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ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

REPORT NO. 83

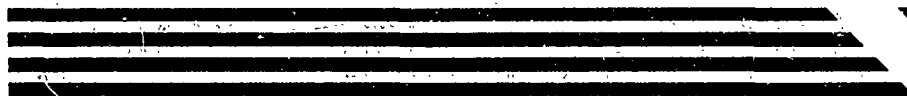
3 June 1952

THE ROLE OF THE LIVER IN THYROID HORMONE METABOLISM
AS STUDIED BY RADIO-IODINE IN INTRA-SPLENIC THYROID
AUTOTRANSPLANTS*

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*Subtask under Environmental Physiology, AMRL Project No. 6-64-12-028, Subtask, A Study of Thyroid Function with the Aid of Radioactive Iodine.



MEDICAL RESEARCH AND DEVELOPMENT BOARD
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY

REPORT NO. 83

THE ROLE OF THE LIVER IN THYROID HORMONE METABOLISM
AS STUDIED BY RADIO-IODINE IN INTRA-SPLENIC THYROID
AUTOTRANSPLANTS*

by

Milton W. Hamolsky, Capt., M. C., O. R. Czerwonka, Biophysicist,
A. L. Botkin, 2nd Lt., M. S. C. and Z. S. Gierlach, Capt., M. C.

from

ARMY MEDICAL RESEARCH LABORATORY
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Subtask, A Study of Thyroid Function with the Aid of Radioactive Iodine.

Report No. 83
Project No. 6-64-12-028
Subtask AMRL S-7
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ABSTRACT

THE ROLE OF THE LIVER IN THYROID HORMONE METABOLISM AS STUDIED BY RADIO-IODINE IN INTRA-SPLENIC THYROID AUTOTRANSPLANTS

OBJECT

To study the role of the liver in the metabolism of thyroid hormone by investigating the effect of preportal transplantation of the thyroid gland on the level of serum protein-bound radioactive iodine as a measure of the removal or inactivation of endogenously labeled thyroid hormone by the interposed liver.

RESULTS AND CONCLUSIONS

The experimental method devised, using radioactive iodine, permits sequential observations in a given animal, of the functional "take" and capacity of thyroid transplants as well as ultimate correlation with the adequacy of the thyroidectomy. Failure of the intra-splenic transplanted thyroid to effect the classical post-thyroidectomy flattening of the weight curve or serum levels of protein-bound iodine comparable to thyroidectomized animals indicate that the liver does not completely remove thyroid hormone. This is further confirmed by lack of any definite evidence of a compensatory thyroid hyperplasia, hyperfunction or adenoma formation. The data do, however, indicate a partial removal or inactivation by the liver of labeled thyroid hormone delivered to it under these experimental conditions.

RECOMMENDATIONS

Studies of the rate and amount of biliary excretion of radioactive iodine, both as the iodide and protein-bound forms, should be carried out as correlative indices of removal of thyroid hormone by the liver. Histological studies of the pituitary and estimation of thyrotropic

hormone release (when a satisfactory method is available) are indicated for a more complete understanding of the effect of hepatic removal of thyroid hormone on the thyroid-pituitary axis.

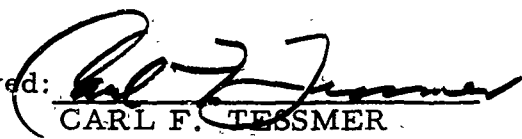
Submitted by:

Milton W. Hamolsky, Capt., M. C.
O.R. Czerwonka, Biophysicist.
A.L. Botkin, 2nd Lt., M. S. C.
Z.S. Gierlach, Capt., M. C.

Approved:


RAY G. DAGGS
Director of Research

Approved:


CARL F. TESSMER
Lt. Colonel MC
Commanding

THE ROLE OF THE LIVER IN THYROID HORMONE METABOLISM AS STUDIED BY RADIO-IODINE IN INTRA-SPLENIC THYROID AUTOTRANSPLANTS

I. INTRODUCTION

The role of the liver in the metabolism of thyroid hormone is not clear. Early studies suggesting such a role (1) used relatively large amounts of exogenous hormonal principles. More recently, Gross and Leblond (2,3), using intravenous injections of radioactively labeled thyroxine in animals, found significant localization in the liver, gastrointestinal tract and the feces. This report deals with the study, in rats, of radioactive iodine (I-131) uptake in thyroid tissue autotransplanted into the spleen and the subsequent levels of plasma protein-bound I-131 as an indirect measure of the removal by the interposed liver of such endogenously labeled thyroid hormone. In independent studies, using similar experimental conditions, Gabe and Arvy (4), basing their conclusions on histological studies, stated that the liver completely removed thyroid hormone whereas Bondy (5) concluded on the basis of protein-bound stable iodine levels, in a small number of animals, that the liver did not destroy and excrete thyroid hormone to a significant extent.

II. EXPERIMENTAL

A. Methods

Four groups of male, albino, Sprague-Dawley rats (150-250 grams), maintained on stock Purina Laboratory Diet, were prepared using intraperitoneal Nembutal anesthesia:- I. Thyroidectomized; II. Thyroidectomized, with thyroid gland implanted in the animal's right axilla, within muscle; III. Thyroidectomized, with the thyroid gland implanted in a splenic pouch; and IV. Sham-operated controls. These four groups were studied, as outlined below, 34 to 39 days post-operatively. A fifth group of 11 animals, thyroidectomized with splenic implants, was followed for over one year before sacrifice. Each animal was injected intra-peritoneally at completion of the operative procedure with 10 c.c. of calcium chloride (10%) in isotonic glucose solution, and was maintained on this solution as drinking water for seven days, followed thereafter by tap water. Weights were recorded periodically.

For preliminary "scanning" studies, 5 μ c of carrier free I-131, in 1 c.c. of either saline or Krebs-Henseleit buffer, were injected intravenously (leg vein). At intervals after injection, external gamma counts were obtained by holding the region of the animal to be counted up to a lead collimated G-M tube (6 mm. window), connected to a count-rate meter.

For plasma I-131 levels and uptake studies, about 15 μ c I-131 were injected intravenously. Blood was obtained at two and twenty-four hour levels by direct cardiac puncture in an immobilized, un-anesthetized animal. The protein-bound I-131 level was determined by Trichloroacetic acid precipitation (6), expressed as
$$\frac{\text{PBI}^{131} \times 100}{\text{Total plasma I}^{131}}$$

Uptake and conversion of I-131 by transplanted tissue were determined by alkaline hydrolysis and fractionation (7). By direct cannulation of the bile ducts of anesthetized animals it was possible to obtain sufficient bile at one and two hour intervals after injection to obtain preliminary data.

At the time of sacrifice a thorough search was made for residual thyroid tissue in the neck, using a G-M tube for scanning purposes plus careful dissection; minute residual fragments could be detected in this way. The presence of adhesions between the spleen and abdominal wall or other organs was noted. The transplant and residual neck thyroid tissue (if present) were removed for I-131 uptake and fractionation determinations, and for radioautography on Eastman NTB plates, 50 mu. thick. Other organ specimens were fixed in Bouin's solution for histological examination.

B. RESULTS

Only one of the 16 splenic transplanted animals died post-operatively. Viable I-131 concentrating tissue was found in 100% of the transplants, including those animals found to have residual thyroid tissue in the neck. There was no gross evidence of thyroid hyperplasia, hyperfunction or adenoma formation in the transplants (Figs. 1 and 2).

The thyroidectomized animals showed the classical "flattening" of the weight curve post-operatively, whereas the weight curves for the axillary and splenic transplant animals were similar to the normal, sham-operated controls (Table 1). The splenic transplanted animals of group V showed a progressive weight gain to values of 520-650 grams at the end of one year.

TABLE 1

Group	No. Animals	24 hour P.B. I-131	Av. Pre-op Wt.	Av. Post-op. Wt.
SHAM	4	29.93 $\pm$ 8.2	233 $\pm$ 20.2	333 $\pm$ 32.7
AUXILLARY TRANSPL. *	3	23.2 $\pm$ 2.9	194 $\pm$ 20.2	316 $\pm$ 20.3
THYROIDECTOMIZED *	6	3.82 $\pm$ 1.6	195 $\pm$ 13.4	207 $\pm$ 7.2
SPLENIC TRANSPL. *	7	12.22 $\pm$ 5.4	163 $\pm$ 21.5	291 $\pm$ 44.0

* = Without demonstrable residual cervical thyroid tissue.

Scanning of body areas by the use of a collimated G-M tube and count-rate meter made it possible to confirm and follow at intervals in the same animal the presence of a functioning transplant and any residual cervical thyroid tissue. In normal animals there was a gradual, progressive rise in counts over the thyroid area for the first 24-48 hours; a comparable rise over the splenic area (attributed to contiguous structures, chiefly kidney) occurred over the first 4-6 hours, followed sharply by a steady decrease. In adequately thyroidectomized animals, the count over the thyroid area showed a sharp, progressive drop after 4 hours; residual tissue was suggested by a persistently high count beyond this time interval and confirmed, in each instance, by post mortem examinations. Similarly a persistently high level of radioactivity over the splenic area beyond 6 hours suggested a functioning transplant, confirmed at time of examination. The PB I-131 values at 24 hours following I-131 injection are presented in Table 1. Residual cervical thyroid fragments were found in five operated animals. In four of these, the PB I-131 values equalled or approached those of the sham-operated controls (av. = 20.9 $\pm$ 5.9). Analysis of the percentage of I-131 organically bound in 24 hours following uptake by transplant tissue in five animals revealed an average of 72.6 $\pm$ 10.9%, a value in general agreement with those found in normal rat thyroids (7).

III. DISCUSSION

The data presented suggest that the liver removes or inactivates, in part, thyroid hormone delivered to it under the conditions outlined. Failure to effect an athyrotic weight curve or to obtain the low PB I-131 values of surgically thyroidectomized animals indicates that the liver does not completely remove the hormone, as concluded from histological studies of the pituitary, liver and adrenals, by

Gabe and Arvy (4), who published no further data relative to the weight curves or metabolic status of their experimental animals. In the paper by Bondy (5), who concluded that there was no significant hepatic destruction or excretion on the basis of control values of serum protein bound stable iodine levels in three surviving animals, the possibility of residual thyroid tissue or adhesions by-passing hepatic circulation was not ruled out. It has been shown by Reinhardt (8) that about 50% of rats subjected to surgical thyroidectomy still contain cervical iodine-concentrating tissue, and we found that seven minute residual fragments resulted in PB I-131 values at or approaching control levels.

It is further to be noted that inasmuch as the PBI level is the resultant of the two processes of delivery to and removal from the vascular compartment, any interpretation of hormonal delivery, based on such levels, rests on the assumption that the "peripheral utilization" of the hormone is not significantly altered. Knowledge concerning this parameter of thyroid function is limited. The data presented herein might thus be interpreted as representing an increased rate of hormonal utilization in the face of an unaltered delivery from the thyroid, through the liver, to the circulation. It is of some interest that the concept of partial hepatic inactivation of thyroid hormone is similar to that most recently proposed for the metabolism of the estrogenic hormones (9).

IV. SUMMARY

The role of the liver in the metabolism of endogenous thyroid hormone, labeled by I-131 in splenic autotransplants has been studied. The method permits serial observations on the functional "take" of the transplants, as well as the determination of uptake and conversion of iodide and the level of labeled protein-bound iodine. The data suggest a partial removal or inactivation of thyroid hormone by the liver under these experimental conditions.

V. RECOMMENDATIONS

Studies of the rate and amount of biliary excretion of radioactive iodine, both as the iodide and protein-bound forms, should be carried out as correlative indices of removal of thyroid hormone by the liver. Histological studies of the pituitary and estimation of thyrotropic hormone release (when a satisfactory method is available) are indicated for a more complete understanding of the effect of hepatic removal of thyroid hormone on the thyroid-pituitary axis.

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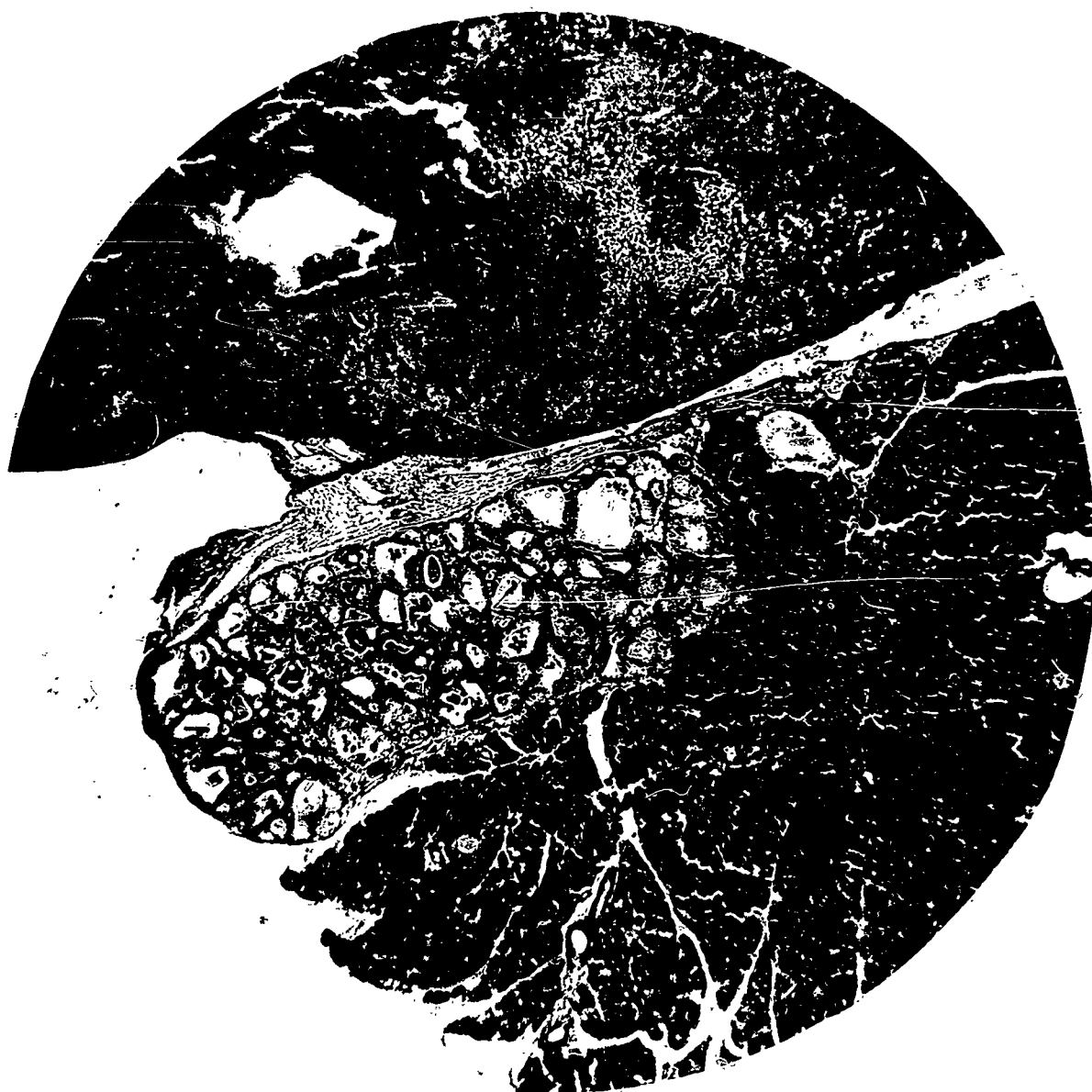


FIGURE 1. TRANSPLANTED THYROID GLAND INTIMATELY ASSOCIATED WITH SPLEEN AND PANCREAS.



FIGURE 2. AUTORADIOGRAPH OF INTRA-SPLENIC THYROID GLAND (EASTMAN NTB PLATE, 5 μ).

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USAMRL-83

Hamolsky, Milton W.; Czerwonka, O.R.; Botkin, A.L. and Others
3 June '52 10pp. photos, table

Thyroxin
~~Thyroxine~~
Liver
Body tissues -
Transplantation

Chemistry (52)
Biochemistry (5)

* Thyroid Hormones
Metabolism

UNCLASSIFIED Surgical Transplantation
Spleen

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