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SUMMARY REPORT

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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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This has been a broad investigation of physiological and morphological changes produced by ^{131}I in the thyroids of animals and man. The work originally began in 1949 under an AEC contract at the Massachusetts General Hospital, Boston. The present principal investigator of this project was one of the responsible investigator under that contract. In 1951 the principal investigator moved to Case Western Reserve University where the present contract began.

Various types of observations have been in progress at the same time. Owing to the variability and availability of special clinical opportunities with respect to the use of ^{131}I, owing to the necessary time intervals between sequential observations on animals and in man, and owing to acute demands on research personnel when unique opportunities arose, efforts have been directed in different areas depending on the circumstances that existed. The personnel engaged in this study are continually occupied with different aspects of the project depending on the variety of circumstances. Indeed, the collection of material from irradiated human thyroids and the study of these individuals has extended over many years in order to collect enough data to permit satisfactory conclusions. We must be continually tooled up to go into detailed study each time an opportunity arises, but remain occupied with other more elective animal studies when circumstances are not pressing.

The study of the morphologic changes grew out of the first observations on ^{131}I treated thyroids in man. A good many years were spent developing various methods of column and paper chromatography for the separation of iodinated compounds at a time when these methods were not available. These methods were used to study sequential changes in the amounts of these compounds in blood and in thyroid tissue in normal and irradiated thyroids. Extensive and detailed observations were made by these methods on selected patients at the time of ^{131}I therapy, and the information was stored for reference as follow-up information was obtained on these individuals through the years

A variety of long term studies on animals have simultaneously been in progress to determine the potentialities of irradiated cells with respect to glandular function, to nuclear structure, to changes in capacity for cell division, to neoplasm formation, and to the life of the cell that is crippled by radiation. These observations have led to information useful in recent national population studies on ^{131}I exposure. The various studies supported by this contract are briefly described in the paragraphs that follow:

The Development and Use of Column Chromatography for the Separation of Iodinated Amino Acids

A method for measuring the formation and disappearance of iodinated compounds in thyroids was desirable not only to study the normal steps in the synthesis of thyroid hormone but to observe the effects of radiation on those steps in synthesis and on thyroid function in general.

When starch column chromatography of amino acids was first described by Stein and Moore in 1948, the principal investigator, while still in Boston, attempted to modify this technique so that it might be applied to the quantitative separation of iodinated amino acids. It was about the time that this method of separation, using an acid medium, first proved effective that the first report by Fink and Fink appeared (1948) on the use of paper chromatography to separate iodinated amino acids.

After the interruption of work caused by the transfer to Cleveland, the method of chromatographic separation was tested in a large variety of experiments to establish the absolute identification of the iodinated amino acids, the completeness of the separation of the compounds and the reproducibility of the separations. There followed a lengthy period of study on the possibility of interchange of the isotopic iodine label among the amino acids during the course of the separation procedures. In the acid medium, which was used in the starch columns and in early experience with paper chromatography, there proved to be some interchange of iodinated atoms, invalidating the method for use in the exploration of biological processes in the thyroid Publications based on chromatography (primarily paper chromatography) using solvents in acid media had begun to appear and their reliability was questioned if interchange took place. A great deal of time and effort was then devoted to trying to find means of inhibiting this interchange without otherwise altering the nature of the biological samples. Sodium thiosulfate proved to be an effective inhibitor of iodine interchange. This was later supplanted by thiouracil, but in the process of exploring these problems the entire solvent system was changed to an alkaline medium which when used with starch columns proved to be a more satisfactory but extremely laborious method of separating iodinated compounds. By careful adjustment of the ratios of the constituents of the solvent and the addition of a small amount of the reducing agent, it was possible to separate thyroxine, iodide, monoidotyrosine and diiodotyrosine quite effectively and interchange of the label did not occur during fractionation. See attached Figure 1. Collection of eluent in 2 ml volumes from starch columns permitted the separation of the compounds by as much as 20 milliliters of solvent. The reproducibility of the separation on starch columns was proven by rerunning single labeled compounds, previously recovered, with other unlabeled compounds to determine under what circumstances complete recovery failed or interchange of the isotopic iodine in compounds occurred.

By using this method, two unknown compounds were repeatedly found in thyroid tissue. For more than 2 years efforts were made to identify the largest and seemingly most important of the 2 unknowns which was repeatedly found on each fractionation of thyroid tissue. When Gross and Pitt-Rivers described 3, 5, 3-triiodothyronine on paper chromatograms, our "Unknown #1" proved to be this compound.

Study of the Hydrolysis of Thyroid Tissue for the Quantitative Recovery of Iodinated Compounds

A method of removing the iodinated compounds from thyroid tissue as a preliminary step to separation and quantitation was required. Methods of hydrolysis for releasing iodinated compounds from thyroid tissue without either degradation or synthesis of new substances was thoroughly explored. Isotopically labeled compounds isolated in pure form by column chromatography were subjected to hydrolysis in the presence of non-labeled thyroid tissue and other types of tissue. This was done to test the reliability of the recovery of each iodinated compound under controlled conditions. A reducing agent was added to the hydrolysis to inhibit interchange of iodine atoms during the process of hydrolysis. The ^{131}I remained bound to each of the original compounds with the exception that as much as 17% of ^{131}I in thyroxine might be freed as iodide when thyroxine was exposed to the hydrolysis of thyroid tissue. The compound, later identified as triiodothyronine, was also slightly unstable under these conditions of hydrolysis. Triiodothyronine sometimes represented as much as 20% of the total yield of iodinated compounds from tissue.

The methods which were developed were used to quantitatively study the biological synthesis of thyroid hormone in thyroid tissue when ^{131}I had been given as a label to rabbits. The work utilizing this method of separation of iodinated amino acids was published in 1953.

Dobyns, B.M., and Barry, S.R.: The Isolation of Iodinated Amino Acids from Thyroid Tissue by Means of Starch Column Chromatography: J. Biol. Chem. 204: 417-531, September, 1953.

This report described the quantitative changes in the iodide and iodinated compounds in the thyroid as time passed following a dose of ^{131}I given to rabbits. It also described the work of the preceding 3 years on the development of the method, the reproducibility of the results, the identification of the compounds and their individual stability in the system, and the nature and control of the hydrolysis of thyroid tissue. The biological turnover of iodine was illustrated in rabbits by studying the thyroid at various intervals of time after ^{131}I had been given. See attached Figure 2. Although the iodide fraction contained a large amount of ^{131}I and the thyroxine fraction contained very little, the iodide and thyroxine ratio shifted in favor of the thyroxine with the passage of time. The mono- and diiodotyrosine fractions were major sites of ^{131}I in the gland during both the early and late periods of observation. The prompt observance and thereafter steady state of the isotope in the mono- and diiodotyrosine fraction prompted the hypothesis that mono- and diiodotyrosine served as a holding mechanism for iodine (storage) before it was converted to thyroxine.

Because of the laborious aspects of starch column chromatography of iodinated amino acids, similar separations were attempted using diatomaceous earth columns similar to the method of Braasch. Some time was saved by somewhat faster flow rates, but in these columns there was a forerunning peak which proved to contain varying amounts of each of the same amino acids that were separated as the chromatogram developed. Under these circumstances the method could not be used quantitatively for iodinated compounds in serum or hydrolysates of tissue. It did, however, permit somewhat more separation of thyroxine and triiodothyronine than did the starch column and was therefore used for this purpose in some experiments. A great deal of time was devoted to resolving the forerunning mixture of unseparated substances. However, this proved fruitless and the method was abandoned.

The Use of ^{14}C Labeled Tyrosine to Explore the Synthesis of Thyroid Hormone

The ^{131}I studies dealing with the synthesis of iodinated compounds in rabbits led to additional work attempting to use a second isotope. Some ^{14}C labeled tyrosine with the label in the carboxyl position was incubated with thyroid slices in a nutrient solution to study the sequence of the synthesis of thyroid hormone. As expected, the ^{14}C labeled tyrosine was recovered in its original form in the thyroid tissue slices but none of the ^{14}C was found in the mono- or diiodotyrosine fractions. It was surprising then that some of the ^{14}C appeared in the thyroxine and triiodothyronine fractions recovered from these slices. Because of the longstanding and universally attractive theory that the synthesis of thyroxine is accomplished by the combination of two iodotyrosine molecules, this apparent by-passing of mono- and diiodotyrosine to form thyroxine was unexpected. See attached Figure 3. It should be pointed out that the yields of ^{14}C in the thyroxine and triiodothyronine fractions were very small but significant. This was reported in the discussion of the paper by Gross and Pitt-Rivers at the Laurentian Hormone Conference in September, 1953.

Dobyns, B.M. In discussion of a paper by J. Gross and R. Pitt-Rivers. Thyroid Hormone Physiology and Biochemistry. Triiodothyronine in Relation to Thyroid Physiology. Recent Progress in Hormone Research 10: 119-121 Academic Press, 1954.

Although the experiment demonstrating the phenomenon of tyrosine by-passing of the mono- and diiodotyrosine to form thyroxine had been successfully repeated before the observation was reported, it was looked upon by others with some skepticism. The experiment has since been performed a total of 7 times confirming the synthesis of a small amount of thyroxine from tyrosine without forming mono- or diiodotyrosine from the labeled tyrosine. These incubation experiments have been performed using ^{131}I to label iodide and ^{14}C to label the tyrosine in the same incubation experiments. The two isotopes were counted in the various fractionated samples and their distribution determined by first counting the ^{131}I and then permitting all of this isotope to decay and then counting the ^{14}C. See attached Figure 4. The ^{131}I iodine was abundant in the mono- and diiodotyrosine but there was no ^{14}C in these compounds. The ^{14}C tyrosine again appeared in the thyroxine and triiodothyronine fractions in small but significant amounts.

Paper Chromatography of Iodinated Compounds

Although it was possible to completely separate the iodinated compounds by the starch column method developed and described, it was so laborious that it required almost a week to run a single separation and count all of the samples from it. It was therefore impossible to use this method of separation for a series of samples containing ^{131}I and collected over a period of hours or a few days because the small amount of isotope in iodinated compounds decayed before it could be measured accurately.

During and following the development of the starch column chromatography, paper chromatography gained increasing popularity. Our method of paper chromatography was developed as a quantitative method using a butanol-dioxane-ammonia solvent in a descending fashion. This we developed for our own use from one of the two steps of the two dimensional paper chromatography originally described by LeBlond and Gross. This general pattern became very popular and was widely used.

The paper method using butanol as a principal ingredient of the solvent in an acid medium was extensively studied in our laboratory because it was rapid and seemed reasonably quantitative. Much time was spent studying this method as it was being used by others. In the light of our experience with the acid medium used originally

in the starch columns, it seemed necessary to determine the circumstances which introduced variability in the paper system. Interchange of the isotopic iodine label again proved to be a serious objection to the method when an acid medium was used. The shift to ammonia in a butanol solvent resolved problems of interchange. It was found that the water-butanol ratio could be adjusted very critically by reducing the temperature of the freshly prepared solvent and thus reducing the solubility of water in butanol. The removal of excess water (by separation) at the lower temperature and the return of the solution to room temperature made the chromatography procedure very reproducible. The separation of all the important compounds was surprisingly clean.

The quantitative paper chromatograms proved to be much less laborious than column chromatography. It was then possible to carry out a series of quantitative determinations on the serum and urine of a patient in a period of a few weeks. This led to a very extensive study of serial quantitative chromatograms on blood, hours, days and weeks after a treatment dose of ^{131}I had been given. These studies will be described in a later section of this report.

Cellular Changes in Human Thyroids Treated with ^{131}I

During the late 1940's the responsible investigator became interested in bizarre nuclear forms found in thyroids of some patients who had previously been treated with ^{131}I. Since the responsible investigator is a surgeon, there has been a unique opportunity to procure samples of previously irradiated thyroids under very strict experimental conditions, indication for surgery usually being the removal of residual masses. With the timing of the procurement of the sample following trace doses of ^{131}I and the analyses being carried out in the laboratory under the supervision of the same person who designed the experimental protocol and procured the samples of tissue, it has been possible not only to obtain, with precise timing, tissues and carry out special histological techniques but also to observe the synthesis of thyroid hormone by these irradiated glands. At the time of the move from Boston to Cleveland a total of 29 human thyroid tissues had been collected. Although much of the work had been done under the former contract in Boston, the preparation of the material and of the publication was accomplished later. The most important observation in this study of radiation effects was the large, bizarre nuclear forms that appeared in the human thyroids long after the radiation from ^{131}I was gone.

Dobyns, B.M., Vickery, A.L., Maloof, F., and Chapman, E.M.: Functional and Histological Effects of Therapeutic Doses of Radioactive Iodine on the Thyroid of Man. J. Clin. Endocrinol. & Metab. 13: 548-567, 1953.

Early Radiation Experiments to Study Changes in Cellular Structure and Function in Animals

When the bizarre nuclear forms were first discovered in man, animal experiments were designed to observe long and short term effects of radiation in thyroids of rats given various doses of ^{131}I. More than 200 young rats were injected with various doses of ^{131}I ranging from 1 μc to 300 μc. The uptakes were determined, and the subsequent functional nature of the thyroids studied. This study became the primary effort of Dr. Farahe Maloof who was a co-worker and now the head of the thyroid laboratory of the Massachusetts General Hospital. Graded impairment to function was demonstrated in the animals. See Figure 5. The two most interesting histologic changes in these thyroids following irradiation were a persistent increase in cell height of the thyroid epithelium even in the slightly irradiated glands and the bizarre nuclear forms as had been seen in some of the human material. The increase in cell height, which was thought to reflect an endogenous stimulus from the animal's pituitary resulting from

subtle functional impairment, was present for 1½ years following doses of 5, 20, 50, and 100 µc of ^{131}I to animals. It was discovered in these studies that the thyroids failed to enlarge in response to the administration of thiouracil in spite of the increase in cell height. The bizarre nuclear forms became apparent after the administration of the thiouracil. Such changes were produced in animals that had received 5 µc and were accentuated in animals that had received 20, 50 and 100 µc. The results of these studies were published.

Maloof, F., Dobyns, B.M., and Vickery, A.L.: The Effects of Various Doses of Radioactive Iodine on the Function and Structure of the Thyroid of the Rat. Endocrinol. 50: 612-638, June, 1952.

Morphological Changes in the Nucleus of Rat Thyroids Following ^{131}I Radiation

One of the primary objectives from very early in this contract has been to study the nature of the large irregular hyperchromatic nuclei in irradiated thyroids. Large numbers of rats were placed on an iodine deficient diet for a number of days and then given various single doses of ^{131}I to study nuclear changes in a variety of ways. As previously observed, changes appeared in several months and were accentuated by the administration of thiouracil. The impairment to the glandular enlargement that is usually produced by the administration of thiouracil, was again evident following doses of ^{131}I which were not large enough to cause architectural distortion. By making more frequent observations it became evident that the impairment to enlargement of the gland in the mildly irradiated gland surprisingly did not occur promptly after ^{131}I had been given but occurred after a considerable lapse of time, long after the ^{131}I had vanished from the gland. See attached Figure 5. The appearance of abundant bizarre nuclear forms and the inability of the gland to enlarge on thiouracil seemed to be temporally related. These observations supported our belief that the irregular nuclear forms represented preparation for but thwarted attempts at cell division. The failure of hypertrophy was suspected to be a consequence.

In order to explore this possibility, animals were divided into several groups depending on the radiation which they had received and were given colchicine. An attempt was made to determine a mitotic index in the previously irradiated thyroids of animals treated in various ways with thiouracil and colchicine. Unfortunately the mitoses seemed to be either sufficiently infrequent or unidentifiable so that these experiments did not yield conclusive results as to the frequency of mitoses. Other more extensive studies of nuclear change will be described.

Observations on the Biochemical Changes Resulting from a Single Therapeutic Dose of ^{131}I in Man

Early in this project enormous amounts of time were devoted to collecting very detailed data, especially kinetic data, on selected patients who were given ^{131}I therapy for hyperthyroidism. Only selected patients could be used because of the enormous amount of time required for the study of a single patient. The sequence of 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. The responsible investigator is in charge of all ^{131}I therapy, as well as other forms of therapy for hyperthyroidism at Cleveland Metropolitan General Hospital. There has thus been an opportunity to study each patient in great detail and to organize all studies in a predetermined fashion so that the largest amount of information could be obtained from each patient, whether he was treated surgically or with ^{131}I.

The following is a brief outline of the observations made on these patients:

1) To determine the amount of the treatment dose taken up by the thyroid, and ith almost daily thyroidal measurements, determine the character of the disappearance urve of ^{131}I from the gland.

2) To follow the concentrations of ^{131}I in the serum at the same intervals as he above observations were being made on the disappearance from the gland.

3) To determine the amount of ^{131}I extractable (with water saturated butanol) rom the serum.

4) To prepare serial quantitative chromatograms to show the amounts of various odinated compounds in whole serum and in extracts of serum (hours, days and weeks) ollowing the administration of the treatment dose. These serial observations not only nitially reflect the abnormalities of the disease process and variations among atients before a substantial radiation effect occurs, but they also reflect changes hat are induced by the radiation.

5) To determine the subsequent function of the irradiated gland by measurement f ^{131}I uptake and hormone production and to relate these to the pretreatment observations.

6) To make observations on the suppression of circulating lymphocytes and granulo-ytes each time the above observations on blood were made. This was done only in the early ears of the study.

7) To relate the biochemical data to the effect of the total dose of radiation elivered and to the ultimate clinical course of the disease.

The rise and fall of various compounds (such as thyroxine and triiodothyronine), and he appearance of certain other compounds (such as mono-iodotyrosine, diiodotyrosine nd thyroglobulin), after the treatment dose reflected not only the effect of adiation, but also probably reflected the features of the abnormal physiology of the isease before the radiation effect becomes significant. Retrospectively, the changing atterns of iodinated compounds in the blood, the alterations in rate of return of the adioactivity from degraded thyroxine to the thyroid, and the subsequent testing of he functional capacity of the gland permitted an analysis of what had occurred.

Because of the large amount of labor involved in the study of each patient for his study, only selected individuals were used. During the early part of this contract he personnel on the study devoted almost all their time to these studies. Gradually s the other long term studies have evolved, more selectivity of patients has been made. total of about 2,500 chromatograms have been prepared on approximately 225 patients erially studied over the course of about 15 years. These very extensive data have ot only been analyzed but also stored for reference and reviewed from time to time in he light of subsequent clinical developments in these patients. Many of the findings re related to other observations described under this contract.

ontribution of the ^{131}I Kinetics Data of the Public Health Service Follow-up Study

When the United States Public Health Service undertook a follow-up study of a arge number of ^{131}I treated patients in various centers particularly interested in hyroid disease, the responsible investigator of this contract was asked to serve on he protocol committee and subsequently became chairman of that committee. Our aboratory became one of the 19 centers in that study. In the course of the review of ata accumulated at various centers, it was found that there was considerable variation

ı the extent of the observations that had been made on ^{131}I-treated patients. lthough percent uptake of the treatment dose, estimate of gland weight, and spot ıecks of urinary excretion were often obtained, detailed data for better under- :anding the kinetics of the ^{131}I such as the long term sequential observations on ehavior of ^{131}I in the gland, the changing levels of radioactivity in the blood ith the changing patterns of the compounds, the daily urinary excretions of radio- :tivity etc., were usually not available. Because neither the observations nor the ethods of making the observations were uniform among the institutions, it was difficult) make comparisons based on fragmentary data between patients in different institutions. : therefore seemed desirable to try to discover patterns of response from very complete ıta on a few patients so that reasonable predictions could be made for missing data ı otherwise similar patients on which only incomplete data were available. The :tailed data which had been assembled in our laboratory over some years under this C contract (including the long term follow-up data) were drawn on rather heavily ıt superficially in an effort to prepare models of kinetics in various clinical ıerapeutic situations. Data on sequential blood levels of ^{131}I, disappearance rates 'er the thyroid, sequential urinary excretion of radioiodine and the appearance and .sappearance of iodinated compounds in the circulation as they relate to the clinical 'fect were furnished. However, time has not permitted their use. Although the :eering committee has felt keenly that data where it might relate to kinetics should : tested thoroughly where the data were available, there have not been personnel fully :quainted with the problem nor funds available to explore this area of the national ıudy. Dr. A. Bertrand Brill who was the USPHS physician and who originally set up the .tional study is now at Vanderbilt and particularly interested in this aspect of the .tional study. To date direction of the national study has been toward leukemia and oplasm development. More will be said of this later.

e Disappearance of ^{131}I from the Thyroid Following a Therapeutic Dose of ^{131}I

In general, there are three types of curves that describe the disappearance of a erapeutic dose of ^{131}I from the thyroid:

1) A rapid decline of ^{131}I from the gland. This is seen where very large doses ^{131}I are given for the purpose of producing rapid complete destruction of the thyroid. ry soon after a large dose of ^{131}I was given (days) the gland was unable to trap e ^{131}I-iodide which became available from the degraded ^{131}I-thyroxine in the circulation. ter the loss of ^{131}I is due to disintegration of the gland.

Occasionally the loss of thyroid function did not follow such a precipitous cline in ^{131}I. The serial quantitative chromatograms reveal that there are occasional tients who make thyroid hormone very rapidly and release it into the circulation so at much of the radiation effect is delivered in the circulation rather than in the yroid. This was shown by the very rapid disappearance of ^{131}I from the gland and the pearance of high levels of ^{131}I thyroxine circulating in the blood.

There were other patients who showed a steep decline in thyroidal radioactivity t who did not show a favorable therapeutic response. In some of these situations ere was found to be an unusually large amount of triiodothyronine produced. One :ticular patient was actually found to be producing more triiodothyronine than yroxine. In this patient the serial chromatograms of urine also revealed considerable ounts of triiodothyronine. It subsequently became clear that triiodothyronine was t well bound to protein in the circulation and that considerable amounts of it spilled t through the kidney. Thus the opportunity for this ^{131}I to return to the thyroid s lost. The extreme example of this phenomenon was observed in a patient through ır therapeutic doses of ^{131}I (with a total of 26 quantitative chromatograms) before e hyperthyroidism was controlled.

obyns, B.M.: The Failure of Radioiodine Therapy Presumably Owing to High Triiodothyronine Production in a Patient with Severe Graves' Disease. Unpublished.

These observations prompted an analysis of data from those patients in which here had been triiodothyronine found in the serial chromatograms. At that time 27 f 52 patients studied had triiodothyronine in the circulation early in the period of bservation. Most of these were Graves' disease. Most were classified as having ore severe clinical manifestations of thyrotoxicosis and almost all displayed a very apid conversion of iodide to thyroxine. Triiodothyronine was also found in a case of oxic adenomatous goiter; in two patients there was a small functioning remnant of hyroid tissue after an almost total thyroidectomy had been performed; and two euthyroid atients were treated for angina pectoris. These findings were summarized in a ublication.

enua, R.S., Dobyns, B.M., and Ninmer, A.: Triiodothyronine in the Serum of Patients reated with Radioactive Iodine. J. Clin. Endocrinol. & Metab. 15: 1367-1378, ovember, 1955.

Another cause for rapid decline of ^{131}I from the thyroid is the presence of unknown ources of ^{127}I which will dilute the ^{131}I when it is returned to the circulation. The tudy of this phenomenon was carried out by the deliberate administration of ^{127}I in some ases following the treatment dose. Contrary to expectation it did not always produce he steep decline that was observed in some of the patients who failed to respond. ubsequent observations suggest that although the ^{131}I may be released from the gland apidly and diluted out of the circulation by an excess of ^{127}I, the excess ^{127}I may low the actual release of hormone from the gland in Graves' disease. The over-all esult is therefore retention rather than loss from failure to recycle the iodide.

2) A high uptake followed by a very slow gradual decline of ^{131}I from the hyroid. This was the natural disappearance curve that represents the biological urnover of iodine. This suggested that there was no disintegration of the thyroid or destruction of those mechanisms which retrap that iodide which is made available rom thyroxine that has been used by the peripheral tissues. In spite of this very low type of disappearance curve, many thyrotoxic patients with this pattern ultimately howed marked reduction in their thyroid function, but the decline occurred gradually ver quite a number of months. A prompt dramatic clinical improvement, followed oon after by hypothyroidism, was seldom seen following this curve.

3) A well sustained ^{131}I level in the thyroid for 7 to 12 days followed by a eak in the curve and a sharp decline. This pattern in almost all instances is orrelated with a fairly precipitous subsequent decline in thyroid function. The erial quantitative chromatograms in such cases suggested that the change in the slope f the curve represented a failure of the gland to retrap iodide. A variety of isappearance curves are shown in Figure 6. Apparently following the break in the urve, ^{131}I was not recycled back into the thyroid. When this break in the disappearance urve was clearly demonstrable, the incidence of myxedema was noticeably increased. hese observations and others were described in a publication.

enua, R.S., and Dobyns, B.M.: Iodinated Compounds in the Serum, Disappearance of adioactive Iodine from the Thyroid, and Clinical Response in Patients Treated with adioactive Iodine. J. Clin. Endocrinol. & Metab. 15: 118-130, January, 1955.

There was a relative increase in the amount of circulating ^{131}I-iodide in roportion to other iodinated compounds shortly after the break in the curve in umerous instances. A coincident rise observed in the excretion of radioactivity in he urine seemed to reflect a failure of retrapping of iodide by the gland.

It had been the policy to prepare butanol extracts of serum from which some f the quantitative chromatograms were made. Concentrating the iodinated compounds nhances the possibility of detecting more unusual substances that might be present nly in minute quantities in whole serum. The butanol used for extraction was aturated with water so that iodide in addition to iodinated organic molecules was ecovered. Other chromatograms were prepared on whole serum for purposes of comparison. he extractability of the radioactivity in the serum using this extraction system was bserved to decline as the radiation effect took place in the thyroid. At the same ime chromatograms from whole serum showed that the radioactivity representing the nsoluble fraction and remaining at the application point increased markedly. This epresented circulating thyroglobulin. In some instances the extractability, which ormally is 85% to 95%, dropped as low as 65% to 70%. Where this occurred, the ubsequent clinical improvement was usually quite prompt and hypothyroidism was a redictable outcome.

he Significance of Mono- and Diiodotyrosine in the Circulation Following ^{131}I Therapy or Hyperthyroidism

Farran, Lea, Goolden and Abbatt had reported that the presence of mono- and iiodotyrosine in the circulation in patients with thyrotoxicosis was an indication hat ^{131}I therapy might fail. Their observations had been made on single samples of lood from 92 patients. In contrast to their observations, when multiple determinations n the form of serial quantitative chromatograms were available on approximately 150 atients with thyrotoxicosis treated with ^{131}I, the observations of the English workers ere not confirmed. It was found that those patients who had mono- and diiodotyrosine n the circulation ultimately displayed a more striking radiation effect. In many atients the mono- and diiodotyrosine began to appear on the fourth or fifth day ollowing the administration of ^{131}I. This appearance was usually accompanied by a educed extractability of the radioactivity from the serum, a greater quantity of adioactivity remaining at the application point when whole serum was chromatographed, prompt subsequent clinical improvement, and a high probability of subsequent ypothyroidism. See attached Figure 7. It was concluded that the presence of ^{131}I n the form of mono- and diiodotyrosine represented products of disintegration of thyroid issue. When found, it was usually in minor quantities in the circulation.

omparison of Two or More Treatment Doses in the Same Individual

We have been on the look out for patients who required further retreatment with adioactive iodine so that it would be possible to compare all of the observations sually made on radiation effect under the two therapeutic conditions. One of two situations may exist in the patient who requires further treatment. Some may have had ittle clinical radiation effect; others may have had an effect which was considerable

but not adequate. Repetition of the detailed studies in both types of patients have been desired, but unfortunately the enormous investment of time and effort to follow a single treatment dose in one patient has been so great that it has only been possible to study selected patients. Thus, it has only been a chance occurrence that an additional therapeutic dose of radioiodine was necessary in a patient who had been completely studied on the first occasion. Partly by chance and partly by design a few patients were given a dose of ^{131}I which was not sufficient to return the patient to a euthyroid state. A second therapeutic dose exactly equal to the first was subsequently given in a few instances and all of the observations repeated. The comparative studies of the two treatment doses have not revealed information that was particularly informative, except that the second dose was often not taken up as well as was the first.

The Use of Paper Electrophoresis to Study the Iodinated Proteins and Polypeptides in the Circulation Following ^{131}I Therapy

It has been pointed out that in serial chromatograms of serum following a treatment dose of ^{131}I there was an increased amount of radioactivity at the application point. Paper electrophoresis was used to explore the nature of the iodinated compounds which remained at the application point. These observations although carried out periodically for a number of years, did not reveal striking results except to show that much of the radioactivity was in the alpha globulin fraction and probably represented the thyroxine-binding globulin. Ultimately when the patient reached the hypothyroid state, considerable ^{131}I was in the albumin fraction.

The Role of Triiodothyronine in Hyperthyroidism

Since we and others had identified triiodothyronine in the circulation of man and its rapid and potent metabolic action had been proven, the possibility that it represented the principle form of thyroid hormone in thyrotoxic patients was entertained by many persons. Among other theories it was thought that thyroxine might be the precursor of triiodothyronine. As previously described, the large number of serial chromatograms on ^{131}I- treated patients probably reflected basic physiology of the disease immediately after the ^{131}I was given and later after hours and days had passed reflected the radiation effect. Triiodothyronine was found in the chromatograms of serum of many of the more thyrotoxic patients. The chromatograms done on the very early samples of blood often showed that triiodothyronine, if present, appeared sooner and in larger quantities than thyroxine (especially in the most toxic patients). This observation tended to discredit the possibility that thyroxine was the precursor of triiodothyronine.

There was in our series an occasional patient treated with ^{131}I for hyperthyroidism caused by a toxic adenoma. Some patients in a euthyroid state have been treated with ^{131}I and studied in the same detailed way. In both situations triiodothyronine was occasionally detected, showing that triiodothyronine was not peculiar to Graves' disease.

Our accumulated evidence has shown that triiodothyronine is not the result of irradiation: first, because it appeared very early before appreciable radiation was delivered to the gland; and second, because it did not increase further with time but was subsequently greatly overshadowed by thyroxine ~~after~~ hours later when the radiation effect should have become more significant. Finally, it has been easier to relate the presence of triiodothyronine to high degrees of clinical toxicity than to subsequent clinical therapeutic success of a single treatment dose of ^{131}I.

In order to get a better perspective of the triiodothyronine metabolism in patients treated with radioactive iodine, considerable time was spent studying the fate of triiodothyronine labeled with ^{131}I and administered to humans. The observations were made in patients with Graves' disease before and after treatment with ^{131}I, as well as in normal individuals. There was special interest in those who were originally known to produce appreciable levels of triiodothyronine. The fate of triiodothyronine was observed in these individuals by quantitative serial chromatograms on samples of serum and urine. In addition, the radioactivity was measured over the liver as it accumulated in this organ. During the first 48 hours following the administration of labeled triiodothyronine, more of the material remained in the circulation in patients with Graves' disease than in normal controls. At the same time more radioactivity was excreted in the urine of Graves' disease than in the normals. This urinary increase was largely due to ^{131}I-iodide. The increased excretion in the patients with Graves' disease occurred regardless of the metabolic state--whether it be untreated hyperthyroidism or hypothyroidism following treatment. Measurements over the liver revealed a maximal concentration minutes after the intravenous injection of triiodothyronine. This was followed by a marked fall. In Graves' disease there was a secondary cyclic rise of radioactivity in the liver but this did not occur in the normal controls. The fate of the triiodothyronine in the peripheral tissues was not studied. The fact that the triiodothyronine levels in the serum of Graves' disease remained higher than that in normal individuals during the first 8 hours seemed to conflict with the observation that more radioactivity appeared in the urine as iodide. The only available explanation was that the triiodothyronine which was removed from the circulation in Graves' disease was deiodinated more rapidly than in the normal subjects. It was further noted that triiodothyronine was retained in larger amounts and for a longer time in the liver of patients with Graves' disease than in normal subjects. (In other studies thyroxine was retained in the liver in a fashion similar to triiodothyronine.) Although there was slightly more triiodothyronine excreted through the kidneys in the severely hyperthyroid individual than in the post-treatment hypothyroid Graves' disease, the difference was less impressive than the difference between all patients with Graves' disease and normals. These observations shed some light on the difference between patients with Graves' disease and normal subjects with respect to triiodothyronine but did not contribute information to an understanding of any possible relationship between triiodothyronine metabolism and the radiation effect on the hyperfunctioning gland.

Gales, I.B., and Dobyns, B.M.: The Metabolism of Triiodothyronine in Graves' disease. J. Clin. Endocrinol. & Metab. 20: 68-80, January, 1960.

The Measurement of Desoxyribonucleic Acid in Individual Thyroid Cells Previously Subjected to ^{131}I Radiation

As this 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. Since ordinary staining methods suggest the presence of excessive amounts of chromatin material in the bizarre nuclear forms that were produced by small doses of ^{131}I plus thiouracil stimulation, it seemed appropriate to try to measure the amount of DNA actually contained in such nuclei. Therefore, a histochemical method for measuring desoxyribonucleic acid in these large nuclei of irradiated thyroids was adapted to this objective.

The method of Pollister for the quantitative microspectrophotometric method of measurement of Feulgen stain within a nucleus was set up in our laboratory with the suggestions and advice of Dr. Pollister of Columbia University. Since the Feulgen stain combines with DNA in a quantitative fashion, the amount of stain contained within a nucleus could be measured by absorbance of transmitted monochromatic light having a specific wave length at which there was maximal absorption for this stain. Rat thyroids were used in these studies. The staining procedure was carried out under highly controlled conditions. The irradiated and nonirradiated tissues which were to be compared were placed side by side on the same microscopic slide so that both tissues were exposed to exactly the same steps of preparation and staining circumstances. Measurements were made under oil emersion magnification. With the use of a calibrated diaphram it was possible to measure the transmission of light through a plug of nucleus which was slightly less than the nuclear diameter. The value for absorbance in the plug was then converted to the sphere, which represented the volume of the nucleus. The mean diameter of each nucleus was determined by direct micrometer measurements. DNA was recorded in arbitrary units. The value for a normal resting nucleus was determined and standardized against rat liver cells. A value approximately equal to the diploid value in the rat cell was obtained from measurement on the liver.

When thiouracil which is a powerful stimulus to hyperplasia of the thyroid was given to an animal, the diameters of the nuclei of normal thyroid cells increased. The DNA also increased toward values which represented two times the diploid value, at which point the cell normally is expected to divide.

Rats were given 10, 30, or 100 µc of radioiodine after a temporary period of iodi-deficiency to inhance the uptake of the ^{131}I. Thereafter a diet containing a normal amount of iodine was resumed. One half of each group of animals was then given thiourac in their drinking water. Some of each group of animals were sacrificed at 16 weeks, the remaining at 26 weeks.

None of the doses of ^{131}I used in this series of experiments was sufficiently great to cause architectural distortion of the thyroid. However, the largest doses were sufficient to interfere with the expected increase in thyroid weight that is usually produced in normal thyroids by thiouracil. The lesser doses of radiation

produced proportionately less impairment to this capacity for glandular hypertrophy. (An example of the effect on the gland weight under the influence of the stimulus is shown from previous experiments in Figure 5.)

Even though there was no obvious evidence of architectural damage to the thyroid, it was believed that these glands had sustained some intrinsic physiological impairment because there was an increase in cell height. The histochemical method showed that in the nonstimulated nonirradiated thyroid the DNA of each nucleus was quite uniform from cell to cell. See Figure 8A. The stimulus of thiouracil alone resulted in some nuclei containing more DNA than the average resting cell but in none did the amount exceed two times the diploid value. Some of these cells were presumed to be preparing to divide. After 10 µc of ^{131}I the DNA content and nuclear size showed slightly more variation. The variation in DNA among individual cells was expressed as a standard deviation of the mean. Some nuclei of radiated thyroids contained far more DNA than two times the diploid value, at which point the cells should have divided. The administration of thiouracil to the animals with slightly irradiated glands caused an increased variation in nuclear size and in the DNA content. The effects of 30 µc of ^{131}I was even more striking. The large irregular nuclei with excessive DNA appeared even without the superimposed stimulus. This was greatly exaggerated when the stimulus of thiouracil was applied.

Further experiments, lasting over several additional years, more animals and a greater range of dosage of ^{131}I were used (0, 5, 10, 20 and 40 µc; followed for intervals up to 1½ years before sacrifice). After doses of as little as 5 µc, there was little if any evidence of nuclear change; but when thiouracil was superimposed, the variation in DNA content in nuclei again appeared. At levels of 20 and 40 µc there was spontaneous variation in DNA content. Examples of occasional nuclei containing multiples of approximately 4, 8, and 16 times the diploid values of DNA were found in some of these large bizarre nuclear forms. It was postulated from these experiments that the cells had the capacity to build up DNA when stimulated but for some reason cell division was thwarted.

This histochemical method of measuring DNA in nuclei may be questioned as to preciseness by some workers. However, if the staining procedure is very strictly controlled when comparisons are made with nonirradiated animals and absorbance is measured in a plug of nuclei that closely approached the size of the whole nucleus, the method has proved in our hands to be quite reproducible and very useful in interpreting the nature of the large bizarre nuclear forms that had previously been observed by ordinary histological techniques in irradiated thyroids. The results of these experiments carried out over a number of years were published in considerable detail.

Dobyns, B.M., and Didtschenko, I.: Nuclear Changes in Thyroidal Epithelium Following Radiation from Radioiodine. J. Clin. Endocrinol. & Metab. 21: 699-720, 1961.

The Large Bizarre Nuclear Forms in Human Thyroid Tissue and the Measurement of DNA in Them

From time to time 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 (previously irradiated with ^{131}I) was to become available. When an opportunity arose to obtain such tissue, plans were made so that it was the responsible investigator of this project who procured the tissue at the operating table. It was placed in fixative in the operating room without delay. 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, there were tissues from 4 thyroids previously subjected to x-ray radiation and 2 controls (exploration for parathyroid adenoma). Ten of the 12 ^{131}I patients were subjected to surgery because of masses which had developed in the thyroid; one patient was operated because of persistence of hyperthyroidism and was receiving an antithyroid drug at the time the tissue was obtained; in two instances tissue was obtained during prompt postmortem examination. All tissues from the various thyroids had been processed and stored in paraffin blocks so that simultaneous staining could be accomplished on all tissue at the same time. The final steps in the preparation and staining of the tissue from these irradiated and control thyroids were concluded simultaneously.

The quantitative measurement of DNA in individual nuclei using Feulgen staining and microspectrophotometry showed considerable variation in nuclear DNA content and nuclear volume in some, but not all, of the irradiated tissues. Measurements indicated that the amount of DNA in some cells was greater than two times the diploid value. This is as was observed in the stimulated thyroids of animals which had previously been given ^{131}I. Here in man as in animals the results are interpreted as a build up in DNA, but thwarted cell division. See Figure 8B.

On review of alternate sections stained with the customary hematoxylin and eosin method, it was found that somewhat fewer of these irradiated tissues displayed bizarre nuclear forms than was observed in our previous irradiated human thyroids described some years before. 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 but who had been given propylthiouracil before the procurement of tissue. 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 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 causes Graves' disease has abated or whether the force is still there, but the thyroid is so damaged that hyperthyroidism is not possible.

Since the opportunity to biopsy irradiated thyroids arose as a result of palpable masses in such thyroids, there was an opportunity to carry out similar measurements on cells comprising adenomas which had also been removed. 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 irradiated 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. Probably the former is true in some cases. Animal experiments to be described later are designed to explore this issue. The observations on human tissue described above are reported in the following publication.

Dobyns, B.M. and Robinson, Leon R. III: Deoxyribonucleic Acid Content Associated with Nuclear Changes in ^{131}I-Irradiated Human Thyroids. J. Clin. Endocrin. & Metab. 28: 875-885, 1968.

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 to result in 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 irradiated cell to divide, although the capacity to build up DNA in preparation to division still exists. 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 may persist for a good many years. However, we now are seeing at 12, 15 and more years after ^{131}I therapy in these human glands, which appeared to have adequate capacity to manufacture hormone, ultimately are failing 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. These observations and hypotheses have led our investigation of the capacity of irradiated thyroid cells to replicate under a variety of conditions.

The Use of Tritiated Thymidine to Study the Mitotic Activity in Irradiated Thyroids

The incorporation of tritiated thymidine into DNA in thyroid nuclei was first studied in this laboratory under this grant a little over nine years ago. It has proved to be a very useful tool to study in another way the effects of ^{131}I radiation on cellular morphology and mitotic activity.

Because the cells which are preparing for mitosis incorporate the thymidine avidly just before they divide and because such cells bearing the label can be demonstrated by radioautography, this method was adapted to the study of mitosis in thyroid cells which had been exposed to ^{131}I. The method for demonstrating DNA synthesis in the rat thyroid was developed in the course of some experiments designed to test the effect of TSH on cell division in weanling and adult rats. One unit of TSH was injected intraperitoneally into these two groups of rats; 16 hours later tritiated thymidine was given to each animal by the same route. The animals were sacrificed 4 hours later and the thyroids were quickly removed. They were placed in Carnoy's fixative for rapid fixation and microscopic sections prepared. After the sections had been deparafinized, they were dipped in photographic emulsion and stored in the dark for various periods of time. Subsequently the film was developed and the sections beneath it stained. The occurrence of labeled nuclei was then determined by noting the blackening in the emulsion over each of several thousand nuclei. It was observed in the weanling animals which had received no TSH that 9% of 12% of thyroid nuclei were labeled. When TSH was given to these animals, the incidence rose to 14%. In the nonstimulated old animals the incidence of

labeled nuclei was only 0.4% but when such animals were injected with TSH, the incidence rose to 7%. Thus, with this technique it was possible to demonstrate that cell division in the untreated young rats was occurring at a rate almost as fast as in the adult animals whose thyroids had been stimulated with TSH. Histologically, in the adult animals, the thyroids were truly in a resting state. After considerable experimentation it was found that the most desirable interval of time between the injection of the tritiated thymidine and the sacrifice of the animals was 4 hours. There was no uptake of thymidine in cells which were resting. Even in this 4 hour interval there were some instances of labeled pairs of nuclei lying in juxtaposition with approximately equal amounts of DNA in each. This gave a general idea of the rate at which cell division took place once the preparation for mitoses had begun. A manuscript describing these observations has been prepared but not published.

Dobyns, B.M., and Sanders, M.A.: A Comparison of the Effect of TSH on the Mitotic Activity in the Thyroids of Young Growing Rats and Adult Rats Using Tritiated Thymidine. (Manuscript not published; later manuscripts incorporated the technique and later findings went far beyond these).

Rat thyroids made hyperplastic by an iodine deficient diet display many nuclei containing the tritiated thymidine. When in a state of iodine deficiency doses of 0, 5, or 50 µc of ^{131}I were given to groups of rats to produce various degrees of radiation damage. Twenty hours after giving 5 µc of ^{131}I (av. uptake 37%; 2.0 day half-life; 2640 Rads), the number of labeled nuclei were noticeably reduced. At this early hour the thyroids of animals given 50 µc showed an almost complete absence of labeling of nuclei, indicating that the process of mitosis had been interrupted. Twenty-two days after these doses of ^{131}I, the administration of thiouracil caused marked increase in labeling in nonirradiated controls and somewhat less labeling in the 5 µc animals, but the 50 µc animals had not recovered the ability for labeling. Recovery from this impairment to mitosis as studied by the labeled thymidine has been of special interest.

After more lengthy periods of recovery following radiation, some of the animals from each group were given thiouracil for 9 to 11 days and tritiated thymidine 4 hours before sacrifice. The results after various amounts of radiation and various periods of recovery are shown in Figure 9. At 2 months following the 5 µc dose of ^{131}I there was an excessive number of labeled nuclei when thiouracil was given. The number of labeled nuclei was considerably greater than that which was produced in a nonirradiated animal. This seemed to be related to the previous observation that gross hypertrophy of the gland could still be produced by thiouracil administration quite some time after radiation has been delivered. At this 2 month interval in this particular series the 5 µc animals showed a moderate increase in thyroid weight suggesting that cell division had actually taken place. The 50 µc animals on the other hand showed essentially no hypertrophy of the gland. Still later in the post radiation period the capacity for excessive labeling could not be produced in the 5 µc group of animals. At this time, these thyroids did not increase appreciably in weight. Even after a longer recovery period (8½ months) following the ^{131}I the extreme degrees of labeling could not be produced with thiouracil in the 5 µc animals. These observations are illustrated in Figure 9.

It was of special interest to find that the large bizarre nuclear forms which appeared when thiouracil was given to these irradiated animals were the nuclei that seemed to have a special predilection for the thymidine. Nuclei of cells which are preparing to divide usually become enlarged, but here the abnormally large nuclei, which presumably have not divided, were making more DNA.

In this series of irradiated animals, one lobe of each thyroid was put in Carnoy's ixative so that the quantitative histochemical (Feulgen staining) measurement of ne total amount of DNA on individual nuclei could be made,as done in earlier xperiments. Since cells normally divide when the DNA of the nucleus reaches two times ne diploid value, the finding of considerably more than this amount of DNA (using the istochemical method) in the large bizarre nuclear forms made the thymidine observations n the same animals of great value.

A complete survey was made of a large number of nuclei in a microscopic slide by easuring and classifying all nuclei by size and determining the distribution of the abel. This showed that the abnormally large nuclear forms contained the label more ften and were thus preparing for mitosis more frequently than other cells.

The parallel histochemical data using the Feulgen stain and microspectrophotometry n these same tissues showed that thiouracil caused a marked increase in DNA in many uclei as well as increase in the nuclear size. When this stimulus was superimposed on reviously irradiated glands, there was an increase in the variability of both DNA and uclear volume of these glands. The greater variability was attributable to the resence of the unusually large nuclei which in the parallel observations were shown o have a predisposition to take up the thymidine. The following publication ummarizes this work:

obyns, B.M., Rudd, Ann E., and Sanders, Mary A.: Desoxyribonucleic Acid (DNA) Synthesis n the Radiated and Stimulated Thyroid Gland, Endocrinology 81: 1-13, 1967.

nomalies of Chromosomes in Circulating Leukocytes in Man Following Large Doses of ^{131}I

When the technique for clearly visualizing chromosomes in human cells by culturing irculating leukocytes began to be developed in this country, Dr. Neil Macintyre of this niversity very early acquired this technique in his laboratory. We solicited his ollaboration in the study of a patient with functioning metastatic carcinoma of the hyroid whom we had treated with large doses of ^{131}I some years before and planned to reat again with very large doses.

Although the patient had been known to us for a long time and had previously been iven large doses of ^{131}I by us, she had had none in the preceding six years. Following dose of 167 millicuries, blood was obtained for cell cultures and chromosome preaparations t very frequent intervals during the first 24 hours and less frequently thereafter for + days. The concentration of ^{131}I in the blood, the leukocyte count and the differential ount were determined at the same time. Spreads of chromosomes from multiple cultures nd from many different individuals cells in those cultures were prepared from each ample of blood. An attached table (Figure 10) shows how the incidence of chromosomal nomalies climbed to as high as 44% between 6 and 12 hours after the dose of ^{131}I was iven. Based on a very extensive experience of observations on 2,885 nonirradiated ontrol cultures there was an anomaly rate among cells from normal individuals of 4.5%. ne chromosomal aberrations were similar to those described by some other workers using ne same technique on patients who had been exposed to other types of radiation. As time assed, following the administration of ^{131}I, the radioactivity in the circulation fell harply because there was very little thyroxine or other iodinated amino acids formed in his individual who had very little functioning thyroid tissue. The chromosomal anomalies

observed in this patient included not only serious deviation from the normal number of chromosomes but numerous breaks, deletions and fragmentations (see attached Figure 11). Occasionally dicentric forms were observed and several examples of 4n nuclei representing reduplication of chromosome sets, but without the exact number of 92. These nuclei were presumably similar to those seen in the thyroids which built up excessive DNA but failed to divide following ^{131}I. These observations were the first reported in this country on chromosomal anomalies in circulating leukocytes of patients treated with ^{131}I.

Macintyre, M. N., and Dobyns, B.M.: Anomalies in Chromosomes of the Circulating Leukocytes in Man Following Large Doses of Radioactive Iodine. J. Clin. Endocrinol. & Metab. 22: 1171-1181, 1962.

One of the most important parts of these chromosome observations was the fact that an 18% incidence of anomalies was found in the circulating leukocytes of this patient's blood before the large treatment dose of ^{131}I was given. Presumably this unusually high rate of anomalies before this treatment was attributable to the previous radiation effect, which had occurred six years and more before. This incidence far surpassed the 4.5% nonmodal chromosome counts observed in normals in this laboratory. Furthermore, the structural anomalies observed in this patient are extremely rare in control bloods.

Fortunately, the usual numerous studies performed under this contract had been carried out on this patient when we had given previous treatment doses. It had been observed then that very significant suppression of circulating lymphocytes had occurred with each of the previous treatment doses 6 years and more before. Repeated chromatograms and other biochemical studies of the blood following these earlier treatment doses showed a marked rise in mono- and diiodotyrosine and a decline in the butanol extractable radioactivity in the circulation. These are all features which we had come to believe were evidences of radiation effect. The value of very long term detailed study and the advantage of being "tooled up" for such studies when the opportunity arises was well illustrated in this case.

The fact that a considerable population of cells with abnormal numbers of chromosomes apparently persisted in this patient for at least 6 years, as shown in the blood drawn before the present dose of ^{131}I was given, seems important. If cell lines stemming from somatic cells which bear such anomalies can remain viable, the same might be true of similarly affected reproductive cells in the gonads. If such a large number of chromosomal changes are demonstrable in tissues such as blood which does not specifically concentrate ^{131}I, then significant alterations in other similar nonthyroidal cells such as the reproductive cells should be considered even with much smaller doses of ^{131}I. It is possible that the less devastating doses of ^{131}I may be more important because smaller doses may not be lethal although perhaps more serious from the standpoint of damage to the cells. The total dosage of ^{131}I given this patient was far greater than that commonly used in the therapy of Graves' disease.

In view of the above observations it seemed appropriate that similar observations should be made on patients who were receiving modest doses of ^{131}I for hyperthyroidism, i.e. 5 to 15 millicuries rather than 167 as in the above described patient. David Patcher, a Ph.D. student with Dr. Macintyre, continued this project to determine whether there is a significant increase in anomalies in patients we selected for treatment of Graves' disease with ^{131}I. Our patients selected were studied in great detail as

reviously described and the bloods for leukocyte cultures were furnished to David atcher. Of 20 patients studied by the serial sample technique, the incidence of ncreased anomalies is equivocal in those patients up to 10 millicure dose range. ncrease is at the limit of significance in the dose range above 10 millicuries. It hould be pointed out that these studies were based on a large series of blood samples n each given patient rather than a "before" and "after" sample as has been reported y others. Mr. Satcher is currently preparing his observations for publication.

tudies of Peroxidase in Thyroid and Other Tissue

Several years ago a study of peroxidase activity in thyroid tissue was undertaken nder this grant with the idea that it might prove to be another way to explore adiation effect. Because at that time there was presumptive evidence that a peroxidase as present in thyroid tissue it was thought that this played some part in the onversion of iodide to iodine and the iodination of organic compounds within the hyroid. Observations previously described under this contract suggested that one of the arly manifestations of radiation effect was a failure of the trapping mechanism.

An experimental method described by Neufeld et al. to measure peroxidase had tilized the rate of oxidation of a leuco dye in the presence of hydrogen peroxide. he reaction mixture of hydrogen peroxide, phosphate citrate buffer and reduced dye olution was used as a substrate mixture into which various samples of homogenized issues were introduced. The rate of the reaction within the mixture was determined by spectrophotometric method using a source of light with a wave length of 645 mμ. Readings ere determined at 5 second intervals after the addition of the homogenate. Catalase ctivity was inhibited by adding 2-4 dichlorophenol. A large variety of rat tissues ere studied in addition to the thyroid. There was a wide range of peroxidase activity ound in these tissues. As anticipated, the small intestine showed the largest amount f peroxidase activity. The thyroid was about equivalent to the lung. Peroxidase ctivity in the thyroids of many different animals was studied by this method. There as a considerable variation in the peroxidase activity in thyroids among the various pecies. Since it was suspected that the variation might be related to the degree of ellularity in the thyroid, microscopic sections of the same tissues (from which omogenates were made) were projected on large pieces of paper and tracings made of the ells, colloid and connective tissue. The areas were cut out and weighed to determined he percentage of cells, connective tissue and colloid making up the tissue. It was urprising to find that although the ratio of the volume of cells to the volume of colloid as very different among different species there was no true relationship between the ell content of the tissue and the peroxidase activity among these species.

The question arose whether the oxidative reaction being measured by the leuco dye as actually related to the production of thyroid hormone and whether the peroxidase ctivity might be under the influence of TSH. Accordingly, TSH was tested both in vivo nd in vitro. It was surprising to find that there was no significant difference etween the oxidative activity of thyroids previously stimulated with TSH and those which ere unstimulated. The effect of TSH on the thyroid was confirmed by the presence of yperplasia demonstrated in microscopic sections of the tissue used.

In other studies the relationship of the peroxidase activity as measured by the euco dye method to the iodination of organic substances in physiological systems was tudied. The basis for the reaction was the iodination of tyrosine. A reaction

xture was used containing tyrosine, carrier-free radioactive (^{131}I) potassium iodide, ucose, glucose oxidase and phosphate buffer. It was observed that crude homogenates sheep thyroid caused organic binding of 14% of the carrier-free ^{131}I added to the stem. In contrast, purified mitochondrial preparations of soluble enzyme (prepared sonification of mitochondria) caused organic binding of 36.7% of the available ^{131}I.

The iodinating properties of a variety of homogenates and soluble mitochondrial zyme preparations from different tissues were then studied. Only very small amounts tyrosine were iodinated by mitochondrial preparations from the small intestine and leen, but homogenates as well as soluble mitochondrial preparations from lung splayed a surprising capacity to iodinate tyrosine. The surprising observation has en repeated in 4 separate experiments. Chromatography revealed that some of the dination produced by preparations from thyroid tissue appeared to be bound to protein, me to tyrosine. It is assumed that the protein had been introduced as intramitochondrial otein that had been freed by sonification. When tyrosine was added to the suspension % of the ^{131}I was bound to it while only 5.4% was bound to available protein. When no rosine was added to the preparation 48% of the ^{131}I was bound to protein. Electrophoretic tterns of the reaction mixture sampled after the incubation had taken place showed at the radioactivity was primarily in the position of albumin if tyrosine had been ded but in the position of gamma gloculin if tyrosine had not been added.

Finally some experiments were performed to compare the radioiodine method and the uco dye method for assaying peroxidase. Spleen and small intestine iodinated very all amounts of tyrosine but very effectively oxidized the leuco dye. Thyroid and ng showed strong peroxidase activity by the leuco dye method and caused iodination of rosine as well. Thus, it appeared that the peroxidase activity measured by the leuco e method was not necessarily identical with the peroxidase activity responsible for dination of tyrosine. The work on the leuco dye method of assay for peroxidase in yroid tissue has not been published. The assay method based on the iodination of rosine was a method which was described before this phase of the work was completed. was hoped that the methods would be useful in radiation studies but has not yet been used, primarily because the interrelationships of reactions being observed in the dination of protein and tyrosine are not understood.

-ray Radiation Effect on the Thyroid

Another part of the study of the effect of irradiation on the thyroid has been search for thyroid masses in 200 patients previously given x-ray therapy to the neck r tuberculous cervical lymphadenitis. All of these individuals had been treated in e tuberculosis clinic of this hospital before 1950 by the same radiologist. Most of ese individuals were children or young adults when treated. All records as to the ount and fields of radiation were available. A total of 67 patients have been traced d called back for examination of the thyroid by us. Twelve of these were found to ve discrete thyroid masses. Nine of these discrete thyroid masses have been removed th the following findings:

3 carcinomas
1 Hurthle cell tumor with capsular invasion
1 Hurthle cell tumor classified as benign
4 with follicular adenomas

Of 60 known to have died, the post-mortem examination was available on 46. Two thyroid neoplasms were described; one was a carcinoma, the other was an adenoma. It has been noted that most of the patients on whom post-mortem examinations were performed died within 5 years of the radiation therapy--a relatively limited interval in which to develop tumors.

Two patients who have very discrete masses continue to refuse exploration. One of these who was recently seen and reluctantly submitted to examination has developed persistent hoarseness and the mass has increased in size strongly suggesting a malignant lesion.

This occurrence of 5 histologically identified carcinomas of the thyroid seems highly significant in this small selected group of patients exposed to known amounts of radiation. A manuscript has been prepared for publication, but it has not been submitted for publication because we have thought it preferable to await a final diagnosis in two patients being followed with suspicious lesions but as yet unwilling to submit to thyroidectomy.

The Life of the Normal Thyroid Cell and Its Potential for Replication as Compared to the Radiated Thyroid Cell:

The long term follow-up of patients treated with ^{131}I is showing that the majority of patients if followed long enough will ultimately suffer from hypothyroidism or myxedema. Our figures show an incidence of only 12% hypothyroidism at the end of one year, but 45 - 50% if patients are observed for fifteen years. Unfortunately, the problem of hypothyroidism, if it does not occur promptly, arises so long after the treatment that the patient has forgotten that he received ^{131}I or he became careless about taking prophylactic supplemental hormone earlier and found that he did not need it. Since the patient has forgotten, subsequent physicians are not alerted to the possibility of hypothyroidism, a diagnosis that is difficult to make if there is no history to suggest its origin. As a result of vigorous follow-up efforts by a few medical centers, a surprising number of patients with unrecognized myxedema have turned up. The symptoms are attributed to, or confused with, senility and considered to be premature aging. It may be that the life span of the "ideally irradiated" cells in diffuse hyperplastic thyroid in man is not greatly different from the non-irradiated cells, the only difference being a failure of replication in the radiated cell.

In order to learn more about the long, latent thyroid failure after ^{131}I, it is necessary first to learn more about the survival and replacement of normal thyroid cells. Initial studies were made in young, middle-aged and old rats quite some years ago in our laboratory, using tritiated thymidine and radioautography to identify the cells that were undergoing mitosis. In the young growing rat, the occurrence of labeling was fairly frequent. The incidence declined with age until, in the rats that were 2½ to 3 years of age, only rare nuclei were found to contain the labeled thymidine. This pilot experiment illustrated the normal replication rate at different points in the life span.

Our first experimental steps to try to determine the life history and rate of replacement of the normal thyroid cells were observations on the labeling of thyroid nuclei with tritiated thymidine. The objectives were to determine how rapidly the labeling takes place, the duration of the stage of DNA duplication in preparation for division and the duration of the total period required for mitosis to become complete.

It has been our routine procedure to sacrifice animals four hours after the injection of tritiated thymidine. Pilot experiments had shown that the labeling was quite satisfactory in four hours. At this interval of time occasional labeled pairs of cells were found showing that division of the cell (with labeling in both nuclei) had taken place. It was known that the availability of labeled thymidine after injection into the circulation was very short, owing to the rapid disappearance by degradation or uptake by nuclei in the process of DNA synthesis. Since only those cells which are in the stage of DNA duplication at the time the thymidine is available will be labeled these nuclei are identifiable.

Several series of young, identical growing rats were injected with tritiated thymidine and sacrificed at 15, 30, and 45 minutes and 1, 2, 3, 4, 6, 8, 10, 12, 14, 20 and 24 hours. Three rats were sacrificed at each time interval. The incidence of labeled nuclei was determined by counting the number of labeled nuclei in a large number of high power fields in microscopic sections of uniform thickness. Paired labeled cells among this large population of unlabeled cells were also recorded.

Within 30 minutes following the injection of tritiated thymidine, the nuclei of cells were found to contain the tritium label. There was a sequential rise in the incidence of these labeled nuclei. This reached a plateau at about 10 hours. In order to determine the duration of available tritiated thymidine in the circulation, the animals were sacrificed by exsanguination,and the radioactivity in the plasma determined.

In order to find out how much of the radioactivity had been reduced to tritiated water in the plasma, samples were evaporated to dryness and counted. The radioactivity in the solid fraction of the plasma was found to be only about 15% of the total radioactivity within 2 hours. A great deal of time was then devoted to trying to identify and quantitate by chromatography the tritium labeled organic metabolic products as well as the thymidine remaining in the blood in order to better assess the availability of the thymidine after an hour or more. These attempts were abandoned because others had shown rapid disappearance of available material and the amounts here in these experiments were not paramount to our objectives.

A continuing rise in the occurrence of labeled nuclei for as long as 10 hours in the face of rapid disappearance of tritiated thymidine from the circulation was found. The increasing number of labeled nuclei up to 10 hours was very clearly demonstrated in two complete series of animal experiments. Pairs of labeled nuclei were first seen with certainty at 4 hours, although some very lightly labeled pairs of nuclei were suspected somewhat earlier. It was assumed that the lightly labeled pair of nuclei had been in the very late stage of DNA synthesis and therefore had an opportunity to take up very little of the thymidine before DNA synthesis terminated and actual division of the cell took place. The occurrence of labeled pairs increased with time and reached a plateau at about 10 hours. Since a thin microscopic section represents a thickness of tissue little wider than a cell, a pair of labeled nuclei would be demonstrated only if the cuts in the tissue happened to pass through both of the daughter nuclei. It can be assumed that random cutting would only reveal an occasional pair where the plain of the cut corresponded with the plain of the pair. It seems likely that the continuing apparent increase of labeled cells, well beyond the time when tritiated thymidine was available in the circulation, can be explained on the continued division of cells and the chance cuts that might catch one or the other or both of a pair.

The increasing occurrence of labeled nuclei in these young growing rats did not progress beyond 10 hours and thereafter declined progressively at 14, 18, 20 and 24 hours reaching a level of only 1/3 of the maximal level. This decline after 10 hours was observed in both series of animals. The explanation for this is not clear. It might be assumed that once the DNA of a nucleus is clearly labeled with sufficient isotope and the two products of mitosis are readily identifiable, labeled cells would not disappear from a gland. Destruction of that cell by radiation from the tritium c not seem plausible in this interval of time.

These experiments have furnished considerable information on the use of tritiate thymidine in studying cell division in the thyroid. They have yielded a rough approximation of the time required for mitosis to take place in the thyroid. They ha clarified some uncertainties and broadened our perspective of experimental designs for studying the capacity for mitosis in the thyroid. Together with earlier experime they have furnished some appreciation of the enormous capacity for cellular replicati in the young vs the old, but they have not yet furnished us with much information on the life of the thyroid cell. The experiments have introduced more questions than th have solved but much of what has been learned can be applied to the study of the irradiated thyroid cell.

OBSERVATIONS ON THE DEVELOPMENT OF THYROID NODULES IN POPULATIONS EXPOSED TO RADIOIODINE FALLOUT AS THEY RELATE TO STUDIES UNDER THIS CONTRACT

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 Marshallese exposed to the fallout, particularly the radioiodines, from the thermonuclear devise detonated on Bikini in March, 1954. The nodularity in the thyroids was first observe 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 requir 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 close to the thyroid; and one on Utirik atoll who had probably received 30 rads. These were brougl 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 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. There were also a vari of observations commonly made on tissues from our animals (not ^{3}H thymidine). Some 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 structur and suggested papillary lesions of multicentric origin. All of these thyroids (except the women from Utirik) showed many examples of the large bizarre nuclear forms repeate observed under this contract in animals and humans that had received ^{131}I. More will said later of study of these tissues under the subject of identification of subtle changes in thyroid tissue produced by ^{131}I.

[illegible] which is probably not compensatory hypertrophy. The writer did not participate in the 1971 survey, but it was reported that this probably was a compensatory nodule for it had shrunken on thyroid supplement.

Conard, R.A., Sutow, W.W., Colcock, B.P., Dobyns, B.M., Depaglia, D.E. Thyroid nodules as a late effect of exposure to fallout. In Radiation-Induced Cancer, P.325-336, I.A.E.A., Vienna, 1969.

Conard, Robert A., Dobyns, B.M., Sutow, W.W., Thyroid Neoplasia as a Late Effect of Acute Exposure. JAMA, 214: No. 2 316-324, October 12, 1970.

Conard, Robert A., Sutow, Wataru W., Bateman, John L., Dobyns, B.M., Riklon, Ezra and Demoise, Charles F., Medical Survey of the People of Rongelap and Utirik Islands Fifteen Years After Exposure to Fallout Radiation. Brookhaven National Laboratory - BNL 50220 (T-562) Upton, New York.

The Utah-Nevada Study

The principal investigator has also participated in the annual examination of the children in the Utah-Nevada fallout area (exposed in 1954) where some endemic goiter is present. A week has been spent in this area each of the past six years in the annual thyroid examinations of children that had been screened from a very large population in the fallout area by a United States Public Health Service screening team. Thyroid abnormalities which prompted concern were referred to a panel of three, of which the writer has been one. Those patients with thyroids judged to contain discrete masses have been sent to the University of Utah Medical Center in Salt Lake City for study. In some instances, the masses have been removed. The application of the same criteria of judgment of radiation effect in thyroids excised from this population has revealed only an occasional thyroid tissue which showed hints of a radiation effect. Reports are in preparation by Dr. Marvin Rallison. Another has been published by Dr. Edward Weiss et al: Thyroid Nodularity in Southwestern Utah School Children Exposed to Fall-Out Radiation, Am. Jour. Pub. Health, 61: 241-249, February. 1971.

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

During the course of participating in the annual survey of the Marshallese people who were accidentally radiated with radioiodine from the first thermonuclear bomb in the Pacific, the principal investigator of this contract had an opportunity to examine many thyroids. It is now known that the exposed people have developed many nodules in their thyroids, some of which have been malignant. In the course of these observations, both exposed and unexposed people were studied. On the island of Rongelap, the impression was gained that young adults (who were born after the fallout had occurred) displayed moderate degrees of hypertrophy of their thyroids. This was a rather surprising observation because this population had lived within a few feet of the sea all of their lives and for protein depended heavily on fish which provides an abundance of iodine. Nodules of the thyroid in the Marshallese population are almost unheard of and the thyroids are considered to be scarcely palpable at any age. The question was raised whether the exposure of adults might effect their thyroid function in such a way as to be reflected in their offspring. If there was a subtle secondary effect on the offspring it might show at adolescence.

Although the number of people in the exposed population was very small and the number of children of the exposed was insufficient to draw any firm conclusions, it appeared that children born of exposed population (after the exposure) had larger thyroids than did the children of the unexposed. If this was a valid observation then the theory was proposed that if the people sustained some thyroid damage (it has been shown that some did) than any fetus whose gestation took place in the environment of the mother's thyroid deficiency might sustain some lasting effect on its thyroid function. Birth records showed that there was a two fold increase in spontaneous abortions and stillbirths in the first several years after radiation exposure, adding further support to the [illegible]

Prompted by these observations, an animal experiment using rats was designed to test this hypothesis. Thirty four female Sprague-Dawley Rats were divided into five groups and given Remington Diet for three days to enhance the uptake of ^{131}I. Based on previous rat experiments, varying degrees of thyroid damage were produced by giving these groups of rats 0, 10, 20, 50 and 100 uc of radioiodine by intraperitoneal injection. The thyroids of these animals were subjected to in vivo counting at 24 hours in order to determine the dose of radiation delivered to the thyroid. Five weeks were allowed to pass at which time all radioiodine disappeared from the thyroids. All rats were then bred, and separately caged. The number of offspring in each litter and the individual body weights of the individuals in each litter were recorded. When the offspring reached approximately 100 days of age, they were given a trace dose of ^{131}I and 24 hours later were sacrificed. An effort was made to kill large numbers of animals at the same time so that any variations in the type of observations would be kept to a minimum. Thyroids were meticulously dissected out, weighed on a Roller-Smith torsion balance and then related to the body weight. The ^{131}I uptake in the gland was measured. The original female rats were rebred following the weaning of each litter.

Unfortunately, not all normal control rats conceived readily nor produced large litters. However, when the radiated animals were arranged in order of microcurie uptake in the gland, there was in general a diminishing rate of conception and size of litters with heavier doses of radiation and the passage of time. Most of the radiated animals irrespective of the dose of ^{131}I became pregnant at the first breeding which was only five weeks following ^{131}I. The 34 animals produced 233 living young in the fir mating, but in a total of 5 matings only a total of 447 were produced. Most of the animals conceived at the second mating at 22 weeks except animals in the two most heavil radiated groups. Only 4 of the 12 in these 2 groups became pregnant and 3 of these litters were very small. On numerous occasions rats appeared to be pregnant following breeding, but did not produce viable offspring. The incidence of stillbirths or early post partum deaths could not be determined because of suspected canabalism.

All breeding animals were permitted to survive for 14 months and through 5 litters. At the conclusion of the experiment, the breeding females were given a trace dose of ^{131}I along with a small amount of tritiated thymidine before they were sacrificed. The thyroids were dissected and weighed.

When a destructive dose of ^{131}I is given, thyroid function usually gradually diminishes, depending on the size of the dose. It is assumed therefore that thyroid function in these mother rats was better in those who received the smaller doses and that function should be better during the earlier matings. This appears to be the case here although the numbers of animals in the experiment are small. At the conclusion of the experiments, the thyroids of the animals which received the largest doses of ^{131}I were entirely replaced by white scar tissue. There was practically no ^{131}I uptake in these animals except in three that were found on gross dissection to have a small surviving nodule of thyroid tissue in the area that had been the center of the isthmus. These 3 animals were the only ones of those in the higher dose range that conceived beyond the first or second litter. These animals had actually received 19 to 28 uc of the original dose of ^{131}I in their glands. Animals which retained 7 to 15 uc similarly had only two conceptions beyond the second breeding. Animals which received 2. to 2.8 uc and 3.3 to 5.4 uc for the most part had rather full litters through the second mating, but thereafter conceived poorly.

The dose of radiation to the thyroid, the average thyroid weights of the mother rats, and the average thyroid weight of the offspring by litters is shown in the attached Figure 12.

It is condluded that animals born of mothers with some thyroid damage do not display thyroid hypertrophy at 100 days of life, which is comparable to adolescence in man.

At the present time, the autoradiographs and histologic interpretation of thyroids of the mother rats have not been evaluated since they remain in preparation.

Since the time that the question was raised concerning the significance of adolescent goiter in the children of radiated Marshallese people, a further recent survey made on Likiep Atoll (remote to the area of fallout) has revealed a few mild adolescent goiters in native Marshallese. This excludes a few other goiters appearing in individuals who have ¼ or more European origin.

The Identification of Subtle Morphologic Effects of ^{131}I on the Thyroid

All during the life of this contract, the principal investigatur and his associates have been concerned with the morphologic changes that are produced in thyroid tissue by radioiodine. The devastating effect of a large dose of radioiodine with the complete loss of thyroid epithelium and replacement by connective tissue is well known. When the hyperplasia of the thyroid of Graves' disease is subjected to radioiodine radiation, the hyperplastic picture of papillary projections into thyroid follicles is changed to a more simple follicular picture. With a large dose of radiation, the follicles are distorted and the general architectural pattern of the thyroid is markedly altered to the extent that there may be only scattered surviving epithelium cells in connective tissue. These changes are well known and easily recognized, but the more subtle changes produced by smaller doses of ^{131}I are difficult to identify with certainty.

Various workers have described a variety of changes that indicate subtle radiation effect. In trying to identify these subtle changes, we have stressed the recognition of the large bizarre nuclear forms containing excessive amounts of chromatin. Other workers have placed more emphasis on lymphocytic infiltration, plasma cells and increased amounts of connective tissue. Unfortunately, changes similar to these may occur in thyroids which have not been subjected to radiation. Lymphocytic infiltration is a common finding in thyroids of patients with Graves' disease. The presence of connective tissue may represent past influences on the thyroid such as necrosis, repeated hyperplasia and involution and varying degrees of nodularity. Objections may be made to using the large bizarre nuclear forms as a means of identifying radiation because somewhat similar changes may occur in the margins of areas that are starting to undergo necrosis. From the considerable experience studying pathologic changes in tissues of patients treated with ^{130}I and ^{131}I and the extensive experience with animal thyroids that are subjected to various degrees of radiation, we have come to the conclusion that a careful consideration of the bizarre nuclear forms is the best clue to the more subtle degrees of radiation effect caused by radioiodine.

To test the hypothesis that subtle radiation effects in thyroids can be identified, a collaborative study was set up with Dr. Robert Conard of Brookhaven National Laboratory. A large collection of thyroid tissues known to have been subjected to varying degrees of radioiodine radiation (including controls) was submitted as unknowns to this writer in order to test the experience gained from the morphologic studies carried on under this contract.

The sources of microscopic slides consisted of the following:1)Tissues from patients in the Marshallese group who were submitted to thyroidectomy and who were known to have been exposed to some reasonable amounts of radioiodine fallout. 2) A group of thyroid tissues obtained from children with nodular goiter in the Nevada-Utah area where fallout was known to exist in 1954, but the extent of radiation to the thyroids of children in this area is very doubtfully significant. 3) Thyroid tissues from nodular goiters removed from children in Safford, Arizona which served as a control for the Utah Study. 4) Thyroids from survivors of the Japanese atomic bomb casualty patients whose radiation exposure is said to have been mostly external, and where fallout is said to contain relatively little radioiodine. 5) A group of thyroids from patients who had thyroidectomy for nodular goiter in several areas of the United States and who so far as could be determined had not received radiation exposure to the region of the neck. All marks of identification were removed from the slides, except for sequential numbering. Multiple slides from the same individual were mixed in the collection. Although the microscopic slides were assembled from a rather large number of hospitals, the techniques of preparation with hematoxylin and eosin and mounting were essentially the same so that no clue as to their source was recognizable.

Primary emphasis for identification of radiation effect was placed on the presence of the bizarre nuclear forms although, in a few instances where more extensive destruction was produced, other evidences of radiation effect were evident. As has been pointed out, studies carried on in this laboratory indicate that the bizarre nuclear forms with increased amounts of chromatin represent cell division in which there has been a build up of DNA, but a failure to divide. A stimulus (such as that from mild impairment to thyroid function or a goitrogen) which will promote mitosis is inclined to bring out the bizarre nuclear forms. It appears from past work that the stimulus may be extremely subtle, so subtle that mild degrees of hypothyroidism which are not readily detectable by modern laboratory methods are sufficient to cause mild degrees of stimulation.

In distinguishing between the nucleus which is participating in normal cell division and the nucleus of a radiated cell, it is important to recognize that, although both nuclei enlarge, the normal nucleus reaches a limit at which it divides into two cells. The nucleus of the radiated cell sometimes continues to enlarge and occasionally becomes irregular in contour. The recognition of these unusually large nuclei is the clue that the thyroid has been subjected to radiation. Thus if the radiation exposure has been enough to produce mild hypothyroidism, the stimulus is present and the occurrence of bizarre nuclear forms is rather noticeable although there may be no architectural distortion of the histologic picture of the thyroid. A fine distinction is, therefore, drawn between universally enlarged nuclei and the occasional nucleus that is significantly larger than all of the others.

On the basis of these considerations, the tissue sections were graded as to positive or negative evidence of radiation effect by the following system:

0 - Nothing to suggest radiation effect
+ - Uncertain category - some features suggested there might be mild radiation effect - often more samples of tissue would have led to a definite decision
++ - Definite radiation effect
+++ - Marked radiation effect; at least some architectural distortion in addition to bizarre nuclear forms

After the observations on each individual slide had been recorded and reported to Dr. Conard, the slides were grouped so that all slides from the same individual could be reviewed and a single grade registered for each thyroid. In some cases, there had been considerable variation in the appearance of the tissue from one area to another. In such cases the evidence for radiation effect was much more convincing in some areas than in others. Where only a single slide was available a reevaluation and grading was all that could be done.

When the code was broken and the judgement as to radiation changes was matched against the radiation exposed (so far as was known), it was found that there was a surprisingly close correlation between exposure and the recognition of radiation effect in the thyroid. See Fig. 13. Tissues from 7 of the 16 Marshallese who were thought to have had the greatest radiation to their thyroid had unequivocal evidence in tissues. Most of the Marshallese were singled out as being at least suspicious. One of these that was definitely positive was considered a Marshallese control (thyroid tissue from post mortem examination from the hospital on Majuro) that later proved to be an individual that had been exposed but had developed no gross thyroid pathology (335 rads).

Changes in the thyroids from the Japanese bomb casualties showed minor or only suspicious change when they were arranged in order depending on their distance from the epicenter. Those nearest seemed to have the most suggestive change. This finding, if significant, was surprising because the amount of radioiodine in that fallout was small considering the presence of other radioactive materials that were in the fallout.

Only debatable changes were seen in the tissues from the Utah group, most of which were not thought to have been exposed to significant fallout. One case in 16 was considered positive for radiation effect. At the present time, it is impossible to determine for certain just how much radiation exposure these individuals received. Certainly the majority had not lived in the fallout area when the largest fallout occurred. Most of these thyroids were adenomatous. Some of those in which suspicion was raised were in glands that contained considerable lymphocytic thyroiditis accompanied by hyperplasia. The only gland that was classified as positive had been considered normal in the pathology report but in this study there seemed to be an occasional large radiation nucleus.

These observations served to illustrate that subtle degrees of radiation changes caused by radioiodine in the thyroid can be identified.

The Development of Neoplasms in Irradiated Animal and Human Thyroids

From the world wide experience now available, it seems clear that the development of a neoplasm, especially a malignant one, is not a common sequella of ^{131}I irradiation to the thyroid in adults. Not enough is known about their occurrence in children because few children have been so treated. The reports of Sheline, Lindsay and their associates suggest that the occurrence of nodules following ^{131}I is considerably more frequent in children than adults. Others such as Crile and his associates and Starr and his associates have no concern about the possibility of the development of neoplasms in children. Because of continued concern, the number of children treated with ^{131}I remains small. Thus adequate experience is not yet available. Although the occurrence of neoplasms may be very low following ^{131}I treatment for hyperthyroidism it may be that the doses used are so large and cellular replication so impaired that the chances of neoplasm formation is almost precluded.

The experimental designs of the studies to be described have taken into consideration several factors about which we must learn more. 1.) The age at which neoplasms are most likely to be produced. 2.) The dose of ^{131}I which is sufficiently disrupting to the cell to induce abnormal replication but not so great that it destroys the capacity for replication. Presumably the most effective doses which produce neoplasms lie between the equivalent of the tracer dose and the therapeutic dose used in man. 3.) Determine what other factors might promote neoplasm formation. 4.) Observe neoplasms during the early stage of their development and the related features in the remainder of the gland which are giving rise to neoplasms.

A number of large series of long-term rat experiments have been set up during the latter half of this contract to study the development of neoplasms of the thyroid in rats treated with various doses of ^{131}I. It is well known from our own experiments and those of others that thyroid neoplasms in the rat require more than a year to develop after continuous administration of a goitrogen or ^{131}I. Hence the problem of housing and the cost of feeding have been seriously limiting factors. In addition, the need for very large series of animals with many controls has been necessary because of natural losses and (in our earlier experience) a rather low incidence of neoplasms. For these reasons and the limitation of funds, only one series of animals has been possible at a time.

In the past there has been controversy concerning the ease with which neoplasms may be produced in animals with ^{131}I. One of the failings in most of the published experiments designed to test neoplasm formation in animals given ^{131}I has been the primary attention to how much of the isotope was given to the animals and not how much and how long it was retained. It has become apparent over a long period of experimentation that the amount retained in the thyroid may be enormously different than that anticipated from the amount injected and the variations in the amounts retained among animals may be very great. As a result we have resorted to individually identifying each animal and measuring its uptake and retention by in vivo and in vitro counting. Weighing the gland of representative animals to determine a mean thyroid weight has contributed to the reliability of calculating rad dose.

The same basic experimental pattern has been followed on 5 series of rats over the past 10 years. The same strain of Sprague-Dawley Rats from Charles River Breeding Colony have been used exclusively through the past 5 years. A Remington diet has been

given for a few days to enhance the uptake of the carrier-free dose of ^{131}I, which is injected intraperitoneally. The series of animals have ranged from 35 to 250 animals. Several dose levels have been used in each series. The animals are individually marked. In vivo counting of the uptake by each thyroid is measured at 24 hours and some animals are sacrificed at this time so that the in vivo measurements may be checked by in vitro counting in representative animals. The thyroids are weighed and an average thyroid weight determined. The dose in rads may thus be calculated for each animal. Animals were grouped according to the doses of ^{131}I administered. Each group was subdivided into those which received chronic thiouracil in the drinking water, those which were given thiouracil only for 5 days before sacrifice and those given nothing but ^{131}I. Controls received no treatment at all.

Aside from those animals initially sacrificed to check precise uptake of thyroid weight, the animals were sacrificed at infrequent intervals for the first year and from time to time up to 2½ years. A small trace dose of ^{131}I in addition to the tritiated thymidine was given to the animals four hours before sacrifice. Autoradiographs promptly prepared from microscopic sections 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 essentially gone after two 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 over the nuclei that were preparing to divide. Subsequent comparisons of the two autoradiographs reveal two types of information about the same cluster of cells which appear in both preparations.

In order to conserve animals in later experiments, some were anesthetized and the thyroid explored about the time neoplasms were first expected to appear. If early nodularity was found, the animals were sacrificed several days later, after the usual presacrifice preparation. This was done not only to conserve valuable animals, but also to give a better idea as to when neoplasms begin and an appreciation of how fast they grow.

The first full series of animals (85 males) were divided into groups and given 0, 5, 10, or 50 uc of ^{131}I, delivering from 30,000 to 300,000 rads to the thyroid. In this series the ^{131}I was not given until the animals had grown to 110 to 135 grams. Unfortunately, there was a very low incidence of neoplasms (6 identified) in this series, although the long term survival was not particularly good. Animals which received the larger doses of ^{131}I had almost complete destruction of the thyroid. In animals that received lower doses, some thyroid tissue could be seen on gross dissection. There was only minimal architectural distortion of the follicles in this range.

A second series of rats similar to the first was prepared. Since the yield of neoplasms had been poor in the juvenile rats approaching maturity in the first series, weanlings were used. By the time ^{131}I was given they weighed 55 to 85 grams. They received 40,000 to 400,000 rads. At about 1 year an epidemic of pneumonitis swept through the animal farm destroying animals more by groups than by random. (It was not necessarily the most heavily radiated animals that died.) Some tumors developed in survivors, suggesting that radiation applied to younger animals would yield a higher probability of neoplasms.

Many collateral observations were made on these first two series of animals in the course of studying the development of neoplasms. Early sacrifices confirmed our former observation that there is a temporary loss of mitotic activity shortly after ^{131}I is given as we previously reported. The abnormally large number of mitoses which appear when goitrogen was given during the recovery phase from ^{131}I occurred again. Although neoplasms do not become manifested until much later, it may be that they started at this time.

In a third series of animals, the age at which ^{131}I was given was even lower being 28 days; 40 to 65 grams. Although a period of iodine deficient diet was again used, th uptake was 25 to 60% of dose, and the graded doses of ^{131}I were reduced so that 5000 to 200,000 rads were delivered to the thyroid, using 5 dose levels. A total of 19 animals developed neoplasms. Although the number of animals that developed tumors seems small, it must be kept in mind that many were sacrificed before one year and tumors were seldo found before 14 months. Only 25 animals were allowed to survive after 14 months; 12 of these ultimately proved to have neoplasms.

A 4th large series of animals was prepared. Since the yield of neoplasms had increased by giving the ^{131}I at a younger age in the preceeding series, this present series was started by procuring pregnant females that had all been bred on the same day. There were 120 offspring. Ninety of these were given ^{131}I on the 16th day of lif by intraperitoneal injection. Thirty served as various kinds of controls. Males and Females were separated after weaning. Iodine deficient diet could not be used here. The actual rad dose delivered to these animals ranged from 300 to 17,000 rads, based on an average thyroid weight of 7 mg. Since the neoplasms tended to appear more often in the lower dose range in the preceeding group of animals, lower doses were used here. Furthermore, these thyroids were extremely small, so that considering the range of the beta ray relatively more of the beta radiation caused ionization outside the gland. A total of 29 discrete neoplasms were found in this series. One of the non-radiated controls given thiouracil chronically developed a neoplasm. There were 8 neoplasms in animals that received only radiation and 20 had been given radiation and chronic thiouracil. Only 49 animals were permitted to live longer than 12 months; 22 of these had neoplasms when sacrificed. Most of the neoplasms that developed without the stimul of thiouracil occurred in the 7,000 to 17,500 rad dose range.

Many collateral observations have been made on these series of animals. Since tritiated thymidine radioautographs have been made on all thyroids in these 4 series, there is a great deal of information that is just now being appreciated retrospectively Some of these observations are as follows: Upon reviewing the non-radiated control animals killed at each period of sacrifice it was found that when the young animals are given a 5 day course of thiouracil, the degree of rise in mitotic index (number of labeled cells per high power field) is quite remarkable. The young unstimulated contro animals showed 0.5 to 1.6 labeled nuclei while those given thiouracil showed 14.0 to 35.0 per high power field. In contrast the same observations on resting animals 1½ to 2 years of age may reveal only one or two labeled nuclei in several cross sections of a whole thyroid lobe. When the latter are stimulated under the same conditions the maxim number of nuclei labeled may be less than one per high power field.

For doses of 2300 to 2800 rads, slight temporary impairment to mitotic activity occurred but ultimately a maximal weight of thyroid could be produced with chronic thio

ateral observations were made on these first two series of animals in studying the development of neoplasms. Early sacrifices confirmed our ition that there is a temporary loss of mitotic activity shortly after as we previously reported. The abnormally large number of mitoses which ɔitrogen was given during the recovery phase from ^{131}I occurred again. lasms do not become manifested until much later, it may be that they is time.

rd series of animals, the age at which ^{131}I was given was even lower being ɔ 65 grams. Although a period of iodine deficient diet was agair used, the to 60% of dose, and the graded doses of ^{131}I were reduced so that 5000 to were delivered to the thyroid, using 5 dose levels. A total of 19 animals plasms. Although the number of animals that developed tumors seems small, pt in mind that many were sacrificed before one year and tumors were seldom 14 months. Only 25 animals were allowed to survive after 14 months; 12 of ely proved to have neoplasms.

rge series of animals was prepared. Since the yield of neoplasms had giving the ^{131}I at a younger age in the preceeding series, this present arted by procuring pregnant females that had all been bred on the same ere 120 offspring. Ninety of these were given ^{131}I on the 16th day of life oneal injection. Thirty served as various kinds of controls. Males and separated after weaning. Iodine deficient diet could not be used here. d dose delivered to these animals ranged from 300 to 17,000 rads, based on yroid weight of 7 mg. Since the neoplasms tended to appear more often in e range in the preceeding group of animals, lower doses were used here. these thyroids were extremely small, so that considering the range of the itively more of the beta radiation caused ionization outside the gland. A liscrete neoplasms were found in this series. One of the non-radiated n thiouracil chronically developed a neoplasm. There were 8 neoplasms in received only radiation and 20 had been given radiation and chronic Only 49 anima s were permitted to live longer than 12 months; 22 of these s when sacrificed. Most of the neoplasms that developed without the stimulus l occurred in the 7,000 to 17,500 rad dose range.

llateral observations have been made on these series of animals. Since midine radioautographs have been made on all thyroids in these 4 series, eat deal of information that is just now being appreciated retrospectively. e observations are as follows: Upon reviewing the non-radiated control ed at each period of sacrifice it was found that when the young animals are y course of thiouracil, the degree of rise in mitotic index (number of s per high power field) is quite remarkable. The young unstimulated control ed 0.5 to 1.6 labeled nuclei while those given thiouracil showed 14.0 to h power field. In contrast the same observations on resting animals 1½ to ge may reveal only one or two labeled nuclei in several cross sections of a d lobe. When the latter are stimulated under the same conditions the maxima clei labeled may be less than one per high power field.

es of 2300 to 2800 rads, slight temporary impairment to mitotic activity ultimately a maximal weight of thyroid could be produced with chronic thiouracil.

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' doses of 3000 to 5000 rads there was some impairment of mitotic activity shortly after 'ing the ^{131}I and some persistent impairment was demonstrable for the remainder of the 'e of the animal. In some animals some temporary impairment was seen below 1000 ls and in some of this group of animals subsequently the maximal enlargement of the und on chronic thiouracil could not be achieved. It must be recognized, however, that ›acity for hypertrophy declines with age so that failure to recover earlier impairment ' not actually be attributable to radiation. Furthermore, the capacity to respond :h increased mitotic activity in radiated animals was more like a non-radiated animal a later age. With 9000 to 20,000 rads the recovery of mitotic activity at 26 days l 5 months was not complete. With 14,000 to 30,000 the capacity for mitotic activity ; only about ½ that of non-radiated animals. It was at about this dose level that 'y little significant increase in thyroid weight could be produced by thiouracil later life until the animal was developing a neoplasm. Among animals sacrificed at 4 weeks :re had been considerable temporary recovery in ability to respond to the stimulus :n after doses as high as 14,000 to 30,000 in one series and 12,000 to 15,000 in the other was at this level of rad dose that neoplasms were most likely to ultimately appear :hout the added stimulus of thiouracil.

When neoplasms were clearly formed, the incidence of mitotic labeling in the lesion ; usually much greater than that in the extranodular tissue. Only in the more :ently prepared autoradiograph for ^{131}I content was the iodine uptake function of the :plasm satisfactorily demonstrated. However, as far as observations go, it appears ıt those neoplasms with the most abundant labeling with tritiated thymidine in nuclei ce up little ^{131}I. Figure 14 illustrates an autoradiograph of a solid cellular tumor with ^{131}I uptake.

All types of histologic patterns of thyroid neoplasms have been found. Mixed ›illary and follicular lesions have been the most common thus far. Such a lesion shown in Figure 15. These are usually found where chronic thiouracil has been superposed on radiation. It has been discovered that most of the lesions found in animals ıt are radiated but not given thiouracil are solid cellular lesions and most appear ite undifferentiated. One such neoplasm is seen in Figure 16. This animal received µc of ^{131}I delivering 5126 rads. No thiouracil was given other than the brief few days stimulate acute mitotic activity. The animal was sacrificed at 18 months. The toradiograph shows an enormous concentration of nuclei bearing the tritiated thymidine ›el at a point where the very undifferentiated neoplasm is breaking through the capsule. : cells that are labeled only had an opportunity to collect thymidine for an hour and ey only collected it when preparing to divide. Therefore, the enormous number of cells ›eled speaks for the rate at which these cells are multiplying at the point of break through.

By making routine tritiated thymidine autoradiographs it has been hoped that we ght learn at what time neoplasms begin to develop following ^{131}I and therefore find em at or near their inception. It has been assumed that any cluster of cells destined be a neoplasm will display a different rate of incorporation of tritiated thymidine its nuclei than will cells of the surrounding tissue as shown in Figure 16 and 17.

After 12 to 15 months radiated thyroids show some areas of variability with respect follicle size and cell size. At this time the labeling of nuclei is not always iformly displayed. Figure 18. Although there may be no discretely encapsulated groups cells which are clearly a neoplasm, the tendency for cell division seems more evalent in some areas than in others. The non-uniform distribution of labeling with itiated thymidine presumably relates to the nodularity that evolves when these glands e stimulated to hypertrophy. It appears that ultimately such glands will be found to ntain lesions whose histologic appearance, degree of encapsulation and discretely

different tendency for mitotic activity will be quite evident. After review of many thyroid preparations it appears that labeling is more often seen in areas where normal follicles have given way to minute microfollicles or to solid cellular areas between follicles. The preliminary impression is gained that these areas represent the origin of "nodules" or actual neoplasms. Such an example is seen in Figure 19 A. A similar histologic pattern was seen in at least 2 of the Marshallese on whom we operated here and studied extensively. It is impossible to judge whether many of the lesions should be classified as malignant. A search has been made in the lung and liver when, on gross dissection, the massive thyroid neoplasm has invaded musculature of the neck and is densely adherent to the trachea and larynx. No proven distant metastases have been found outside of the neck thus far. Figure 20 illustrates a very extensive lesion invading structures of the neck.

There are so many interrelated observations in these experiments that all relationships are not yet appreciated. Several conclusions thus far seem quite clear.

1. There is an increased incidence of thyroid neoplasms following the administration of ^{131}I to rats.

2. Neoplasms are more readily produced when ^{131}I is given at a younger age.

3. A dose level of ^{131}I must be small enough that it does not seriously cripple the capacity for replication but it must be large enough to produce some intrinsic damage to the cell.

4. Neoplasms are produced more readily in the 7000 to 18,000 rad dose range. Although rat thyroids are not totally destroyed with doses up to 80,000, an occasional tumor has occurred above this level.

5. With increasing doses of ^{131}I there is a decreased labeling with tritiated thymidine. There is an immediate cessation of mitotic activity for a brief period of time. With recovery from non-destructive doses there is a supranormal rate of mitotic activity. As time passes there is then a decline below the normal level.

6. The permanent decline in inducable mitotic activity occurs over weeks or months depending on the magnitude of the original radiation insult.

7. The capacity to develop a hypertrophied gland under chronic thiouracil stimulation is gradually impaired or lost depending on the size of the dose of ^{131}I.

Participation in Other Work on Radiation Effects:

Coincident with the long term studies carried on under this contract, the principal investigator (along with some others) has devoted a great deal of time to several national problems concerned with radiation effects on the thyroid. These are as follows:

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 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.

The background of experience provided by this contract has provided an opportunity to participate, in a major way, in studies of these issues. The study and analysis that goes into dealing with these problems has in turn given a unique perspective for the experiments to be carried out in the laboratory. For example the work with the Marshallese has contributed in several ways. First, it gave a first hand review of the problem by seeing and examining the people. Second, it brought these people to us which proved to be extremely valuable material. Being prepared to carry out the surgery with a special view toward the problem and doing it in conjunction with studies to be done in the laboratory immediately afterward made the most of this unique human situati Thirdly, the question of the effect of radiation in the mother having a subsequent effect on offspring was raised. Laboratory experiments in animals resulted.

As Chairman of the Steering Committee, the principal investigator has devoted much time to analyzing the data and summarizing the results of radioiodine therapy and tumor formation 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 owing to the opportunities furnished by this contract (See earlier part of this report.)

It has been thought that such data could be the basis from which to devise and test models that would reflect the true nature and the extent of the radiation effect on the thyroid in these patients. In this way it might be possible, now that we have the long time results, 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 thyroid of still others. From data which we furnished him, Dr. A. Bertrand Brill of Vanderbilt University has carried out some preliminary analysis. He feels encouraged about gaining meaningful interpretations on the kinetics. (See Proposal for Continuation of Work.)

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