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GRADUATE COLLEGE

EFFECT OF HOMOTRANSPLANTS OF THYROID AND PITUITARY ON THE METABOLIC RATE OF THE LABORATORY RAT

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

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degree of

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WALTER EUGENE CROWDER

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EFFECT OF HOMOTRANSPLANTS OF THYROID AND PITUITARY ON THE METABOLIC RATE OF THE LABORATORY RAT

APPROVED BY

Harriet Harvey

Elroy L. Rice

W. M. Davis

A. P. Brown

H. A. Ellis

DISSERTATION COMMITTEE

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EFFECT OF HOMOTRANSPLANTS OF THYROID AND PITUITARY ON THE METABOLIC RATE OF THE LABORATORY RAT

CHAPTER I

INTRODUCTION

Endocrinology, immunology, and genetics have been enhanced by information obtained by transplantation of tissue, and all three fields are involved in endocrine transplantation. The transplantation of endocrine tissue occurred long before the formulation of the concepts of endocrinology and was a leading factor in the development of these concepts.

In 1771, Hunter transplanted testes of cocks from the normal site to various visceral organs, and also into the abdomen of young hens. In 1849, Berthold presented the first classical demonstration of the endocrine nature of an organ by replacing testes in caponized cockerels (Krohn, 1959). In 1889, Brown-Sequard injected macerated testes into his own body in an attempt to produce a rejuvenated physical state (Turner, 1955).

It has been well established that thyroid and pituitary secretions can elevate the metabolic rate of mammals. Magnus-Levy, in 1895, used a calorimeter to show that orally administered thyroid tissue raised the metabolic rate of patients (Turner, 1955). Smith (1926) and Loeb and Bassett (1929) demonstrated the thyrotropic effect of the pituitary in mammals. Anderson and Collip (1933) were able to increase the metabolic rate

of both rats and guinea pigs with injections of thyrotropic hormone of the pituitary. Boothby, et al. (1938) produced a higher metabolic rate in laboratory mammals with injections of thyroxine.

Severinghous, Smelser, and Clark (1934a,b) demonstrated the inhibitory effect of the thyroid on the pituitary. This, in addition to the work of Smith (1926) and Loeb and Bassett (1929) on the thyrotropic effect of the pituitary, led to the development of the pituitary-thyroid axis concept by Salter (1940). This concept is concerned with the "feedback mechanism" of the thyroid and pituitary in maintaining an equilibrium in the metabolic rate of the organism.

Shortly after the discovery of thyrotropin, workers began to report a lack of response of laboratory mammals to prolonged injection of pituitary extracts. Loeb and Friedman (1931) reported the thyroids of young guinea pigs no longer responded to extracts of cattle anterior pituitary after a long series of injections. Anderson and Collip (1934a) reported the typical increase in metabolic rate following the initial injections of thyrotropin, but a prolonged treatment reduced the metabolic rate to that of the hypophysectomized rate. It was believed to be due to the production of antithyrotropic substance. Lerman (1942) demonstrated a reduction in oxygen consumption following successive injections of thyroglobulin. It was believed the reduction in the metabolic rate was due to the formation of antibodies against the thyroglobulin. Crowder (1956) was able to demonstrate a lowering of oxygen consumption following the homotransplantation of thyroid into the eyes. Anigstein, Eklund, and Whitney (1957) injected rat thyroid into rabbits and then injected the anti-rat thyroid serum of the rabbits into rats, thus producing lower metabolic rates.

The study of the destruction of transplants also began early in this century. Many ideas concerning the host-transplant reaction have evolved since. The first such concept, concerning the transplant's rection to the host, was the "Law of Endocrine Deficiency" formulated by Halsted (1909). The "law" of the endocrines involved the concept that an autotransplant (tissue transplanted within the same organism) of endocrine tissue would not be successful unless there was a deficiency of at least fifty per cent for the particular hormone or hormones of that gland.

The concepts concerning the host's reaction to the transplant are probably more far-reaching than the transplant's reaction to the host. The most striking reaction of the host is its destruction of homotransplants (tissue transplanted from one organism to another of the same species) and heterotransplants (tissue transferred between animals of different species).

Various approaches have been utilized to determine the mechanism involved in the destruction of the transplants, and also the mechanism for inhibiting this destruction. The possible role of lymphocytes in the destruction of transplants was first proposed by Wade (1908). Murphy (1914) demonstrated that X-irradiation of lymphoid tissue permitted the growth of heterotransplants of carcinomas in rats. Loeb (1926a,b) presented evidence that the destruction of homotransplants of thyroid and parathyroid in guinea pigs and rats was due to lymphocytes and not to the hormonal level in the organism. Weaver, Algire, and Prehn (1955) demonstrated that a homotransplant would survive if the lymphocytes could not

make contact with it. However, if the lymphocytes were permitted contact with the homotransplants, the transplants were destroyed.

Whitelaw (1951) reported that adrenocorticotropic hormone (ACTH) increased the longevity of homotransplants of skin on a burned patient. This report initiated much research on the effect of ACTH and adrenal corticoids on transplants. Billingham, Medawar, and Krohn (1951) administered cortisone to rabbits and prolonged the life of skin homotransplants. Toolan (1954a,b) used cortisone to maintain human neoplasms in rats. Woodruff (1954) found that large doses of cortisone increased survival time of thyroid homotransplants in guinea pigs. Medawar and Sparrow (1956) reported that both cortisone and hydrocortisone prolonged the life of skin homotransplants in rabbits and mice. Their findings also revealed that these two corticoids were the only two that were effective in increasing longevity of homotransplants.

The question arises as to the response of a host to an intact thyroid that was capable of responding to humoral stimuli. Would the additional thyroid or pituitary become involved in the normal pituitary-thyroid axis, thus maintaining a constant metabolic rate? Would these transplants increase the metabolic rate as the initial injections of thyroid and pituitary, or initiate the production of anti-thyroid or pituitary substances and thus reduce the metabolic rate?

Apparently the only published attempt to determine the effect of the homotransplantation of whole thyroid glands on the metabolic rate of rats has been that of Crowder (1956). This study is a continuation and expansion of the former one. The daily oxygen consumption of the host was used as a criterion for determining the effects of the addition of

the following on the metabolic rate: (1) whole thyroid homotransplants; (2) whole pituitary homotransplants; (3) a combination of whole thyroid and pituitary homotransplants; and (4) thyroid homotransplant encapsulated so as to protect it from lymphocytes. The last phase of the problem, which did not involve oxygen consumption, was the effect of hydrocortisone upon the survival of thyroid homotransplants of rats.

CHAPTER II

MATERIALS AND METHODS

Description of the Apparatus

The respirometer used in this problem was designed by members of the Department of Pharmacology, University of Oklahoma School of Medicine. It was used in the work published by McArthur, Lhotka, and Hellbaum (1957), but its description has never been published, so a brief description with photographs will follow. The apparatus includes six spirometer type respirometers, with each respirometer consisting of two units, an animal chamber (A) and a spirometer (B) (Fig. 1,2,3).

Each spirometer was composed of an outer cylinder filled with water and an inner gas cylinder, the position of which was determined by the volume of gas supporting it. A wire from the top of the gas cylinder passed over a circular gauge (C) to the balance weight (D). The gas cylinder was so balanced that it remained stationary so long as the internal and external gas pressures were equal. The circular gauge (C) consisted of a wheel, 28 cm in circumference, with a shallow groove on the side of the periphery to guide the balance wire. The remainder of the peripheral surface of the gauge was covered with a tape graduated from 1 to 28 cm, with each centimeter graduated into millimeters.

The upper water bath (E), animal chambers (A), and cylinders of the spirometers were constructed of aluminum to permit more rapid heat transfer. The animal chambers and outer cylinders of the spirometers

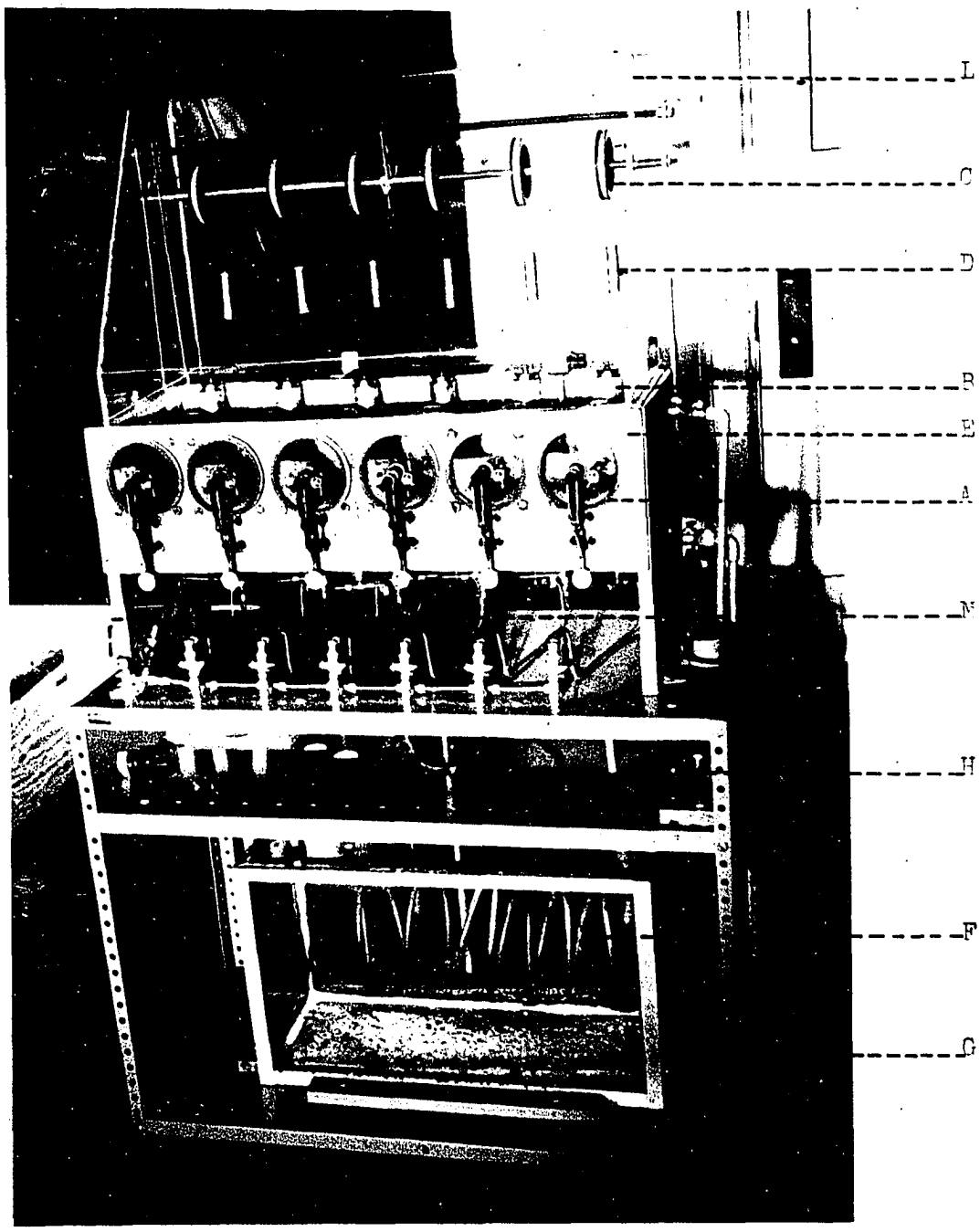


Fig. 1.--Apparatus used to measure the oxygen consumption of the rats.

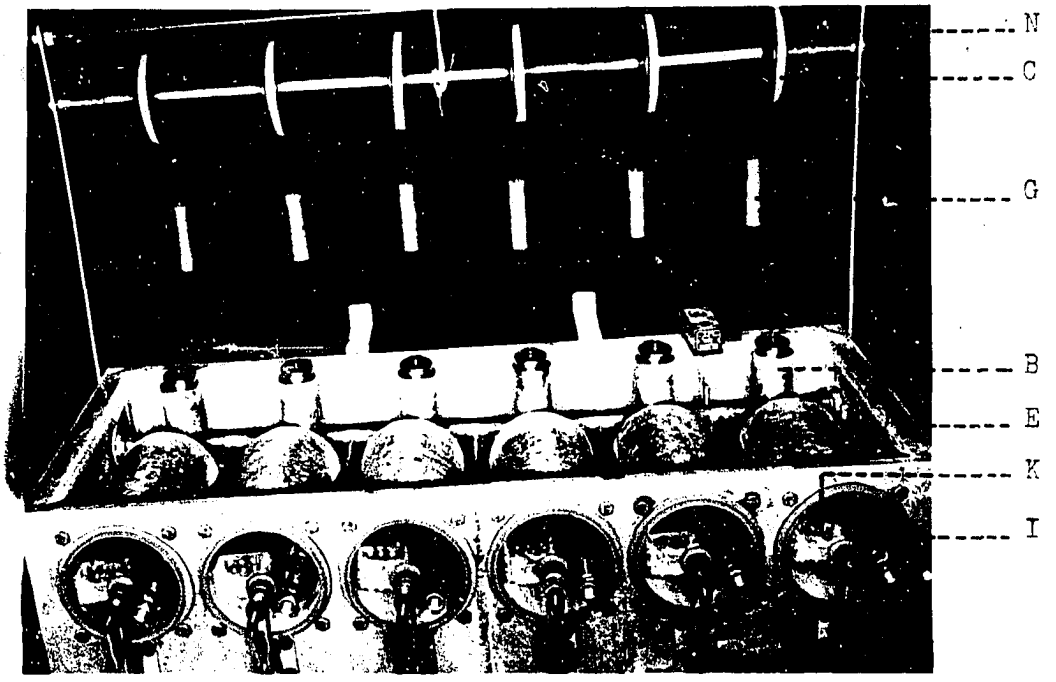


Fig. 2.--Upper water bath, animal chambers, respirometer, and circular gauges. Hood removed.

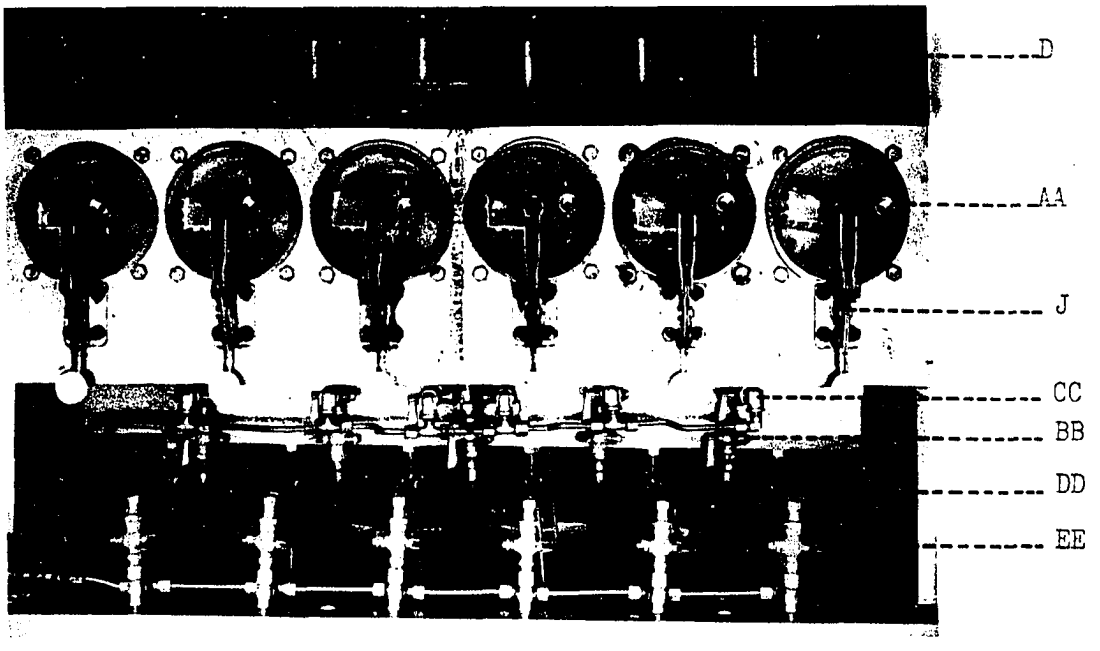


Fig. 3.--Front view of the upper water bath showing chambers and copper tubing connecting oxygen tank to animal chambers and respirometers.

were covered with water in the upper water bath (E) to facilitate this heat transfer. The apparatus was employed in a constant temperature room that maintained the water temperature at 24 $\pm$0.2° C. and the humidity at 93 $\pm$1 per cent.

A horizontal wire (Fig. 2) was placed in front of the gauges at eye level. The reference wire (N) was used as a pointer to determine the position of the gauges before and after the test.

Water from the upper bath was circulated by gravity flow into the copper coil (F) in the lower water bath (G) and pumped back into the upper bath by a centrifugal pump powered by a 1/15 horsepower electric sewing machine motor (H). The lower bath (G) was an eighteen gallon aquarium.

The animal chambers (Fig. 2) were cylinders 9.9 cm in diameter and 19.05 cm in length. The cylinders were open at the front but they could be closed by securing the plexiglass door (I) with the clamp lock (J). The inner surface of the door supported the soda lime box (K) which was made of plexiglass and measured 17/16 x 15/16 x 14/16 inches. The sides and bottom of the box were perforated to permit a better circulation of the gases in the soda lime. It was found that the box filled with 4-8 mesh reagent soda lime was sufficient for six fifteen-minute tests.

There were two outlets to each animal chamber (Fig. 3). The anterior outlet was a 1/4 inch gas cock (AA) set off-center in the door and the posterior opening communicated by 1/8 inch I.D. copper tubing to the spirometer by passing through stop cock (BB) or to the outside through stop cocks (CC) and (DD) or through stop cocks (BB) and (EE).

A removable plexiglass hood (L) rested upon the upper bath. This prevented currents of air from moving the gauges, balance weights or reference wire.

The respirometers were filled with oxygen by the following procedure. The oxygen cylinder, using a high pressure gas regulator, was connected by a rubber hose to point (M) in Figure 1. The gas cocks were so arranged as to permit the flow of gas into either the spirometers or the chambers or both. The respirometers were filled by introducing the oxygen at point (M) with stop cocks (DD), (CC), and (AA) open, and stop cocks (EE) and (BB) closed (Fig. 3). The chambers were flushed with oxygen and then stop cock (EE) was opened for each of the spirometers until they were filled. At this time the index wire (N) was at approximately zero on the circular gauges. Following the filling of the spirometers, all gas cocks were closed and the oxygen supply was turned off. Then stop cocks (BB) were opened to permit the communication of gases between the chamber and the spirometer.

A closed circuit type respirometer, as used in this study, uses pure oxygen and a carbon dioxide absorbant, such as soda lime. As the oxygen is utilized by the animal, the carbon dioxide is given off and absorbed, the pressure inside the respirometer is reduced, allowing the gas cylinder of the spirometer to fall. The displacement of gas by the gas cylinder should be a measure of the oxygen consumed by the animal.

Small wire cages, constructed from 1/2 inch hardware cloth, housed the animals while they were in the respirometer. These held the animals out of the feces and urine that would collect in a two-hour period and restrained some movement without frightening the animals.

Calibration of the Respirometers

Each respirometer was calibrated in a manner similar to that used by Holtkamp et al. (1955) except in this study a 50 cc syringe was used instead of a 10 cc syringe. The respirometers were filled with oxygen until the dials read approximately zero. The syringe was connected to the outlet stop cock (AA) by a polyethylene tube and copper screw fitting. Fifty cc of oxygen were removed from the chamber and the amount of the spirometer drop was measured. The above procedure was triplicated for each respirometer and an average of the three was taken for each individual unit.

The following readings, corrected to 760 mm Hg were derived for the six respirometers. The drop of a spirometer for a distance of one centimeter was equivalent to the following number of cubic centimeters of gas.

Respirometer number	A drop of one centimeter equals the following in cc of oxygen
1	8.339
2	8.367
3	8.521
4	8.422
5	8.353
6	8.205

The actual calculations to determine the oxygen consumption were made by multiplying the spirometer drop, measured to the nearest .02 of a centimeter, times the value obtained by the calibration.

Calculations of Oxygen Consumption

The volume of oxygen consumed by the animals was adjusted to standard temperature and pressure, and expressed as liters of oxygen

consumed, per square meter of surface area, per hour (Holtkamp et al., 1955). The following formula was used to determine the oxygen consumption:

$$L/M^2Hr. = \frac{V}{t} \times \frac{P}{760} \times \frac{273}{T} \times \frac{10,000}{(Wt)^{2/3} \times 10} \times \frac{1}{1,000}$$

$L/M^2/Hr.$ = Volume of oxygen consumed per square meter of surface area per hour.

V = Volume of oxygen consumed (expressed in cc).

t = time in hours.

P = barometric pressure expressed in millimeters of mercury.

760 mm = mean barometric pressure at sea level.

273° abs. = freezing point of water. 0° C.

T = absolute temperature at the time of test.

Wt = weight of animal expressed in grams.

$\frac{10,000}{(Wt)^{2/3} \times 10}$ = formula for computing approximate surface area in square meters from body weight (Hill and Hill, 1913).

The temperature (T) and time (t) were kept constant at 24° C and 0.25 hours. The relative humidity in the room was constant at 93 $\pm$ 1%.

Substitution of values for the above symbols in the formula produces the following equation.

$$L/M^2/Hr. = \frac{V}{0.25} \times \frac{P}{760} \times \frac{273}{297} \times \frac{10,000}{(Wt)^{2/3} \times 10} \times \frac{1}{1,000}$$

Since the temperature (T) and time (t) are constant, the following formula was derived and used for each individual reading. Volume (V), barometric pressure (P), and animal weights changed from day to day.

$$L/M^2/Hr. = \frac{V \times P}{(Wt)^{2/3}} = 0.00484$$

$(Wt)^{2/3}$ was calculated by multiplying 2/3 x common log of the weight of the animal and then finding the antilog. A table was prepared showing $(Wt)^{2/3}$ for the complete range of weights that were used in this problem.

The following is an example of how the final determination of oxygen consumption was made. The cubic centimeters of oxygen consumed, barometric pressure, and weight of the animals were substituted for V (69.22), P (743), and $Wt^{2/3}$ ($326^{2/3}$ = 47.368).

$$L/M^2/Hr. = \frac{69.22 \times 743}{47.368} \times 0.00484 = 5.26$$

Calculation of the Volume of Surviving Transplants

The approximate volume of the surviving tissue of the thyroid transplants was determined in a manner similar to the following method used by Angervall (1959). He cut serial sections ten microns thick, of various glands and calculated the volume of every tenth section. A plane mirror was mounted on the microscope tube which reflected the enlarged image onto a table. The image was traced and square area determined with a planimeter. A stage micrometer was used to determine the degree of linear enlargement. Knowing the thickness of the sections, and the square area of one tenth of the sections, the volume of the gland could be determined.

All of the serial sections were projected by means of a micro-projector, (Bausch and Lomb), onto a good grade, non-water-marked, typing paper. The outline of the tissue was traced with a sharp 4-H pencil. In cases were epithelial cells of the thyroid transplants were replaced by

connective tissue only the tissue that appeared to be functional thyroid epithelium was outlined. The lens of the microprojector was maintained at a constant height, so that a linear distance of 22.5 mm on the tracing paper represented one mm on the micrometer. The volume of all of the thyroid transplants and five normal thyroids was determined in this manner.

A standard was made by projecting a two mm linear measure from a stage micrometer onto the paper and constructing squares with each side representing four mm or an area of 16 mm^2. The mean standard was derived by determining the mean of the weights of standards constructed from four different sheets of paper.

Weights of the four standards in grams:

1. = 0.4974
2. = 0.4915
3. = 0.4998
4. = 0.4944

$\underline{X}$ = 1.9841

$\overline{X}$ = 0.4956 or 0.496 g of paper represented 16 mm^2 of paper.

The tracings were cut with scissors so no graphite was showing on the traced area. Forceps were used to place each individual tracing in a manila envelope so it would not be touched by the hands. A separate envelope was used for the tracings of each thyroid gland and standard.

Approximately one-half of the tracings were weighed on a given day. Twenty-seven hours before the tracings were weighed, the envelopes containing the tracings were placed in a dry heat oven at 67°C for 24 h. The envelopes were then removed and placed in a desiccator for three

hours to permit cooling. The tracings of each individual transplant were removed from the envelope by forceps and placed on the balance pan and weighed immediately on an analytical, chainomatic, Christian Becker Type AB-4 scale which read directly to 0.1 mg. The weight of the tracings enabled the computation of the square area of the sections of the transplant. Since the sections were cut to a thickness of 10 microns, the volume of the glands, in cubic millimeters, would be the square area of the sections times .01 mm.

The formula for determining the volume of follicular tissue was derived as follows:

Wt^1 = weight, in grams, of the tracings of a gland.

Wt^2 = weight, in grams, of the mean of the standard squares.

.01 = .01 mm. The tissues were sectioned at ten microns or .01 mm.

16 = square area of standard weighing .496 g.

$\frac{Wt^1}{Wt^2}$ X standard mm^2 x .01 = Volume of transplant.

Example:

$\frac{.345}{.496}$ X 16 X .01 = 0.1109 or 0.111 mm^3 of follicular thyroid.

General Procedure

Forty-eight male rats of the Sprague-Dawley strain and 24 of the Holtzman strain were used as the experimental (host) animals and 37 females of the same age and strain were used as donors. There was no cross-strain transplantation. The host animals were divided into six major groups designated as Groups I through VI. Groups I through IV were obtained from the animal colony (Sprague-Dawley) of the Department

of Zoology, University of Oklahoma, while the last two groups were from the animal colony (Holtzman strain) of the College of Pharmacy of the University of Oklahoma.

Mature male rats were used as the host animals to prevent the fluctuation of oxygen consumption due to the estrous cycle of the female or rapid growth of the immature rat. The animals were housed at approximately 24°C and fed and drank ad lib. during the experiment. Each group was kept together in a large cage and separated only during the actual tests.

Each group was divided into subgroup A, tested from 7:00 AM to 9:30 AM, and subgroup B, tested from 10:00 AM to 12:30 PM. The utilization of subgroups A and B was necessary since the capacity of the respirometer was six animals. Six 15-minute tests were performed daily on each animal to determine the oxygen consumption before and after the transplantation. The respirometers were refilled with oxygen after each 15-minute test. The testing of a group was completed, tissues collected and preserved, before another group was started.

The terms pre-operative and post-operative refer to the periods of testing before and after the transplantation of the tissue. The length of the pre-operative period was indefinite with the transplantation being performed after the average reading of a group was approximately the same for three consecutive days. An exception to this was Group V in which the pre-operative period was continued for fourteen days to determine the effect of prolonged exposure to high concentrations of oxygen.

The post-operative period began the day following the operation and continued for 9 to 14 days. On the final day of the testing, the animals were sacrificed and the normal thyroids and pituitaries were recovered and weighed on a Roller-Smith torsion balance accurate to 0.2 mg. The transplants were recovered and immediately fixed in modified Zenker formol (Bensley), later sectioned in paraffin at ten microns, and stained with Mallory's Triple Connective Tissue stain.

Transplanting Operation

The method used in the transplanting of tissue was similar to that used by Woodruff and Woodruff (1950) except the vitreous chamber of the eye was used instead of the anterior chamber. The transplantation required that both the host and donor be prepared for surgery at the same time. Ether was used as the anesthetic. The operation required two workers, one to prepare the host and perform the transplantation while the other removed the tissue from the donor and presented it to his co-worker on saline-moistened cotton.

The host's eye was prepared by applying pressure to it with the thumb and forefinger until the corneo-scleral junction was visible. An incision, approximately one-fourth the circumference of the eyeball, was made with a number 12 curved scapel blade. A straight forcep was used to remove the lens and most of the vitreous humor to facilitate the placement of the transplant. The transplant was placed in the area of the retina and sutures were used, if necessary, to close the eye. In less than two hours the eye was filled with humor and appeared normal except for the visible transplant. Group I did not have the lens removed and the transplant was placed behind the lens.

Thyroid Gland

Thyroidectomy was performed by making a mid-line, longitudinal incision in the throat area. The submaxillary glands, sublingual glands, and sternohyoid muscles covering the trachea were separated and moved laterally leaving the thyroid exposed. A lobe was removed as needed, and all donor animals were sacrificed immediately after removal of desired tissue.

Hypophysis

The vertebral column was severed with bone clippers and the head separated from the trunk with scissors. Bone clippers were used to cut through the occipital bone by inserting one blade into the foramen magnum. Cuts were made on either side of the skull through the occipital and parietal bones. Forceps were used to lift the roof of the skull until it broke at the coronal suture, thus exposing the cerebrum. Curved forceps were passed in front and under the frontal lobes and the entire brain was lifted to expose the pituitary. The pituitary was removed from the sella turcica with small forceps and handed to the coworker in the same manner as the other tissues.

Experimental Groups

Group I, serving as a control on the operation, received skeletal muscle (gluteus maximus) with the assumption that this tissue was metabolically inert in influencing the over-all oxygen consumption of the host. Each host received two portions, one in each eye, approximately the size of a lobe of a rat thyroid. Tissue from a single female

donor was used for all of the hosts in this group. The post-operative period was ten days.

Groups II and III were similar groups but tested at different times. Subgroup A of each of these two groups received a single lobe of thyroid gland and subgroups B received a single pituitary gland. The right eye was used for thyroid and left eye for the pituitary transplants. The mixing of treatments of these two groups permitted the use of a single donor to supply one host with a thyroid and another host with a pituitary. The post-operative period for Group II was fourteen days and for Group III, nine days.

Group IV received both a thyroid lobe in one eye and an entire pituitary gland in the other eye. Each host required a single donor to supply the tissue to be transplanted. The post-operative period was 14 days.

Group V received a single lobe of thyroid capsulated with a methyl cellulose filter. The material used in this problem has been used in construction of filter chambers by Algire, Weaver, and Prehn (1954), Weaver, Algire and Prehn (1955), Sturgis and Castellanos (1957) and others. In the present study, Millipore filter, type HA, was used. This came in discs measuring 47 mm in diameter, 150 microns thick, with a pore size of .45 $\pm$0.02 microns. It was also imprinted with a 3.80 mm grid, which permitted the construction of capsules of uniform size. The post-operative period was ten days.

A strip of the filter 2 x 2 1/2 squares measuring 9.50 x 7.60 mm in size was used for each capsule. The strip was folded over on itself and one end and the edges opposite the fold were cemented together. The

cement was made from methyl-cellulose and acetone. Care was taken not to crack the filter while folding. The capsule at this point was a flattened cylinder measuring approximately 7.60 x 4.75 mm and open at one end. Just before using, these capsules were autoclaved at fifteen pounds of pressure for fifteen minutes and placed in a small dish of sterile saline.

It was necessary to cut the thyroid lobe into two or three pieces in order to get it into the capsule. After the capsule was filled, the open end was sealed by holding the millipore together with forceps and applying the cement with a teasing needle. The capsule was then put back into the saline to permit the cement to set and keep the tissue moist while the final preparations were made on the host. The capsule was then placed in the eye and one or two stitches taken to close the slit in the eyeball.

The size of the capsule was the best size found that could be placed in the eye of the host and also hold the tissue of a thyroid lobe.

Group VI received a single lobe of thyroid in the same manner as Group II-A and III-A and in addition it received subcutaneous injections of hydrocortisone. Each animal of this group received two mg of hydrocortisone at the time of the operation and two mg every other day until four injections or eight mg of the corticoid had been given. No oxygen consumption tests were made with this group. They were sacrificed 14 days after the operation.

Statistical Analysis

Three different analyses were utilized in an attempt to better understand the significance of the data obtained. Analysis of variance

was applied to the data on oxygen consumption, per cent body weight of normal thyroid and pituitaries, and volume of thyroid transplants. The variation in oxygen consumption within groups was so great that significant difference between the pre- and post-operative conditions could not be demonstrated using the analysis of variance.

In view of the variation described above, an approach recommended by McNemar (1955) was used to make statistical evaluation of the change shown by the experimental groups when compared with that shown by the control group. McNemar has summarized very clearly the factors involved in an analysis of data in this type of problem. Following his suggestion, a small sample statistic for correlated means was used to analyze the pre- and post-operative data for each of the five groups. To determine if there was a significant difference between the differences of the control and experimental group, the animals were rank-ordered on the basis of pre-operative oxygen consumption and then matched. This permitted the use of McNemar's comparison-of-change statistic.

CHAPTER III

RESULTS

Oxygen Consumption

Metabolic rate of the laboratory rat, as determined by oxygen consumption measurements, does seem to be influenced by the homotransplants, but a well-established homeostasis is also evident. These fluctuations and stabilities are graphically shown in Figures 4 through 8. The straight solid line represents the pre-operative mean, the value of which is the mean of the last three pre-operative days. The solid plotted line represents the daily mean value while the broken lines above and below the solid line represent the highest and lowest individual extremes respectively. Each plotted point of the daily mean represents approximately 66 readings of 15 minutes each, while the points on the extremes represent five, or usually, six readings.

The pre-operative mean oxygen consumption for the group receiving muscle transplants was 4.9 liters. The first noticeable change was an increase of about .5 liters of oxygen which occurred the second post-operative day. On the sixth day the daily mean dropped to near the pre-operative mean and by the ninth and tenth days the readings appear to be back to normal (Fig. 4).

In the group receiving a single lobe of thyroid the pre-operative mean was 4.8 liters. The post-operative oxygen consumption dropped gradually until the sixth day where it leveled off to 4.2 liters (Fig. 5).

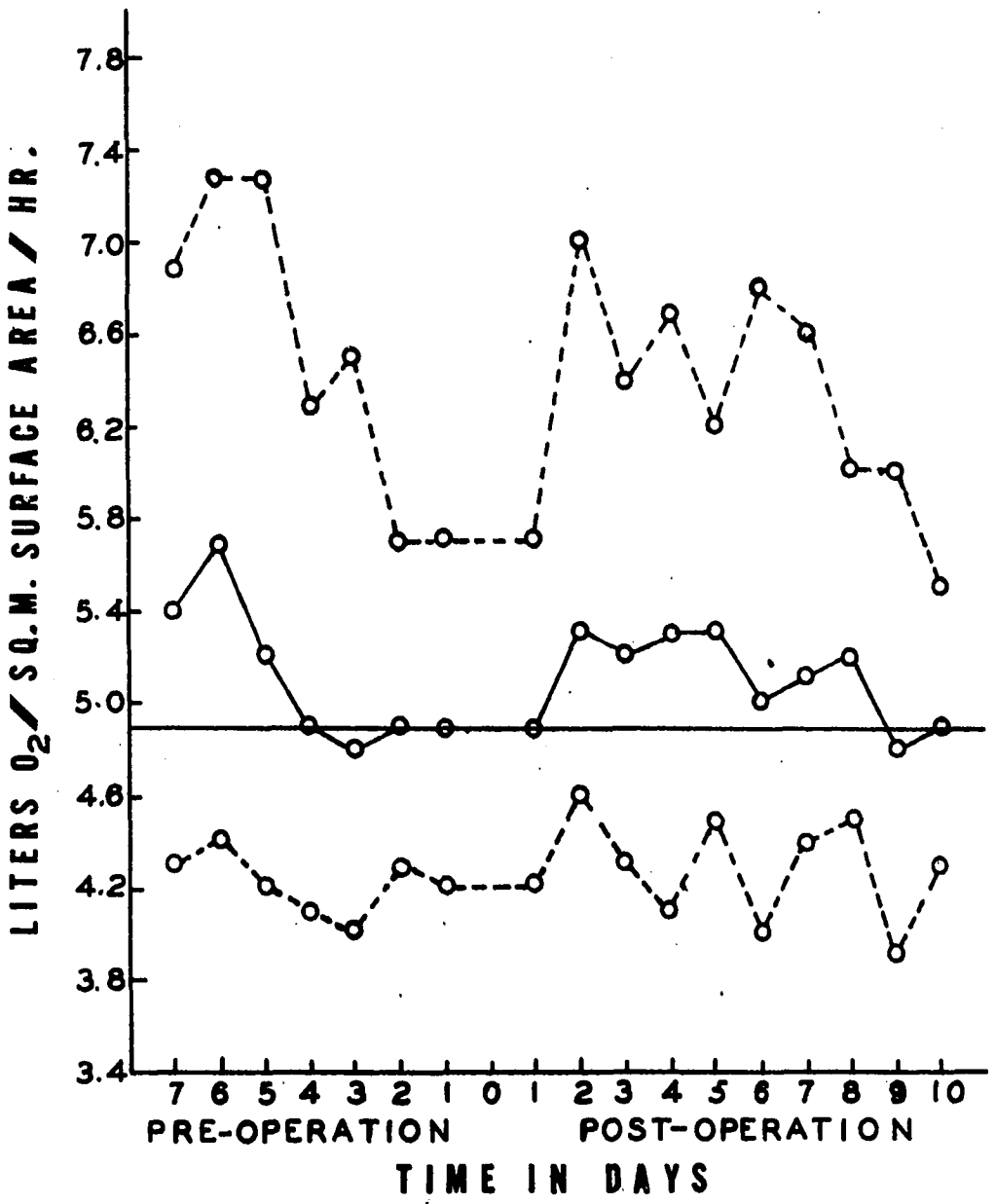


Fig. 4--Oxygen Consumption of Group I measured in liters of oxygen per square meter of surface area per hour. The solid plotted line represents the mean oxygen consumption of the group. The broken plotted lines represent the individual extremes. The numbers on the abscissa represent the days before and after the operation. Day 0 represents the day of operation.

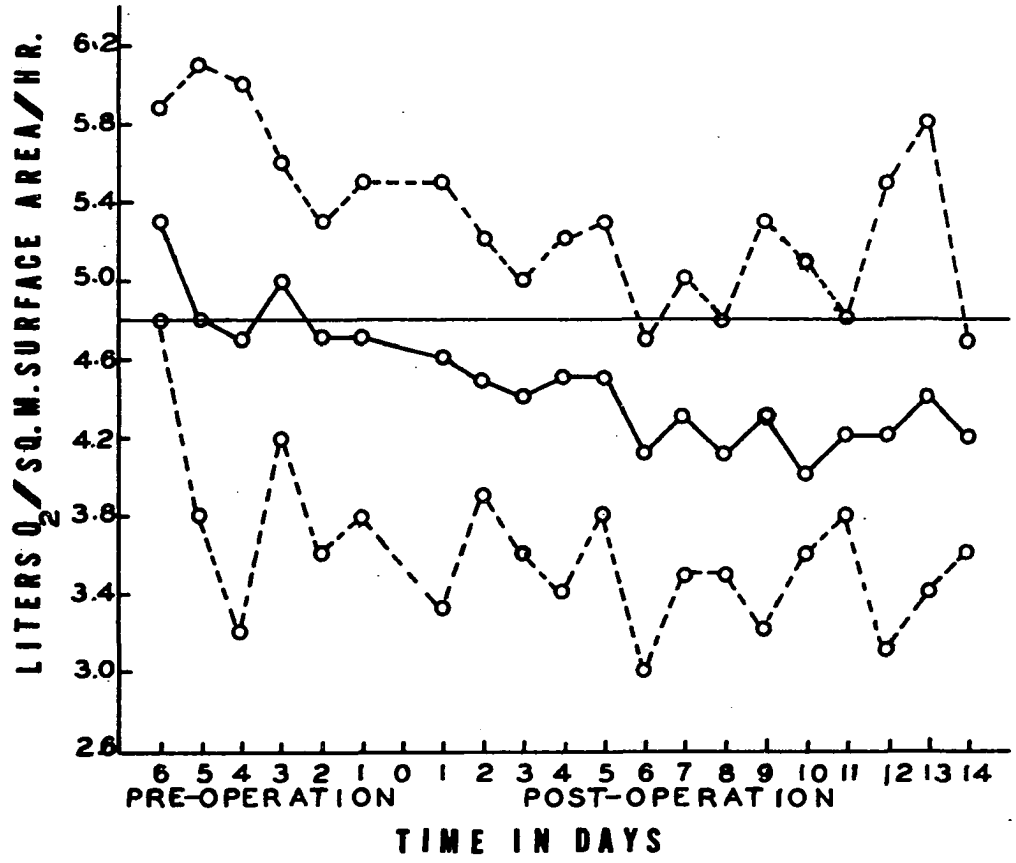


Fig. 5--Oxygen consumption of Groups II-A and III-A measured in liters of oxygen consumed per square meter of surface area per hour. The solid plotted line represents the mean oxygen consumption of the group. The broken plotted lines represent the individual extremes. The numbers on the abscissa represent the days before and after the operation. Day 0 represents the day of operation.

The group which was given transplants of an entire pituitary gland into the left eye showed a pre-operative mean of 4.5 liters of oxygen. The daily means following the operation dropped to approximately 4.2 liters through the seventh day, and then returned to the pre-operative state the eighth and ninth days. On post-operative days 10 through 14 the oxygen consumption of Group II-B fell again, but returned to the pre-operative condition (Fig. 6). This reduction is similar to that seen in Fig. 5.

The transplantation of both thyroid and pituitary appears to produce a different physiological response than does either of the two endocrine organs transplanted separately. The thyroid was placed in the right and the pituitary gland in the left eyes of the animals of Group IV. The pre-operative mean of this group was 4.5 liters. The pattern of oxygen consumption in this group was similar to that of Group I except in Group IV the consumption rose on the day following the operation and remained about .4 liter above the pre-operative mean until the last day. The last day shows as unusual increase in oxygen consumption (Fig. 7).

The results from Group V indicated that the encapsulated thyroid did not cause any appreciable change in the post-operative oxygen consumption over the pre-operative mean. This group had the highest pre-operative mean of 5.7 liters (Fig. 8). An unusual sequence in pre-operative data shows that the first two days of testing were the lowest.

Analysis of Oxygen Consumption

The analysis of variance, as shown in Table 1 reveals very little significant difference in the oxygen consumption data. A three-way

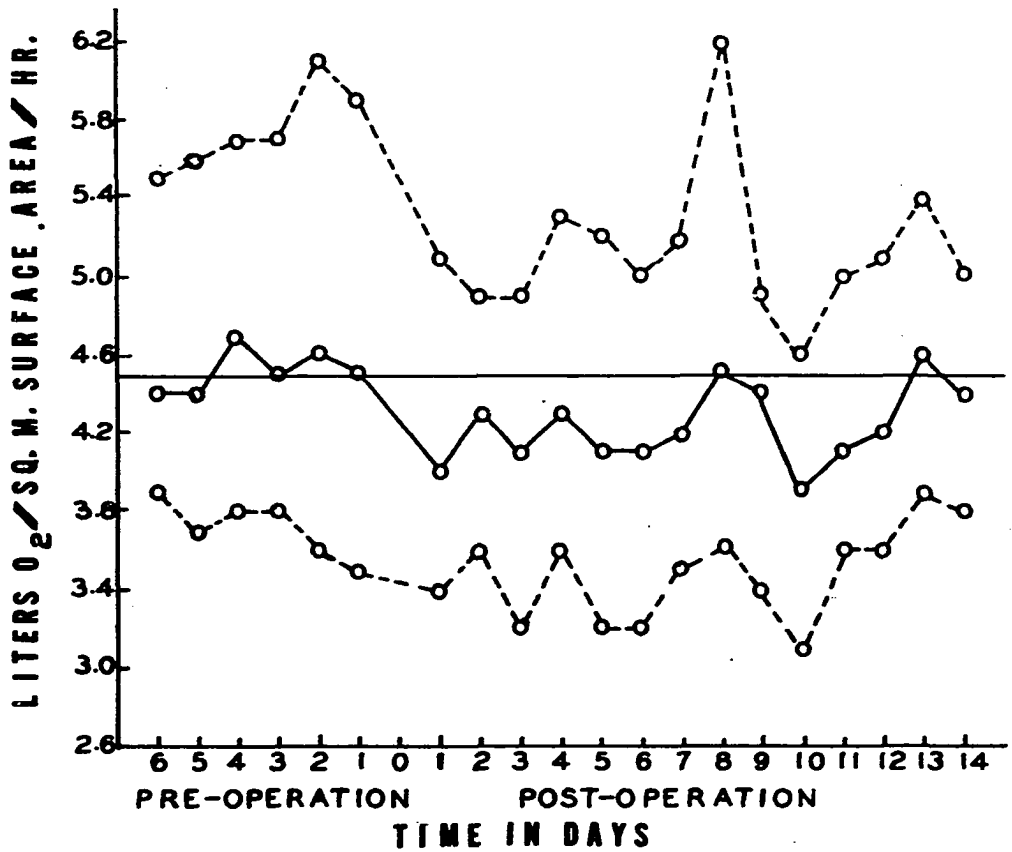


Fig. 6--Oxygen consumption of Groups II-B and III-B measured in liters of oxygen consumed per square meter of surface area per hour. The solid plotted line represents the mean oxygen consumption of the group. The broken plotted lines represent the individual extremes. The numbers on the abscissa represent the days before and after the operation. Day 0 represents the day of the operation.

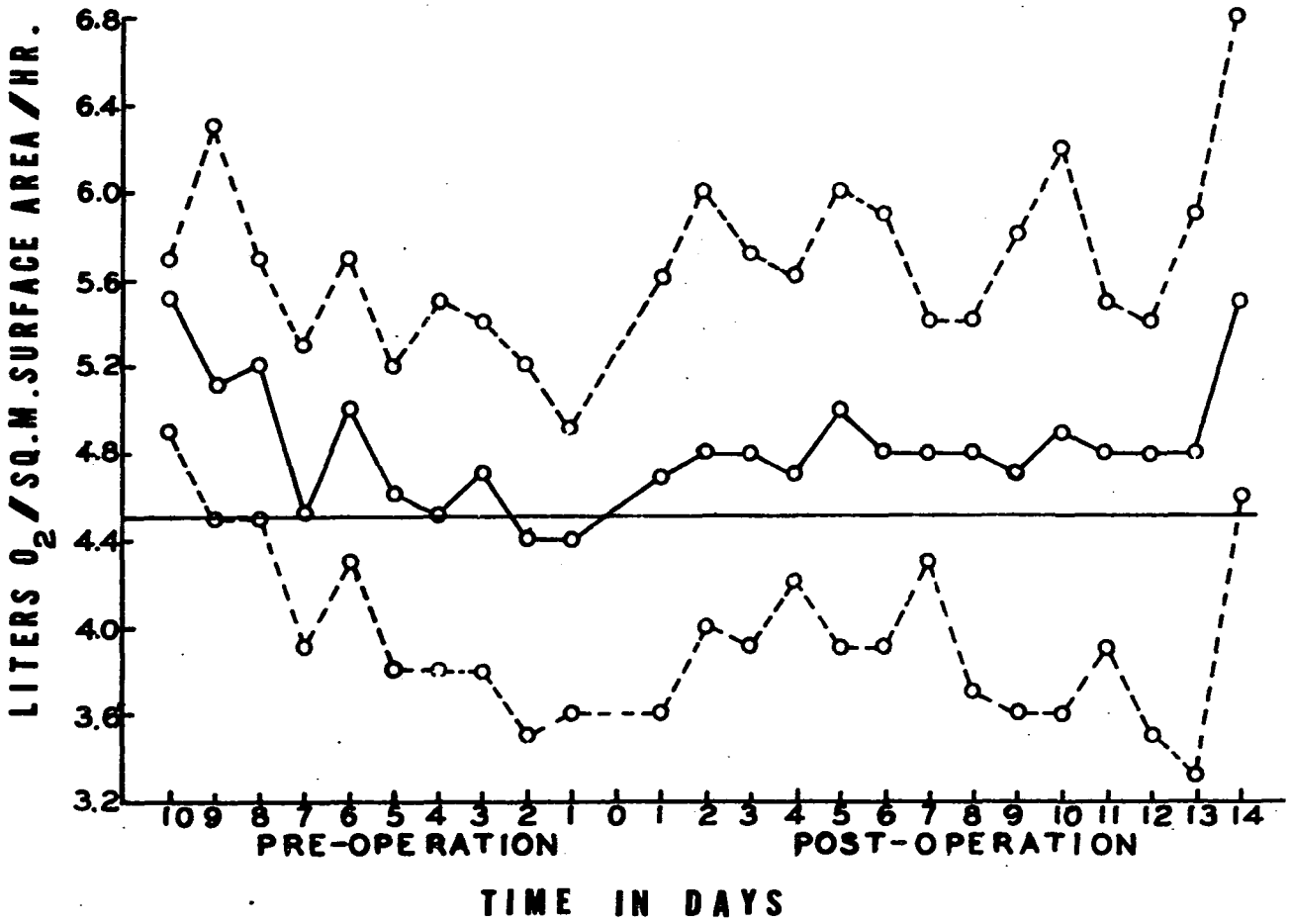


Fig. 7--Oxygen consumption of Group IV measured in liters of oxygen consumed per square meter of surface area per hour. The solid plotted line represents the mean oxygen consumption of the group. The broken plotted lines represent the individual extremes. The numbers on the abscissa represent the days before and after the operation. Day 0 represents the day of the operation.

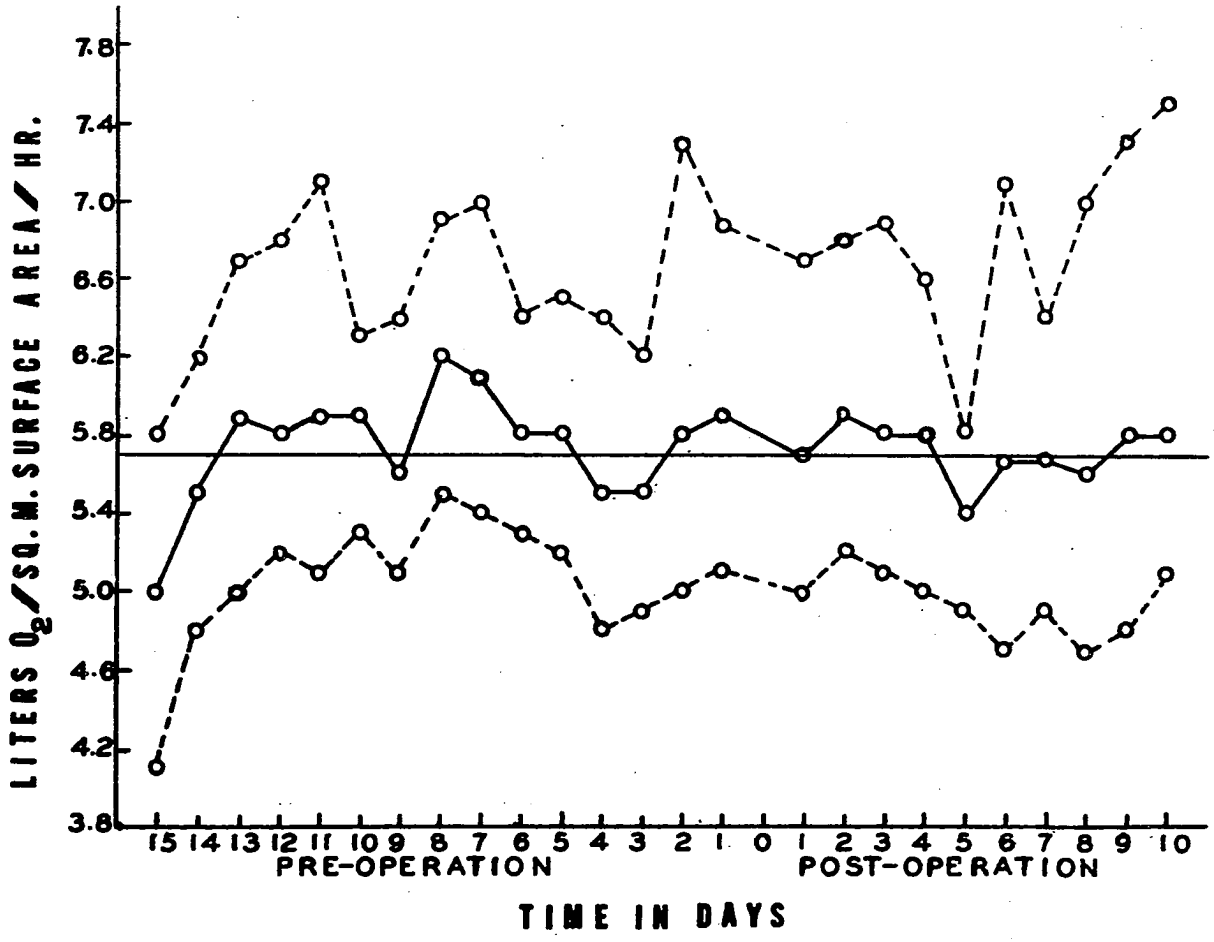


Fig. 8--Oxygen consumption of Group V measured in liters of oxygen consumption per square meter surface area per hour. The solid plotted line represents the mean oxygen consumption of the group. The broken plotted lines represent the individual extremes. The numbers on the abscissa represent the days before and after the operation. Day 0 represents the day of operation.

TABLE 1

ANALYSIS OF OXYGEN CONSUMPTION DATA[a]

Animals	Groups-A (Early)					Groups-B (Late)				
	I	II	III	IV	V	I	II	III	IV	V
	Pre-Operative Data									
1	4.251	4.616	. . .	4.659	5.479	4.613	4.074	4.872	5.060	5.040
2	4.735	3.861	4.854	. . .	5.880	4.622	3.661	. . .	4.531	6.430
3	4.645	4.626	. . .	3.773	5.965	4.621	4.592	4.275	4.822	6.680
4	5.211	4.625	5.230	3.966	5.065	5.878	5.879	4.183	4.420	5.587
5	5.177	4.350	4.824	4.386	5.208	4.942	4.986	4.201	3.767	5.784
6	5.052	5.313	4.852	4.878	5.809	4.760	4.360	4.722	5.019	5.821
$\bar{X}$=	4.845	4.565	4.940	4.332	5.568	4.906	4.592	4.451	4.603	5.890
	Post-Operative Data									
1	4.611	4.049	. . .	5.390	5.502	5.213	3.702	4.351	5.174	5.130
2	5.213	3.491	4.118	. . .	5.867	4.830	3.662	. . .	4.895	6.586
3	4.900	4.119	. . .	4.274	5.616	4.719	4.221	4.056	4.966	6.287
4	5.478	4.789	4.568	4.083	5.587	6.132	4.950	3.988	4.954	5.591
5	4.942	4.058	4.299	4.545	5.754	5.892	4.513	3.851	4.469	5.553
6	4.760	4.610	4.845	4.818	6.008	4.708	4.268	4.294	5.319	4.953
$\bar{X}$=	4.984	4.186	4.465	4.622	5.722	5.249	4.216	4.108	4.963	5.683

Items	Analysis of Variance					
	Source	SS	DF	MS	F	Significance
1	Pre vs. Post	.0336	1	.0336	. . .	Less than Unity
2	5 Expt. Groups	27.1103	4	6.7780	31.38	$P < .001$
3	Early vs. Late	.0493	1	.0493	. . .	Less than Unity
4	Interaction 1x2	2.4777	4	.6194	2.87	$P < .05$
5	Interaction 1x3	.0001	1	.0001	. . .	Less than Unity
6	Interaction 2x3	1.5310	4	.3827	1.77	Not significant
7	Interaction 1x2x3	.3012	4	.0753	. . .	Less than Unity
8	Within Groups	19.8756	92	.2160	. . .	Less than Unity
	Total	51.3788	111			

[a]The readings for the Pre-operative group represent the three days prior to the operation and are the mean based on the eighteen trials per rat. The readings for the Post-operative period represent the first nine days following the operation and are the mean based on fifty-four trials per rat. The values are expressed in Liters of Oxygen / Sq. Meter Surface Area / Hour.

analysis of variance was utilized to determine: (1) the differences between the pre-operative and post-operative conditions, (2) the differences among the experimental groups, and (3) the effect of early and late testings. The only statistically significant difference of the three conditions studied was among the five groups (condition 2). Further computation of the F test showed that the significant difference within the groups was between Group V and the rest of the groups.

The E test in the small sample statistic was to determine the degree of change between the pre-operative and post-operative data within each group. Group I showed a post-operative rise. Readings of Groups II-A and III-A and II-B and III-B indicated a post-operative decrease. Group IV showed an increase, while Group V showed a slight drop that was not significant since the critical ratio was less than unity (Table 2).

Analysis of the differences between the differences of the control and the other experimental groups showed that all groups except IV varied significantly (Table 3). The apparent reason for the significance between Groups I and V was not due to a change in Group V but to the post-operative change in Group I.

Per Cent Body Weights

An analysis of variance was performed on the data of the per cent body weights of the normal (*in situ*) thyroid and pituitary glands (Tables 4 and 5). The missing values in these two tables, indicated by the dotted lines, represent the animals that died during the operation.

TABLE 2

ANALYSIS OF OXYGEN CONSUMPTION BETWEEN PRE AND POST CONDITIONS FOR EACH GROUP[a]

Animals[b]	Groups									
	I		II & III-A		II & III-B		IV		V	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	4.251	4.611	4.616	4.049	4.074	3.702	4.659	5.390	5.479	5.502
2	4.735	5.213	3.861	3.491	3.661	3.662	. . .	. . .	5.880	6.586
3	4.645	4.900	4.626	4.119	4.592	4.221	3.773	4.274	5.965	5.616
4	5.211	5.478	4.625	4.789	5.879	4.950	3.966	4.083	5.065	5.587
5	5.177	4.942	4.350	4.058	4.986	4.513	4.386	4.545	5.208	5.754
6	5.052	4.760	5.313	4.610	4.360	4.268	4.878	4.818	5.809	6.008
1	4.613	5.213	. . .	. . .	4.872	4.351	5.060	5.174	5.040	5.130
2	4.622	4.830	4.854	4.148	. . .	. . .	4.531	4.895	6.430	6.586
3	4.621	4.719	. . .	. . .	4.275	4.056	4.822	4.966	6.680	6.287
4	5.878	6.132	5.230	4.568	4.183	3.988	4.420	4.954	5.587	5.591
5	4.942	5.892	4.824	4.299	4.201	3.851	3.767	4.469	5.784	5.553
6	4.670	4.707	4.852	4.845	4.722	4.294	5.019	5.319	5.821	4.953
$\bar{X}$=	4.876	5.116	4.752	4.326	4.522	4.162	4.468	4.762	5.729	5.702

Small Sample Statistic

Groups	Critical Ratio	(n)	Probability (Two Tailed Test)	Shift of the Post Mean from the Pre Mean	
				Increment	Decrement
I	2.410	12	.05	X	
II & III-A	7.798	10	.001		X
II & III-B	6.644	11	.001		X
IV	4.373	11	.01	X	
V	0.630	12	not significant		X

[a]The readings from the Pre-operative group represents the three days prior to the operation and are the mean based on the eighteen trials per rat. The readings for the Post-operative period represents the first nine days following the operation and are the mean based on fifty-four trials per rat. The values are expressed in Liters of Oxygen / Sq. Meter Surface Area / Hour.

[b]The first six animals are early groups and the second six are late groups, except in groups II & III-A and B. The first six are II and the second six are III.

TABLE 3

ANALYSIS OF THE DIFFERENCES BETWEEN THE DIFFERENCES OF THE CONTROL AND THE EXPERIMENTAL GROUPS[a]

Animals[b]	Pre-Operative Groups					Post-Operative Groups				
	I	II III-A	II III-B	IV	V	I	II III-A	II III-B	IV	V
1	4.251	3.861	3.661	3.767	5.040	4.611	3.491	3.662	4.469	5.130
2	4.613	4.350	4.074	3.773	5.065	5.312	4.058	3.702	4.274	5.587
3	4.621	4.616	4.183	3.966	5.208	4.719	4.049	3.988	4.083	5.754
4	4.622	4.625	4.201	4.386	5.479	4.830	4.789	3.851	4.545	5.502
5	4.645	4.626	4.275	4.420	5.587	4.900	4.119	4.056	4.954	5.591
6	4.735	4.824	4.360	4.531	5.784	5.213	4.299	4.268	4.895	5.553
7	4.760	4.852	4.592	4.659	5.809	4.708	4.845	4.221	5.390	6.008
8	4.942	4.854	4.722	4.822	5.880	5.892	4.148	4.294	4.966	5.867
9	5.052	5.230	4.872	4.878	5.821	4.760	4.568	4.351	4.818	4.953
10	5.177	5.313	4.986	5.019	5.965	4.942	4.610	4.513	5.319	5.616
11	5.211	. . .	5.879	5.060	6.430	5.478	. . .	4.950	5.174	6.586
12	5.878	. . .	. . .	. . .	6.680	6.132	. . .	. . .	. . .	6.287
$\bar{X}$=	4.876	4.752	4.522	4.468	5.729	5.116	4.326	4.162	4.762	5.702

Analysis of McNemar's Comparison of Change Statistic

Experimental group compared to control	Critical Ratio	N's	Probability (Two Tailed Test)	Experimental Group Increment	Experimental Group Decrement
II & III-A	4.270	10	.01		X
II & III-B	4.870	11	.001		X
IV	Less than one	11	Not significant	X	
V	5.950	12	.001		X

[a]The data for this analysis is the same as that in Table 2 except in this analysis the animals were rank-ordered on basis of Pre-operative exygen consumption (Liters of O_2 / Sq. M. Surface Area / Hr.) and then matched from control (Group I) to each experimental group.

[b]These numbers represent the animals in an ascending order of Pre-operative oxygen consumption.

TABLE 4

ANALYSIS OF THE PER CENT BODY WEIGHTS OF THE NORMAL THYROIDS

Animals	Groups					
	I	II & III-A	II & III-B	IV	V	VI
1	.497%	.457%	.384%	.585%	.408%	.684%
2	.484%	.553%	.484%	. . .	.508%	.355%
3	.439%	.551%	.384%	.524%	.443%	.342%
4	.483%	.446%	.535%	.393%	.466%	.452%
5	.503%	.448%	.383%	.441%	.449%	.354%
6	.622%	.542%	.457%	.500%	.434%	.458%
1	.588%	. . .	.497%	.410%	.442%	.352%
2	.385%	.400%	. . .	.607%	.458%	.373%
3	.615%	.523%	.492%	.523%	.301%	.299%
4	.472%	.442%	.315%	.478%	.480%	.341%
5	.550%	.420%	.498%	.473%	.520%	.336%
6	.654%	.388%	.466%	.500%	.534%	.385%
$\bar{X}$=	.524%	.470%	.445%	.494%	.454%	.394%

Analysis of Variance

Source	DF	SS	MS	F	Significance
Between Group Variance	(k-1)= 5	.1671	.02342	4.14	P <.01
Within Group Variance	(N-k)=63	.3559	.00565		
Total	(N-1)=68				

Groups	I	II & III-A	II & III-B	IV	V	VI	$F^{b,c}$ for N's of
I	. . .	.054[c]	.079[c]	.030[c]	.070[c]	.130[c]	11 .05=.020 .01=.026
II & III-A		. . .	.025[b]	.024[b]	.016	.076[c]	
II & III-B			. . .	.049[c]	.009	.051[c]	12 .05=.019 .01=.025
IV				. . .	.040[c]	.100[c]	
V					. . .	.060[c]	11 & 12 .05=.019 .01=.026

[a]The first six animals are early groups and the second six are late groups, except in groups II & III-A and B. The first six are II and the second six are III.

[b]P < .05 [c]P < .01

TABLE 5

ANALYSIS OF THE PER CENT BODY WEIGHTS OF THE NORMAL PITUITARIES[a]

Animal	Groups					
	I	II & III-A	II & III-B	IV	V	VI
1	.306%	.322%	.287%	.293%	.290%	.345%
2	.317%	.332%	.245%	. . .	.289%	.367%
3	.368%	.334%	.350%	.253%	.214%	.347%
4	. . . .	.261%	.257%	.282%	.199%	.368%
5	.315%	.312%	.236%	.243%	.264%	.354%
6	.279%	.325%	.307%	.231%	.295%	.361%
1	.303%	. . .	.322%	.280%	.245%	.340%
2	.273%	.268%	. . .	.270%	.358%	.355%
3	.304%	.253%	.287%	.278%	.311%	.293%
4	.324%	.324%	.251%	.228%	.246%	.324%
5	.306%	.256%	.282%	.254%	.241%	.282%
6	.332%	.241%	.276%	.337%	.262%	.285%
$\bar{X}$=	.312%	.293%	.282%	.268%	.268%	.335%

Analysis of Variance

Source	DF	SS	MS	F	Significance
Between Group Variance	(k-1)= 5	.0407	.00814	5.898	P <.01
Within Group Variance	(N-k)=62	.0731	.00118		
Total	(N-1)=67				

Groups	I	II & III-A	II & III-B	IV	V	VI	$F^{b,c}$ for N's of
I	. . .	.019	.030[b]	.044[c]	.044[c]	.023	11 .05=.029 .01=.038
II & III-A		. . .	.011	.025	.025	.043[c]	12 .05=.027 .01=.036
II & III-B			. . .	.014	.014	.053[c]	
IV				. . .	.000	.067[c]	11 & 12 .05=.028 .01=.037
V					. . .	.067[c]	

[a]The first six animals are early groups and the second six are late groups, except in groups II & III A and B. The first six are II and the second six are III.

[b]P <.05 [c]P <.01

Per Cent Body Weights of Thyroid Glands

The F test indicates that the per cent body weights of the thyroids of Group I were significantly higher than the other groups. Group VI had the lowest average and was significantly lower than all other groups. Group IV was second highest in thyroid per cent body weights and significantly greater than Groups II-A and III-A, V and VI (Table 4).

Per Cent Body Weights of Pituitary Glands

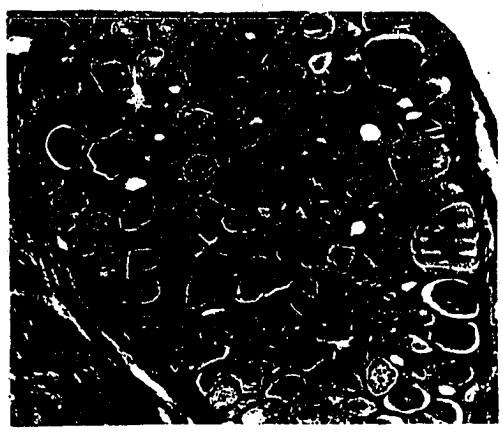
The per cent body weights of the pituitaries shows considerable variation from that of the thyroids. Group VI instead of being the lowest, was the highest, and Group I the second highest. Group VI was significantly higher than all groups, except Group I. Group I was significantly higher than Groups IV and V (Table 5).

Histological Observations

No recognizable pituitary homo-transplant tissue was recovered, hence a description of the tissues will concern only the thyroid homotransplants. To help illustrate the histological observations, six photographs were selected to show specific points or ideas and do not represent a random selection, nor do they represent all of the groups that received thyroid transplants (Fig. 9).

Free Thyroid Homotransplants

The only major observable difference among the transplants of the various groups was the amount of surviving tissue, which will be described later.



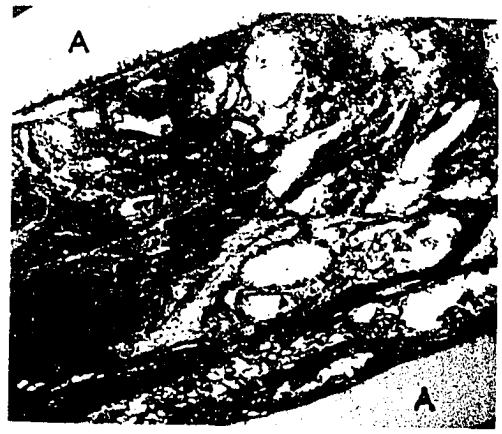
a (65x)



d (37x)



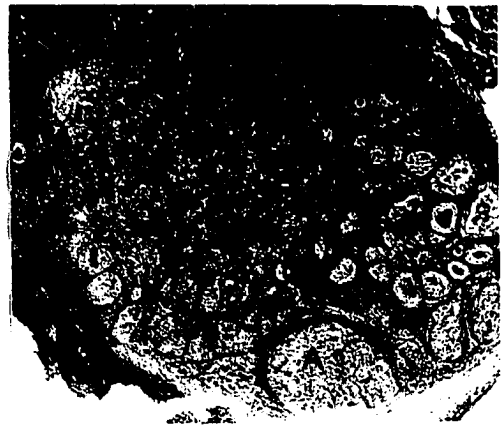
b (313x)



e (87x)



c (60x)



f (62x)

Fig. 9--Micro-photographs of the thyroid transplants.

If follicles were present, they appeared normal, with the lumen filled with colloid. The cells of the follicles appeared to be normal cuboidal parenchyma. In most of the transplants the surviving follicles were located on or near the periphery. Examples of these are shown in Figure 9a, b, c, and f. Figures 9a, b, and c were of tissues removed from animal A-6 of Group IV. The total length of the follicular transplant was 1.19 mm. Figures 9a and b represent a section 0.24 mm from one end of the thyroid lobe. Figure 9b is an enlargement of the enclosed area in Figure 9a. The follicles and cells of these thyroid transplants appear to be normal. Figure 9c was a section located 0.30 mm from the opposite end of the lobe. Sections in Figures 9a and c were separated by a distance of 0.65 mm.

Figure 9c shows the replacement of follicles by connective tissue. The area A is a large follicle and B is the parathyroid. The better developed follicles are near the periphery. Figure 9f is of tissue recovered from animal B-5 of Group VI. This is a good example of the replacement of thyroid follicles by connective tissue. A is the remnant of one of the parathyroid lobes. C is an isolated group of follicles near the edge of the transplant. B is the general area of connective tissue.

Encapsulated Tissue

The follicles of the tissue placed in the Millipore capsule were compressed into elongated oval shapes. The relationship of the transplant to the capsule are shown in Figures 9d and e. In Figure 9d, A marks the Millipore capsule and B indicates the parathyroid. In Figure 9e, A again marks the capsule and the oval spaces inside are the follicles. The parenchyma cells are not as thick as in normal thyroid.

In Group V, there were three encapsulated thyroids heavily infested with lymphocytes. The leucocytes entered through breaks in the capsule wall. These were animals A-1, A-6, and B-6. The first two had no follicles and the last had only a trace.

Analysis of the Tracings

The success or failure of a thyroid transplant is very difficult to evaluate and impossible to study statistically without some values such as the size of follicles, number of follicles, size of cell, or volume of the entire transplant. The tracing method was used to determine the absolute volume and the comparative volume in reference to normal thyroids.

TABLE 6

VOLUME OF THYROID TRANSPLANTS

Group	Mean Volume in Cubic Millimeters	Greatest Individual Volume in mm^3
II-A and III-A	0.120	0.368
IV	0.352	0.710
V	0.079	0.192
VI	0.162	0.375
5 normal rat thyroids	1.171	1.588

Group V had the least volume of the surviving thyroid transplants and also the least number of follicular transplants since only six had distinguishable follicles. Group IV had the highest average volume and the highest individual volume as well. The mean volume of Group IV is

approximately one-third of the mean volume of the normal thyroids and the greatest individual volume in Group IV was approximately one-half of the greatest individual volume of the normal thyroids (Table 6).

The analysis of variance of the weight of the paper tracings of the thyroid transplants indicate that Group IV was significantly higher than the other groups (Table 7). The data were not converted to volumes because it was believed that the fewer calculations made after the weights were established, the more accurate the values. Only transplants that had follicles at the time of autopsy were used in the analysis.

Tables Showing Daily Oxygen Consumption

Tables 8 through 14 give the daily mean oxygen consumption of all of the readings taken during the experiment. Each index represents five or, usually six, readings. Due to the longer periods of testing, A and B of Groups IV and V are placed on separate pages.

TABLE 7

ANALYSIS OF THE WEIGHTS IN GRAMS OF TRACINGS OF THE THYROID TRANSPLANTS[a]

Animal	Groups			
	II & III-A	IV	V	VI
1	0.8896	0.7954	0.0000	0.3446
2	0.0344	0.0000	0.0314	0.0452
3	0.2238	0.3772	0.4976	0.4395
4	0.0000	0.0000	0.1335	0.4234
5	0.1153	1.4156	0.0000	0.3591
6	0.0398	1.9605	0.0000	0.7101
1	0.0000	0.0816	0.0000	0.8712
2	0.2042	0.8397	0.5930	0.1544
3	0.1522	0.4800	0.0000	0.7643
4	0.2815	1.3594	0.0000	0.3462
5	1.1334	1.3398	0.1325	1.1564
6	0.6277	2.1873	0.0907	0.3678
$\bar{X}$=	0.3702	1.0836	0.2464	0.4785

Analysis of Variance

Source	DF	SS	MS	F	Significance
Between Group Variance	k-1= 3	3.7240	1.2413	6.458	P <.01
Within Group Variance	n-k=34	6.5417	0.1922		
Total	n-1=37	10.2657			

Groups	II & III-A	IV	V	VI
II & III-A	. . . .	0.7134[c]	0.1238	0.1283
IV		. . . .	0.3872[c]	0.5851[c]
VI			. . . .	0.2521

$F^{b,c}$ for n's of

10
.05=0.3840
.01=0.5054

10 & 12
.05=0.3677
.01= 0.4840

10 & 6
.05=0.4435
.01=0.5838

12 & 6
.05=0.4294
.01=0.5653

[a] The first six animals are early groups and the second six are late groups, except in groups II & III-A and B. The first six are II and the second six are III.

[b] P <.05 [c] P <.01

TABLE 8

AVERAGE DAILY READINGS FOR GROUP I[a]

Days before and after operation O=Day of operation	Animals					
	1-A	2-A	3-A	4-A	5-A	6-A
7	4.178	4.880	4.643	5.554	5.830	5.560
6	4.417	5.085	5.734	6.499	7.034	5.547
5	4.498	5.038	5.410	5.371	5.313	4.929
4	4.522	4.819	4.642	5.594	5.156	4.905
3	4.049	5.137	4.469	5.213	5.189	5.334
2	4.527	4.294	5.112	5.389	4.900	5.001
1	4.177	4.772	4.355	5.029	5.441	4.820
0	. . .	. . .	. . .	. . .	. . .	. . .
1	4.171	4.735	5.080	5.262	5.000	5.080
2	4.642	5.003	4.786	5.947	4.996	5.222
3	4.610	5.499	5.705	4.917	4.282	4.870
4	4.122	5.339	5.128	5.594	4.881	5.026
5	5.108	5.624	4.667	5.666	5.415	4.971
6	4.438	4.861	4.501	5.018	4.984	4.377
7	4.681	5.558	4.667	5.565	4.989	4.403
8	5.064	5.096	4.690	5.378	5.183	4.537
9	4.751	5.204	4.871	5.959	4.751	4.347
10	4.560	4.827	5.360	5.473	5.481	4.491
	1-B	2-B	3-B	4-B	5-B	6-B
7	5.568	5.838	4.453	6.914	6.256	5.197
6	4.973	4.367	4.968	7.315	6.725	5.794
5	4.334	4.998	4.185	7.265	6.023	4.959
4	4.527	4.284	4.260	6.293	5.541	4.102
3	4.581	4.304	4.162	6.505	5.464	4.405
2	4.509	4.498	4.540	5.466	5.631	5.195
1	4.751	5.064	5.162	5.662	5.207	4.613
0	. . .	. . .	. . .	. . .	. . .	. . .
1	4.543	4.433	5.078	5.655	5.633	4.378
2	4.938	5.105	5.218	6.368	6.984	4.950
3	4.766	4.936	4.981	6.418	5.995	5.341
4	5.734	5.007	5.239	6.665	5.930	4.702
5	5.080	5.324	4.801	6.175	4.825	4.505
6	6.790	4.767	4.028	6.171	5.757	4.362
7	5.102	4.654	4.380	6.582	5.728	4.512
8	5.657	4.604	4.856	5.959	6.030	4.972
9	4.312	4.645	3.875	5.185	5.148	4.650
10	4.485	4.664	4.303	5.149	4.832	4.620

[a]Each reading is a mean of six daily readings.

TABLE 9

AVERAGE DAILY READINGS FOR GROUPS II-A AND III-A[a]

Days before and after operation O=Day of operation	Group II					
	1-A	2-A	3-A	4-A	5-A	6-A
5	4.862	3.759	5.121	4.495	4.015	5.403
4	4.988	3.206	4.474	5.789	4.020	5.069
3	4.752	4.159	4.682	5.566	4.642	5.489
2	4.653	3.649	4.851	5.316	4.175	4.982
1	4.443	3.777	4.345	5.139	4.233	5.469
0	. . .	. . .	. . .	. . .	. . .	. . .
1	4.090	3.260	5.461	4.853	4.814	5.027
2	4.278	3.871	4.230	5.189	4.601	4.937
3	3.690	3.591	4.300	5.018	4.601	4.937
4	4.063	3.390	4.543	5.160	3.983	4.877
5	4.624	3.750	4.157	4.524	4.240	4.696
6	3.900	3.010	3.715	4.204	3.460	4.207
7	3.818	3.862	3.462	4.472	3.910	4.617
8	3.867	3.526	3.627	4.344	3.707	4.127
9	4.102	3.158	3.578	5.344	3.760	4.194
10	3.772	3.822	3.940	5.063	3.567	3.795
11	4.420	3.859	3.683	4.770	3.807	4.503
12	4.144	3.076	4.773	5.520	3.595	3.893
13	5.825	3.369	3.685	4.950	4.037	4.284
14	4.309	3.879	4.660	4.563	3.567	3.928
	Group III					
	1-A	2-A	3-A	4-A	5-A	6-A
6	died	4.912	not	5.931	5.395	4.821
5	. . .	4.117	used	6.050	4.676	4.170
4	. . .	4.013	. . .	6.009	5.013	4.754
3	. . .	4.710	. . .	5.252	5.472	5.207
2	. . .	4.728	. . .	4.976	4.648	4.672
1	. . .	5.126	. . .	5.463	4.363	4.676
0	. . .	. . .	. . .	. . .	. . .	. . .
1	. . .	4.038	. . .	4.655	4.582	4.537
2	. . .	4.000	. . .	4.843	4.385	4.627
3	. . .	4.194	. . .	4.464	4.187	4.748
4	. . .	3.894	. . .	4.898	4.400	5.080
5	. . .	4.057	. . .	4.681	4.656	5.261
6	. . .	4.398	. . .	4.401	3.966	4.671
7	. . .	4.448	. . .	4.219	3.770	5.024
8	. . .	3.743	. . .	4.380	4.308	4.766
9	. . .	4.560	. . .	4.566	4.438	4.897

[a]Each reading is a mean of six daily readings.

TABLE 10

AVERAGE DAILY READINGS FOR GROUPS II-B AND III-B[a]

Days before and after operation. 0=Day of operation	Group II					
	1-B	2-B	3-B	4-B	5-B	6-B
5	3.674	3.677	4.178	4.985	4.926	4.035
4	3.768	3.880	4.712	5.713	4.890	3.962
3	3.926	3.791	4.314	5.678	4.822	4.351
2	4.078	3.649	4.687	6.057	5.107	4.085
1	4.218	3.543	4.773	5.902	5.028	4.645
0	. . .	. . .	. . .	. . .	. . .	. . .
1	3.977	3.359	4.354	5.112	4.739	4.238
2	3.865	4.617	4.601	4.872	4.666	4.340
3	3.285	3.159	4.104	4.584	4.134	4.225
4	3.718	3.882	4.429	5.269	4.042	4.613
5	3.614	3.431	4.070	4.965	5.189	4.445
6	3.528	3.602	4.349	5.004	4.350	4.184
7	3.720	3.549	3.992	4.635	4.622	4.140
8	3.936	3.575	4.070	5.235	4.397	5.292
9	3.678	3.792	4.019	4.867	4.478	3.937
10	3.131	3.862	4.054	4.553	4.376	3.581
11	3.930	3.637	3.756	4.438	5.018	3.833
12	4.044	3.693	3.644	5.104	4.908	3.784
13	3.975	3.879	4.150	5.391	4.739	4.264
14	4.405	3.982	4.334	5.007	4.664	3.778
	Group III					
	1-B	2-B	3-B	4-B	5-B	6-B
6	4.202	died	3.908	4.080	4.270	5.530
5	4.485	. . .	3.870	4.575	3.976	5.587
4	4.639	. . .	4.896	5.006	4.920	5.405
3	4.794	. . .	4.443	4.375	4.518	4.283
2	5.211	. . .	4.410	4.178	3.869	5.201
1	4.612	. . .	3.971	3.998	4.214	4.682
0	. . .	. . .	. . .	. . .	. . .	. . .
1	3.901	. . .	3.892	3.546	3.384	4.032
2	4.201	. . .	3.908	4.092	3.569	4.459
3	3.920	. . .	4.880	3.510	4.119	4.644
4	4.614	. . .	3.699	4.353	3.636	4.555
5	4.279	. . .	3.880	3.916	3.223	4.133
6	4.426	. . .	4.477	3.659	3.248	4.663
7	3.903	. . .	3.769	4.643	5.163	4.332
8	6.184	. . .	4.408	3.773	4.911	4.196
9	3.724	. . .	3.587	4.409	3.403	3.631

[a]Each reading is a mean of six daily readings.

TABLE 11

AVERAGE DAILY READINGS FOR GROUP IV-A[a]

Days before and after operation. 0=Day of operation	Animals					
	1-A	2-A	3-A	4-A	5-A	6-A
10	5.184		6.025	5.071	5.604	5.461
9	4.814		4.575	4.938	6.280	4.935
8	5.496		5.070	4.829	5.051	5.254
7	4.063		4.535	5.152	4.500	5.057
6	5.171		4.279	5.742	5.123	5.386
5	4.422		3.758	4.690	5.149	5.239
4	5.214		3.807	3.861	4.400	4.834
3	5.370		3.750	3.848	4.145	4.978
2	3.761		3.490	3.714	4.170	4.728
1	4.845		4.078	4.336	4.843	4.926
0	. . .		. . .	. . .	. . .	. . .
1	5.430		3.600	4.363	5.268	4.826
2	5.975		3.987	4.478	4.570	4.558
3	5.671		3.945	4.375	4.778	4.803
4	5.563		5.039	4.199	4.495	4.360
5	5.982		4.008	3.874	4.760	5.436
6	5.163		4.640	3.937	4.232	4.747
7	4.676		4.776	4.251	4.402	4.593
8	5.239		4.345	3.662	4.096	5.218
9	4.812		4.125	3.610	4.209	4.821
10	4.817		4.314	3.620	4.551	4.886
11	4.768		4.538	3.935	4.533	4.652
12	4.526		4.409	3.494	4.948	5.124
13	5.094		4.701	3.258	4.736	4.771
14	5.622		5.305	5.059	5.515	5.681

[a]Each reading is a mean of six daily readings.

TABLE 12

AVERAGE DAILY READINGS FOR GROUPS IV-B[a]

Days before and after operation O=Day of operation	Animals					
	1-B	2-B	3-B	4-B	5-B	6-B
9	5.407	4.943	5.474	4.721	4.454	5.987
8	5.731	5.328	5.206	5.219	4.467	5.548
7	5.264	5.945	4.267	4.242	3.879	4.590
6	4.769	4.786	4.926	4.897	4.732	4.766
5	4.704	4.475	4.974	4.640	4.060	4.994
4	5.460	4.472	4.162	4.643	3.946	4.892
3	5.374	4.871	5.416	4.494	3.769	5.348
2	5.207	4.763	4.612	4.501	3.887	5.166
1	4.596	3.961	4.462	4.264	3.643	4.540
0	. . .	. . .	. . .	. . .	. . .	. . .
1	5.255	4.334	4.358	4.603	4.424	5.573
2	5.023	4.392	5.045	4.835	4.159	5.422
3	4.703	5.400	4.753	4.974	4.235	5.455
4	4.737	4.344	5.227	4.668	4.651	4.770
5	5.380	5.379	4.810	4.887	4.659	5.420
6	5.356	5.112	5.186	5.911	4.664	5.134
7	5.364	5.306	5.158	5.250	4.419	4.936
8	5.396	5.119	5.153	4.600	4.402	5.337
9	5.358	4.670	5.012	4.862	4.594	5.803
10	5.485	6.188	5.586	4.859	4.216	5.253
11	5.212	4.904	5.187	5.134	4.433	5.546
12	5.024	4.837	5.343	5.382	4.344	5.407
13	5.925	4.837	5.132	4.924	4.124	4.836
14	5.916	5.891	6.758	4.853	4.619	5.266

[a]Each reading is a mean of six daily readings.

TABLE 13

AVERAGE DAILY READING FOR GROUP V-A[a]

Days before and after operation O=Day of operation	Animals					
	1-A	2-A	3-A	4-A	5-A	6-A
15	4.071	5.057	5.051	4.686	5.137	4.620
14	4.844	4.987	5.442	5.389	5.926	5.602
13	5.216	5.700	5.879	4.965	6.180	5.950
12	5.353	5.779	5.550	5.162	6.231	5.820
11	5.416	5.522	6.285	5.123	5.462	6.096
10	5.422	6.182	5.980	6.060	6.156	6.329
9	4.659	5.712	5.123	5.195	5.552	6.386
8	5.721	6.223	5.812	6.232	6.772	6.882
7	5.631	6.064	6.341	5.825	6.457	5.980
6	5.433	5.666	5.872	5.276	6.200	6.425
5	5.316	6.025	5.546	5.587	6.540	6.352
4	4.794	5.619	5.491	4.952	5.272	5.769
3	5.195	5.557	6.005	4.872	4.899	5.837
2	5.682	6.132	6.004	5.090	4.953	6.024
1	5.558	5.950	5.888	5.235	5.773	5.568
0	. . .	. . .	. . .	. . .	. . .	. . .
1	5.574	6.340	5.279	5.823	5.498	6.097
2	5.809	5.630	5.418	6.037	6.832	6.925
3	5.655	5.828	5.789	6.056	4.872	6.015
4	5.634	5.838	6.170	5.611	6.186	6.079
5	5.176	5.529	5.355	5.107	5.649	5.814
6	5.405	5.999	5.416	5.491	5.875	5.948
7	5.312	5.998	6.056	5.204	5.618	6.054
8	5.338	5.724	5.788	5.474	5.053	6.224
9	5.614	5.920	5.279	5.479	5.204	5.815
10	5.398	5.929	5.665	5.766	5.486	6.282

[a]Each reading is a mean of six daily readings.

TABLE 14

AVERAGE DAILY READING FOR GROUP V-B[a]

Days before and after Operation O=Day of Operation	Animals					
	1-B	2-B	3-B	4-B	5-B	6-B
15	5.040	5.829	4.751	5.320	5.467	4.695
14	5.687	6.204	5.039	5.687	5.590	4.758
13	6.391	6.041	6.706	5.730	5.708	6.460
12	6.173	6.811	5.012	5.675	5.678	5.725
11	5.761	7.065	5.696	5.766	6.128	6.149
10	5.390	6.221	5.816	6.072	5.873	5.307
9	5.725	6.275	5.811	5.845	5.344	5.982
8	5.548	5.989	6.609	6.504	5.830	5.625
7	5.665	6.711	7.018	5.788	6.186	5.420
6	5.256	6.243	6.198	5.770	6.028	5.539
5	5.236	6.441	6.125	5.734	5.932	5.274
4	5.312	6.355	6.410	5.486	5.329	5.158
3	4.956	5.959	6.207	5.534	5.689	5.800
2	5.026	6.430	7.301	5.615	5.273	5.546
1	5.139	6.899	6.523	5.611	6.394	6.117
0	. . .	. . .	. . .	. . .	. . .	. . .
1	5.414	6.050	5.740	5.439	5.274	4.965
2	5.498	6.448	6.015	5.707	6.806	5.151
3	5.202	6.046	6.323	5.689	5.439	5.062
4	5.114	6.643	6.255	5.575	5.763	4.979
5	4.943	5.719	5.802	5.564	5.432	4.989
6	4.682	7.073	6.315	5.644	5.630	4.742
7	5.236	6.080	6.389	5.295	5.830	4.873
8	4.746	5.976	6.213	5.554	5.140	4.981
9	5.334	7.349	6.525	5.858	5.800	4.835
10	5.106	7.491	6.278	5.500	5.335	5.055

[a]Each reading is a mean of six daily readings.

CHAPTER IV

DISCUSSION

The reduction of the oxygen consumption by the transplantation of a single pituitary or thyroid gland could be interpreted as due to the formation of antibodies. If this be true, the antibody production and its reflection in the metabolic rate is more rapid than past work has indicated.

Uhlenhuth, in 1903, is reported to have recognized the antigenic properties of an organ rather than of the species. He demonstrated the presence of an organ specific antigen in the lens of the eye. (Witebsky, Rose and Shulman, 1955). Fleisher, Hall, and Arnstein (1920), Fleisher and Arnstein (1921) and Fleisher (1922) discuss three categories of antigens in animals: (1) antigens present in all organs of a species; (2) antigens characteristic of specific organs and not of species; and (3) antigens common to several different organs.

Aron (1930) observed that the normal hyperthyroid condition produced by the injection of pituitary extracts may revert to hypothyroidism following prolonged injections of the extract. Loeb and Friedman (1931) found that following long-term treatment with pituitary extracts the thyroids of guinea pigs failed to respond. Collip and Anderson (1937) injected thyrotropic hormone and increased the metabolic rate the first week to a plus 28 per cent. The second and third week the rate had dropped to normal and by the fifth week the metabolic rate was minus 29

per cent or the level of the hypophysectomized rat. This was believed to be due to the formation of an anti-thyrotropic substance.

Lerman (1942) reported mammalian thyroglobulin to be strongly antigenic and the antibodies were organ-specific. The continued injection of thyroglobulin increased the metabolic rate to a plus 47 per cent the first week, followed by a gradual decline to a plus 20 per cent the third week and after omission of injection the metabolic rate dropped to a minus 31 per cent.

Witebsky and Rose (1956) reported rabbits producing antibodies against extracts of their own thyroids as well as extracts of thyroids of other rabbits. Anigstein, Eklund, and Whitney (1957), after injecting rats with 0.5 ml of anti-rat-thyroid serum for three days a week for two weeks reported a lowering of oxygen consumption in eight days. Witebsky et al. (1957) and Witebsky et al. (1957-58) reported thyroid antibodies against human thyroid extracts in chronic thyroiditis in man. Terplan et al. (1960) demonstrated experimental thyroiditis in rabbits, dogs and guinea pigs following immunization with auto- and hetrologous thyroid extracts. Ehrich and Harris (1942) detected antibodies in the efferent popliteal lymph two days after subcutaneous injections of typhoid vaccine, sheep erythrocytes, and egg albumen into the feet of rabbits. McKenna and Stevens (1957) injected rabbits with bovine serum albumen and detected high titers of antibodies in the spleen after twelve hours. These antibodies were not detected in the serum or blood this early. Crowder (1956) found that the oxygen consumption of rats was lowered within 48 hours after the transplantation of a lobe of homothyroid into each vitreous chamber. These findings support the rapid production of antibodies as postulated in this study.

Lawrence (1957-58) compared the antigen-antibody reaction of the tuberculin-type allergy to the destruction of homotransplants. Two of these similarities may be pertinent to this phase of the problem: (1) there seems to be no parallel between the degree of sensitivity and concentration of serum antibodies; and (2) transfer of sensitivity from one animal to another can be accomplished by the transfer of cells and not serum. Possibly this could also apply to inhibition of the physiology of endocrine glands as well as the destruction of the homotransplants. It may be that the inhibition can be initiated by an antibody titer too low to detect, or possibly some substance other than antibodies.

The duration of survival and pattern of destruction of thyroid homografts are well established and historically related to the study of organ specific antibodies. These studies are now believed to be closely related.

Manley and Marine (1916) found that thyroid homotransplants in rabbits were usually destroyed between 10 and 30 days. Loeb (1930) reported that similar transplants lasted about 30 days in guinea pigs. Crowder (1956) found no trace of follicles in thyroid homotransplants in rats after 20 days. Loeb (1930) and Woodruff and Woodruff (1950) have described the pattern of destruction of thyroid homotransplants. A necrosis in the center of the transplant occurs during the first three days following transplantation leaving viable tissue only around the periphery. New cells and follicles begin to develop about the third day. Marine and Rosen (1934) reported the destructive processes against thyroid homografts begin about the eighth day. All of the thyroid transplants in my study were recovered within the destruction period. It was

obvious that most of the follicles were on the periphery and the remaining portion of the transplant was composed of connective tissue, with a large number of lymphocytes.

The relationship between the lymphocytes, destruction of homotransplants, and antibodies is not clearly understood. The destruction of transplanted sarcoma by lymphocytes was proposed by Wade (1908). Murphy (1914), Murphy and Morton (1915), Murphy and Taylor (1918), Murphy and Sturm (1919), and Loeb (1930) demonstrated the role of lymphocytes as the destroyer of homografts. That the formation of antibodies, or acquired immunity, is also involved in the destruction of transplants was indicated by Gibson and Medawar (1943) when they reported that second skin transplants were destroyed more rapidly than the first. Ehrich and Harris (1945), Mitchison and Dube (1955) and Scothorne and McGregor (1955) demonstrated that the primary site of antibody formation is the lymph nodes and the secondary site the spleen, indicating a close relationship between lymphocytes and antibodies. Medawar (1953) stated the problem of this relationship in this way:

> As Loeb and Murphy have long since insisted, the infiltration of tissue homografts by lymphocytes is a regular accompaniment to their breakdown. What are they doing? Are they seats of hypothetical homotoxins, or the vehicles by means of which antibodies are brought to the neighborhood? Or is their presence merely a by-product of the general inflammatory changes that accompany tissue destruction?

Merwin and Hill (1954) using subcutaneous transplants and Prehn, Weaver, and Algire (1954) using diffusion chambers demonstrated that the host did not produce an immunity against a homotransplant unless the leucocytes of the host made contact with the transplant. Prehn, Weaver, and Algire (1954) also demonstrated that tissues in diffusion chambers

could survive in both non-immune and immune homologous hosts. Weaver, Algire, and Prehn (1955) showed that tissues protected by "cell-impenetrable" chambers survived in homologous hosts but were destroyed if in a "cell-penetrable" chamber. This seems to indicate that the homograft destruction is produced by the host's cells and not by circulating antibodies since it was demonstrated that antibodies could pass through the pores of the "cell-impenetrable" chambers. It was believed that the antibodies were transported by the lymphocytes. Medawar (1959) in regard to homograft destruction states, "The homograft reaction belongs to that demimonde of immunologic responses in which "classical" antibodies seem to play no necessary part."

The effect of the encapsulated thyroid on the oxygen consumption may be explained by the lack of formation of antibodies. Prehn, Weaver, and Algire (1954) have shown that if the homologous tissue is protected from the cells of the host, it does not produce an immunity. In most cases the encapsulated thyroid did not permit the entrance of host cells, and therefore prevented the formation of antibodies, and consequently no reduction in oxygen consumption. If this proposal is correct, it seems that there is a close relationship between the production of a substance that causes a reduction in oxygen consumption and the production of the immunity against the homograft.

The results of the transplantation of both thyroid and pituitary into each individual show an increase in the oxygen consumption and an increase in the volume of surviving thyroid transplant. Why does the combination of the bi-ocular transplants reverse the effect on oxygen consumption of either of the glands transplanted separately?

Martini et al. (1959), Khazin and Reichlin (1961) and Knigge (1961) have reported the production of Thyroid Stimulating Hormone (TSH) from pituitary transplants. The increase in the metabolic rate of the bi-ocular group could be due to such an increase of TSH acting on both the intact and transplanted thyroids. The metabolic rate increased within 24 hours following the transplantation. This is compatible with the findings of Smith and Brown (1952) and Barker and Klitgaard (1952) who reported an increase in oxygen consumption in rats within 24 hours subsequent to the injection of thyroid extracts. That the increase in the bi-ocular group is noted within a similar time period suggests that it may be in response to elevated thyroid hormone levels caused by increased TSH levels.

The apparent reduction in the destruction rate of the thyroid transplant in the bi-ocular group probably was not due to the increased TSH. Woodruff (1954) and Dempster and Doniach (1955) could find no effect of thyrotropin on the rate of destruction of thyroid homografts. It would be more likely to have been an increase in adrenocorticotropic hormone (ACTH) acting on the intact adrenal cortex. Silberberg (1934) found that extracts of anterior pituitary aided in the survival of thyroid homotransplants. Turner (1939) was able to prolong the survival of homo-adrenal cortex transplants by injecting sliced pituitaries into the same eye with the transplant.

Billingham et al. (1951) used cortisone to prolong the life of skin homografts of rabbits. Toolan (1954a, b) used cortisone to help maintain human neoplasms in rats. Woodruff (1954) reported cortisone increased survival time of thyroid homografts in guinea pigs. Medawar and Sparrow (1956) reported that hydrocortisone and cortisone were the

only two corticoids effective in increasing the longevity of skin homografts in rabbits and mice. The method or methods by which the corticoids inhibit host reaction are not understood, but Heller (1955) demonstrated that cortisone appears to hinder the ability of the reticuloendothelial cells to phagocytize and regenerate.

The group receiving hydrocortisone (Group VI) was used only to determine the effect of the compound on the survival of the transplant and oxygen consumption was not taken. There was slightly more surviving thyroid tissue in Group VI than in the groups receiving only thyroid. It would appear that the presence of the pituitary transplant in addition to the thyroid transplant had more effect on the thyroid transplant than did the injections of 2 mg of hydrocortisone every other day until the total of 8 mg was given. The volume of surviving thyroid transplant was significantly greater in Group IV than in either Group II-A and III-A or Group VI.

No satisfactory explanation is presented concerning the per cent body weight of the thyroid and pituitaries. The addition of either thyroid or pituitary appears to affect the weight of the normal (in situ) thyroid. The group that received the muscle transplant (Group I) had the thyroids with the largest per cent body weight. This could be due to the absence of specific thyroid or pituitary antibodies or an increase in the hormonal level due to the transplant which could inhibit the thyroid (in situ).

The per cent body weight of the pituitary does not follow the pattern of the thyroid per cent body weight. It probably should be noted that Group I was the second highest in per cent body weight of the

pituitaries. This may indicate that both the thyroid and pituitary per cent body weights are greater if there are no thyroid or pituitary antibodies present in the organism.

The actual mechanism by which the formation of antibodies act against the thyroid or other endocrine glands is not known, but Rose and Witebsky (1956) demonstrated a histological change in the thyroid (in situ) following injections of anti-thyroid serum. Cellular elements had partially replaced some of the thyroid tissue. Possibly the presence of anti-thyroid or pituitary antibodies would cause the replacement of the normal glandular tissue with connective tissue that may be lighter in weight.

The failure to recover pituitary tissue from any of the pituitary homotransplants makes it appear that pituitary homografts are destroyed more rapidly, when placed in contact with the circulatory system in non-hypophysectomized hosts, than thyroid homografts. There are many cases where pituitary transplants have been recovered when transplanted into protected areas or hypophysectomized animals as discussed by Medawar (1959). Knigge (1961) also reports that the use of either chloroform or ether to sacrifice the donor had some deleterious effect upon the survival of pituitary transplants. Perhaps the combination of site, non-hypophysectomized host, lack of inbred strain, and use of ether to sacrifice the donors could account for the total destruction of the pituitary transplant.

The oxygen consumption of two groups showed a strong homeostasis. Group I (muscle) had a slight increase in oxygen consumption following the transplantation and a return to normal before the termination of the

experiment. This increase may have been due to activity of white cells against the transplant. The group receiving the pituitary transplant showed a return to normal following the reduction. Medawar (1959) described a very brief period of immunity following the injection of soft tissue such as dissociated homologous spleen cells. The reduction in oxygen consumption could be due to the presence of pituitary specific antibodies but a rapid decline in the antibodies following total destruction of the transplant may have permitted the normal oxygen consumption.

It appears that the presence of thyroid transplants can affect the oxygen consumption of the rat in two major ways: (1) stimulation of the metabolic rate through the action of thyroid hormone; and (2) inhibition of metabolic activity through the formation of thyroid antibodies. A third possible action that may be involved is the pituitary-thyroid-transplant axis, which returns the organism to its normal level.

The hypothesis presented here involves the relationship between hormones and antibodies of the thyroid and pituitary glands. If an intact thyroid or pituitary homograft is placed in contact with the circulatory system, the production of antibodies begins soon after transplantation, but the circulating hormone of that particular gland is not increased since its release seems to be controlled humorally. The antibodies are able to express themselves within 24 hours as in Groups II-A and III-A (thyroid) and II-B and III-B (pituitary). If, however, extracts of these glands are injected, the blood level of the respective hormones goes up with a consequent rise in metabolic rate. This rise would occur even in the presence of antibodies since the anti-thyrotropic substance does not seem to antagonize the action of thyroxine (Anderson and Collip,

1934b). The prolonged injection of extracts of thyroid or pituitary brings about a lowering of the metabolic rate, probably due to the inhibition of the thyroid or pituitary (in situ), thus lowering the blood level of their hormones.

If the transplant is protected from the leucocytes by the methylcellulose capsule, or white cell activity hindered by increased amounts of adrenal corticoids, the transplant is free to express itself as an endocrine gland. The absence of a decrease in the metabolic rate of Group IV (thyroid plus pituitary) could be due to the production of ACTH that retards the production of antibodies through its effect on the adrenal cortex and its production of cortisone and hydrocortisone. The increase in oxygen consumption could be due to the production of TSH by the pituitary homotransplant.

CHAPTER V

SUMMARY

Six groups of 12 male rats had homotransplants placed into the vitreous chamber of the eye. A group received one of the following transplants: skeletal muscle (gluteus maximus); entire thyroid lobe; entire anterior pituitary; both a lobe of thyroid and of pituitary; thyroid lobe encapsulated in Millipore filter; or a thyroid lobe transplant plus 8 mg of hydrocortisone administered subcutaneously. Females of the same age and strain were used as donors. The muscle was used to control the effects of the operation. Encapsulation was employed to determine if the effects of grafting differed when the thyroid was protected from leucocytes. Oxygen consumption was measured in a battery of six closed circuit respirometers before and after the transplantation to determine the effect of the transplants. Five of the groups were measured in this manner while the sixth received hydrocortisone and no oxygen measurements were made. The animals were autopsied 9 to 14 days following the operation and the volume of the thyroid transplants was determined by the paper weight method. The per cent body weight of the normal thyroid and pituitary glands of the hosts was determined.

It may be concluded that:

1. Homotransplants of muscle produce a slight increase in oxygen consumption, but this returns to normal in less than 10 days.
2. Either thyroid or pituitary homotransplants produce a reduction in oxygen consumption. This is interpreted to be due to the

formation of antibodies acting against the respective endocrine glands and reducing the production or the release of the hormone.

3. If leucocytes are permitted contact with thyroid or pituitary homotransplants, organ specific antibodies are apparently formed within 24 hours.
4. If thyroid homotransplants are protected from the leucocytes of the host, there is no significant change in oxygen consumption.
5. A combination of thyroid and pituitary homotransplants produces a significant increase in oxygen consumption. It is believed that the production of ACTH by the transplanted pituitary inhibits the production of antibodies, and the increased production of TSH by the transplanted pituitary causes an increase in oxygen consumption.
6. The immunity produced by pituitary homotransplants exists only a short time following the destruction of the transplant.
7. Under the circumstances found in this study, muscle and pituitary homotransplants are destroyed within 10 days while thyroid homotransplants survive up to 14 days.
8. Pituitary homotransplants produce something that aids in the survival of thyroid homotransplants. This substance, probably ACTH, is more effective in maintaining thyroid homotransplants than is 8 mg of hydrocortisone.
9. The weight of the thyroid (in situ) is greater in rats receiving muscle tissue than those receiving thyroid or pituitary. The thyroid or pituitary antibodies probably cause the reduction in weight.

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