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STUDIES OF ENDOCRINE METABOLISM AND
FUNCTION IN DOMESTIC ANIMALS UTILIZING
RADIOACTIVE ISOTOPES AS TRACERS

MASTER

FINAL PROGRESS REPORT

Contract No. At(11-1)1484

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5-28-68
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STUDIES OF ENDOCRINE METABOLISM AND
FUNCTION IN DOMESTIC ANIMALS UTILIZING
RADIOACTIVE ISOTOPES AS TRACERS

FINAL
Progress Report
June 1, 1967 - May 31, 1968

Submitted by the
Department of Veterinary Biological Structure
College of Veterinary Medicine
University of Illinois
Urbana, Illinois

Contract Number AT(11-1)1484

1. Studies of endocrine metabolism and function in domestic animals utilizing radioactive isotopes as tracers.

2. Department of Veterinary Biological Structure
(Formerly Veterinary Anatomy and Histology)

College of Veterinary Medicine

University of Illinois

Urbana, Illinois

3. Prepared May 23, 1968, for the period June 1, 1967 through May 31, 1968.

4. Project Final Progress Report

The primary goals of the project were the same as described in the renewal proposal. New areas of investigation were designated only when pertinent data or literature results indicate their feasibility.

- A. Determinations of daily thyroid secretion rate, by in vivo replacement techniques, provide a base line from which various treatment variation could be recognized. Identical procedures of in vivo blocking with both thyroxine (T_4) and triiodothyronine (T_3) are used to determine daily thyroid secretion rate.
- B. Oral administration of L-thyroxine, L-triiodothyronine and protamone (a thyroactive casein) were made in swine. The results will be compared to those recorded from parenteral routes of administration. Analysis of these data has not been completed.

C. A study of the effects of thyroxine and triiodothyronine administration of body weight gains in swine has been completed. Three groups of swine containing eleven animals each and having a mean individual weight of 160 pounds were treated as follows: All groups were housed in the same quarters to equalize competition and intake of food, water and shelter, Group I received daily injections of L-thyroxine at approximately 3 times normal daily secretion rate; Group II received daily injections of L-triiodothyronine at approximately 3 times normal daily secretion rate and Group III, the placebo controls, received daily injections of physiological saline. The animals received these treatments daily for a period of 21 days. The mean daily ambient temperatures ranged from the mid 60's to high 70's. Maximal daily temperatures were above 90 degrees three consecutive days during the third week of the test.

Data on treatment were as follows: After seven days, group I lost 13.5 pounds, group II had lost 25.5 pounds and group III had gained 39.5 pounds. After fourteen days group I had gained 44 pounds, group II had lost 11 pounds and group III had gained 107 pounds. After 21 days and a loss of an animal from each of Group I and Group II, all groups showed gains. Group I gained 103.5 pounds, group II gained 107.5 pounds and group III gained 143.5 pounds. All groups became acclimated to the excessive treatments and were showing comparable gains after 21 days.

A paper, on this work, was presented by F. E. Romack, in 1967 at the Third Midwest Conference on the Thyroid at the University of Missouri, Columbia, Missouri.

- D. Biological half-lives of L-thyroxine and L-triiodothyronine have been ascertained. Data on physiological action of these hormones are very difficult to obtain without great variation. Observations have shown that in vivo metabolism of thyroid hormones proceeds at a much more rapid rate than was previously indicated. Information obtained from research workers participating in the Third Midwest Conference on the Thyroid at the University of Missouri 1967 indicate, that a rapid rate of their depletion from the biological system is to be expected. Further observations will precede summarization.
- E. The effects of dietary nitrate on thyroid secretion rate in lambs have been observed. Nitrate toxicity in plant foods results when excessive nitrate is absorbed from the soil by growing plants. These nitrates are converted, through intermediate compounds, into plant proteins. Accumulation of these undesirable compounds in plants have been known to adversely affect the nutrition and health of animals. It has been shown that thyroid secretion rate is affected by the presence of nitrates in the diet.

This study, utilizing young growing sheep, was in cooperation with the Department of Animal Science, University of Illinois.

Twenty-eight lambs were utilized. These animals were housed in a temperature controlled laboratory maintained at 21° C to eliminate effects of variation on thyroid secretions. Lighting was controlled to remove this variable.

The lambs were divided into three groups. Group I received a complete balanced ration including four percent potassium nitrate and were allowed to adapt to this ration before thyroid secretion rates were measured. Group II also received a complete balanced ration but did not receive potassium nitrate until thyroid secretion rates were to be calculated. Group III were controls and received only the complete balanced ration. Observations were made on thyroid uptake of radioactive iodine (^{131}I), daily thyroid secretion rate, kidney concentrations of ^{131}I , urinary levels of ^{131}I and background radiation.

Conclusions from these data suggest that potassium nitrate at this level does not inhibit, but may enhance, iodine uptake and thyroid function.

These studies were reported in a Master's thesis by S. P. Arora. University of Illinois, 1966.

- F. Effects of high levels of radioactive iodine (^{131}I), over a period of time up to one year, on body tissues of swine have been initiated. Fifteen swine were included in this study. Observations have been made on the blood changes and histological specimens of body tissues are being prepared. Summation of these results are not complete.

- G. Movement of in vivo labeled thyroid hormones from maternal to fetal tissues have been studied in both rats and swine.

Preliminary results in rats indicate restricted passage across the placental tissues until day 19 when movement to fetal tissues was evident. Data have been obtained from swine periodically throughout the 115-day gestation period. Observations were made on the 25, 50, 75, 100 and 110th days of gestation. Complete analysis of these data has not been accomplished.

- H. Comparative studies of tissues localization of radioactive iodine labeled thyroid hormones by autoradiographic analysis have been conducted in rabbits, rats, guinea pigs and swine. These studies included in vivo tagging of thyroid secretions with both ^{131}I and ^{129}I , injections of exogenously tagged L-thyroxine ^{131}I and L-triiodothyronine ^{131}I in non-treated animals of the above species as well as tapazole (goitrogen) treated subjects.

Autoradiographs, using modifications in the dipping technique, were used for comparing tissue specimens and blood smears. Tissue sections from treated animals were exposed to photographic emulsion for various time periods and improved methods of clearing the stained autoradiographs were devised. Nuclear definition was enhanced by modifying nuclear fast red-indigo carmine staining techniques. Data from this study were summarized for a doctoral dissertation by Prem Prakash, 1966.

- I. Ablation of pituitary tissue by surgical placement of stainless steel seeds containing various radioactive

materials are in progress. Observations on the effect of radon (^{222}Rn) and iridium (^{192}Ir) have been partially completed. Histologic observations are made on specimens after treatment with various energy levels and exposure times of pituitary tissue, in situ, to these isotopes. Yttrium (^{91}Y) in stainless steel sheaths, is now being implanted for observation. Summarization of results will not be completed until observations of the effects of yttrium have been terminated and treated tissue specimens have been completely analyzed.

These studies are being conducted in cooperation with the staff members of the Department of Veterinary Physiology and Pharmacology, University of Illinois.

Hypophysectomized swine are desirable for further elucidation of thyroid function and other pituitary-controlled endocrine action.

J. Cooperative studies with the Department of Veterinary Clinical Medicine, has resulted in the observation of liver function in studies of normal equine subjects and by utilizing carbontetrachloride interference. These observations were made with labeled Rose Bengal. Intravenous injections of rose bengal ^{131}I were made following a background and normal non-isotope count of the environmental area and the subject. Rate of liver uptake and depletion were recorded, plotted and summarized. These data will be compared to known liver condition as determined by histopathological examination of liver

biopsy specimens. Preliminary observations indicate this procedure to be a valid clinical test for equine live function.

- K. Attempts to remove single swine fetus from the intra-uterine environment during gestation resulted in problems that lead to cooperative studies of anesthesia in swine. The use of barbiturates resulted in abortion in each trial when a quantity sufficient for surgical anesthesia was used. Preliminary observations of electroanesthesia and gaseous anesthetic procedures have resulted in an apparently successful **anesthetic** procedure for pregnant swine. These trials were made primarily with halothane, a gaseous hydrocarbon. Pre-anesthetic agents used to date are acepromazine (Ayerst Labs) and My-301 (a guariacol Glycerol ester of ether) both have given acceptable results when used in conjunction with halothane. Many more observations will be made before this study is summarized.
- L. Attempts have been made to identify the nuclei resulting from rapid cellular division during implantation (day 7 to 10) in swine. Tritiated thymidine has been used with autoradiographic techniques in both rats and swine. Observations have not reached the point where analyses can be drawn at this report. Replicated observations will be scrutinized.

COOPERATIVE STUDIES

Free association in interdepartmental research activities within the College of Veterinary Medicine and with other disciplines on the University of Illinois Campus have occurred. Sharing of radiation detection equipment; liquid scintillation counter, ratemeters, scalers and probes are commonplace and have been accomplished to enhance, rather than deter, research investigations. Some areas of cooperation are listed:

- A. F. E. Romack, Ph.D., principal investigator, Studies of Endocrine Metabolism and Function in Domestic Animals Utilizing Radioactive Isotopes as Tracers. AT(11-1) 1484.
- B. A. R. Twardock, Ph.D., principal investigator, Placental Transfer of Minerals. (NIH, HD00727).
- C. R. D. McQueen, Ph.D., principal investigator, Histamine Synthesis and Action on Mammalian Uterine Tissue. (General Research Support Project).
- D. E. E. Hatfield, Ph.D., Effects of Dietary Nitrates on Thyroid Function. (Agriculture Research Station Project).
- E. F. E. Romack, H. E. Garner and J. C. Thurmon, Co-Investigators, Surgical Anesthesia in Domestic Animals (Multi-Support, Research Project).
- F. J. E. Lovell and F. E. Romack, Autoradiographic Observations of Developing Embryo. (General Research Support Project).
- G. T. N. Monfort, M. Patel, H. Hardenbrook and F. E. Romack, Liver Function Studies in Equine Subjects with Carbontetrachloride Interference. (General Research Support Project).

- H. F. E. Romack, L. E. St. Clair, J. J. B. Anderson and A. R. Twardock, Hypophysectomy of swine by tissue ablation with radioactive isotopes. (General Research Support)
- I. F. E. Romack, A. M. Watrach and M. Watrach, Studies of Tritiated-Uridine on in vitro cell cultures via, liquid scintillation and electron microscopic autoradiographic observations. (General Research Support).
- J. F. E. Romack and D. K. Normandin, Electron microscopic autoradiographic observations of mouse duodenal epithelial crypt cells labeled with Tritiated-Thymidine.

STATEMENT OF TOTAL PROJECT COSTS
AND

UNEXPENDED FUND STATEMENT
UNIVERSITY OF ILLINOIS
CONTRACT NO. AT(11-1)1484
FOR THE PERIOD JUNE 1, 1967 THROUGH MAY 31, 1968

Total Project Level	\$17,410.00
Total Project Cost for Current Period	
To April 30, 1968	<u>9,894.00</u>
Balance	\$ 7,516.00
Estimate of unexpended funds to be incurred during remainder of period	\$ 7,516.00
Estimate of unexpended funds at the end of the current period	00.00

STATEMENT OF AEC FUNDS UNEXPENDED

Total AEC Funds June 1, 1967 through May 31, 1968	\$15,000.00
Costs from June 1, 1967 to April 30, 1968	\$ 9,113.00
Estimate of Costs, April 30, 1968 through May 31, 1968	5,887.00
	Total <u>\$15,000.00</u>
Estimate of Funds Unexpended	\$ 00.00

NOTE: Some expenditures have not cleared our accounting offices and exact figures are not available. However, the principal investigator is exhibiting extreme care, in closing this project, to prevent deficit spending.

Equipment Report
University of Illinois
Contract No. AT(11-1)1484
For the Period June 1, 1967, through May 31, 1968

Equipment Item	Purchase Date	Acquisition Cost
1 - Survey Meter	5-29-67	\$ 355.00
1 - Scintillation Well Detector	7-18-67	1175.00
1 - Cliniscaler	7-18-67	1250.00
1 - Dual Recorder	7-18-67	<u>1185.00</u>
	Total	<u>\$3965.00</u>
Estimated expenditures for this period		\$3550.00

PUBLICATIONS AND PAPERS

- Romack, F. E., 1967, Effects of T_4 and T_3 Administration on Body Weight Gains, (Abstr.) Paper presented at the Third Midwest Conference on the Thyroid, University of Missouri, Columbia.
- S. P. Arora, E. E. Hatfield, F. E. Romack and U. S. Garrigus, 1966, Effect of Dietary Nitrate upon I^{131} Uptake by Thyroid in Lambs. (Abstr.) J. Animal Sci., 25: 1253 (S. P. Arora presented paper at Am. Society of Animal Sci. Chicago, 1966).
- S. P. Arora, E. E. Hatfield, U. S. Garrigus, F. E. Romack and H. Motyka, 1967, Effect of Dietary Nitrate on Thyroid Secretion Rate in Lambs. (In Press) J. Animal Sci.
- F. E. Romack and M. J. Kelly, Radioactive Diffusion Studies Between Maternal and Fetal Tissues in Rats. (In Press) Illinois Veterinarian, 1968.
- F. E. Romack and L. Seidenberg, The Effects of Increased Quantities of L-Thyroxine and L-Triiodothyronine on Body Weight of Albino Laboratory Rats. (In Press) Illinois Veterinarian, 1968.

Graduate Studies

- Master's Thesis, Effect of Dietary Nitrate on Thyroxine Secretion Rate in Lambs, by S. P. Arora, 1966.
- Doctoral Dissertation, Tissue Localization of Radioactive Iodine Labeled Thyroid Hormones by Autoradiographic Analysis, by Prem Prakash, 1966.
- Master's Thesis, (in Progress) I^{131} Movement Across the Placental Barrier in Swine, L. A. Calsyn.

Special Problems

The Effect of Increased Amounts of L-Triiodothyronine (T_3) and L-Thyroxine (T_4) on Albino Laboratory Rats, by Lew Seidenberg, 1966.

Radioactive I^{131} Diffusion Studies Between Maternal Thyroid, Fetal Thyroid and the Placental Barrier in Rats, by M. J. Kelly; 1966.

Effect of ^{131}I in Swine Over Long Periods of Time on Circulating Blood Cells and Lymph Nodes, by L. A. Calsyn, 1967.