marshallese and that when found, such as on Likiep, it was often in individuals whose origin was half German or Portuguese.

THE STUDY OF MORPHOLOGIC CHANGES IN HUMAN THYROIDS PREVIOUSLY TREATED WITH ^{131}I

Unfortunately for our work, in the past year no patients who had previously been treated with ^{131}I for hyperthyroidism and studied extensively in our personal series have had clinical reason for thyroid surgery, which would permit study of the thyroid tissue. Opportunities to obtain such tissues for study occur in a random fashion and only when a mass develops which causes concern. There were, however, the Marshallese patients who furnished material of this kind during the past year.

THE RELATIONSHIP OF BIZARRE NUCLEAR FORMS TO THE PERSISTENCE OF LACTS 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, the unknown driving force that makes the thyroid overactive probably continues for a time. The result of this driving force 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 presence of bizarre nuclear forms in some ^{131}I treated human thyroids and not in others might be attributable to the continued stimulus of Graves' disease

to produce hyperplasia in some 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 of Graves' disease during the past year and to carry out our usual studies on thyroid tissue with special attention to the occurrence of bizarre forms in such patients, when the opportunity does arise we are prepared to see whether the presence of LATS in the serum has any relationship to the bizarre nuclear forms found in some human thyroids.

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 a very large dose of ^{131}I. Not only was there an acute and marked rise in the incidence of anomalies in chromosomes of the circulating leukocytes, but residual effects in the form of anomalies from previous massive doses of ^{131}I had persisted six years. Since that time, we have attempted 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, there has been 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 blood samples, 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. David Satcher, an M.D., Ph. D. candidate working under Dr. Neil Macintyre (with whom we published the first work), has been studying this problem of chromosomal aberrations in some of the ^{131}I treated patients who we were studying in detail. Although he has studied relatively few cases he has studied them in great detail as implied above. His thesis entitled, "The Effects of ^{131}I and X-radiation on the Chromosomes of Peripheral Blood Leukocytes", has just been completed and his degrees are being awarded. He has studied the effects of doses of ^{131}I ranging from 6 to 19.7 mc. An increase in the frequency of chromosomal aberrations was found which reached a peak at 24 hours and fell to almost control levels by one week post treatment. He has not found a relationship between the dose of ^{131}I ingested and the increase in frequency of chromosome aberrations in time. Some patients have shown a greater response than others without a readily available explanation. He has concluded from the dose-response curves for aberrations that only leukocytes already in the blood stream at the time of ^{131}I therapy were involved and that precursor cells were not significantly damaged. He has very carefully analyzed a suspected source of error that may have contributed to misinterpretations of data produced by some others. The question is logically raised whether the ^{131}I circulating in the blood at the time a sample is taken and transferred to the culture contributes to the chromosomal aberrations while these cells are in culture. To test this question, a goodly amount of serum was obtained from the patient before the ^{131}I was administered. This non-radioactive serum was then substituted for the patient's radioactive serum when cultures were prepared subsequent to giving the patient ^{131}I. Conversely the "hot" serum was added to pretreatment control cultures. It was shown that residual ^{131}I carried into the culture continued to damage chromosomes while the culture was in progress. Hence most

of these studies were made using pretreatment serum in the culture with irradiated cells that had been collected at various intervals after the ^{131}I had been given. It can be concluded that the dose of ^{131}I commonly used to treat Graves' disease does cause chromosomal aberrations such as dicentrics and ring forms, which are the result of the radiation and not attributable artifacts. It is anticipated that this work will be published.