DIFFERENCES IN THYROID ACTIVITY OF SEVERAL STRAINS OF INBRED AND F_1 HYBRID MICE

by

ABOLGHASSEM AMIN

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology and Pharmacology

Year 1956

Approved______________________________________

ABOLGHASSEM AMIN ABSTRACT

Strain differences in thyroid function were studied in A/Jax, BALB/c, C57BR/cd, C57BL/6 mice and their F_1 hybrids, CAF_1 (BALB/c ♀ x A/Jax ♂) and BBF_1 (C57BL/6 ♀ x C57BR/cd ♂). These strains of mice were provided from the Jackson Memorial Laboratory and were produced by brother x sister matings for more than 20 generations and are, therefore, considered to be quite homogeneous.

Radioiodine uptake, its turn-over rate and the thyroid secretion, which involves the determination of the amount of l-thyroxine required to prevent output of iodine from the thyroid of individual mice previously given 5 microcuries of I-131, were estimated.

Statistically significant differences between sexes in either of the parameters measured, except in C57BR/cd strain, were not found. The average per cent I-131 uptake and per cent output, respectively, for the various genotypes were: A/Jax 16.1, 15.3; BALB/c 17.4, 18.0; CAF_1 19.4, 13.8; C57BR/cd 10.4, 26.0; C57BL/6 7.9, 34.1; and BBF_1 10.4, 32.3. Per cent uptake was significantly higher and output rate was significantly lower in the first three groups than in the others. The thyroid secretion values were 2.09, 1.77; 2.56, 1.76; 2.07, 1.65; 3.67, 3.04; 3.97 and 2.85 for the males and females of strains A/Jax, CAF_1, BALB/c, C57BR/cd, BBF_1 and C57BL/6, respectively.

A reciprocal relationship was observed between the per cent uptake and the output rate. The results clearly show the strain differences among the strains studied.

Although these data do not indicate the number of genetic factors involved in control of thyroid secretion level, they provide strong evidence that it is a heritable character.

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ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. E. P. Reineke for his stimulating guidance and helpful criticism throughout the course of this investigation.

Special thanks are due to Dr. C. K. Chai, Jackson Memorial Laboratories, Bar Harbor, Maine, for his active collaboration in the research.

The aid of Dr. William D. Baten in the statistical analysis of these data is gratefully acknowledged.

The author wishes to express his gratitude to Mr. Jack Monroe and Mr. Howard Hardy for their help and to Dr. Ellen J. Monkus for her technical assistance.

Thanks are due to Dr. B. V. Alfredson for the use of the facilities of the Department of Physiology and Pharmacology and to the Michigan Agricultural Experimental Station for support of the project under which this work was done.

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REVIEW OF LITERATURE

Iodine was discovered in the thyroid gland in 1896 by Baumann (1), and the ability of the gland to absorb iodine selectively from the blood in relatively large amounts was demonstrated in 1915 and 1916 by Marine and his associates (2,3,4,5). Since then the importance of iodine in thyroid metabolism has been amply demonstrated.

The purely chemical investigation of iodine and its compounds in the thyroid has yielded outstanding results, culminating in the isolation (6), chemical identification and synthesis of the active principles of the thyroid gland, namely, thyroxine by Harrington and his coworkers (7,8,9) and triiodothyronine by Gross and Pitt-Rivers (10, 11,12).

Physiologically, the relationship which exists between the thyroid and the anterior lobe of the hypophysis (13,14) plays a major role upon thyroid function. The anterior pituitary secretes a hormone called thyrotropin or the thyrotropic principle (13), which regulates iodine trapping (15,16,17,18,19,20,21), its organic binding and its release into the circulation (22).

These different phases of the iodine cycle are interdependent, but can be classified into: 1) iodine trapping,

2) incorporation of iodine into organic binding, and 3) release of the thyroid hormone.

Before goitrogenic compounds were discovered, thyroidal activity was measured by such methods as measurement of oxygen-carbon dioxide exchange (23,24,25), rate of metamorphosis in tadpoles, or by chemical methods such as the estimation of the protein-bound iodine in the thyroid gland or the blood plasma (26,27,28,29).

When goitrogenic compounds became known, another technique for measuring the thyroid secretion was added to those already present. Goitrogens inhibit the formation of the thyroid hormone, which permits increased secretion of TSH by the pituitary; this in turn stimulates the thyroid and causes hypertrophy and hyperplasia of the gland (30,31, 32,33). Enlargement of the thyroid can be prevented by the administration of thyroxine in sufficient amounts to bring the pituitary and thyroid back into normal balance.

Dempsey and Astwood (34) observed that the decrease in thyroid weight in thiouracil-treated rats given thyroxine bore a quantitative relation to thyroxine dosage. The relationship between thyroid weight and thyroxine dosage is a straight line. The point where the response curve intercepts the normal thyroid weight represents the amount of thyroxine required to maintain the normal thyroid pituitary balance.

In recent years the thyroid secretion rate has been measured in several species of animals. Of special interest are the findings by Hurst et al. (35) that three strains of mice showed differences in thyroid secretion rate. They demonstrated that Schwing female mice had the highest thyroid secretion rate, whereas Rockland and C_3H females had almost the same secretion rate. Mixner et al. (36), Schultze and Turner (37) and Mixner and Upp (38) observed differences in the thyroid secretion rates of several strains of chickens.

With the discovery of artificial radioactivity by Joliot and Curie (39), the building of the cyclotron (40, 41) and the development of the uranium chain reacting pile (42), a valuable tool became available to biologists.

In 1937, a cooperative investigation for the study of thyroid physiology with radioactive isotopes of iodine was started (43). Since then the isotopes of iodine, and especially I-131 with 8-day half-life, have been used in the study of thyroid gland physiology.

Shortly after radioactive iodine is administered it is trapped by the thyroid gland, and incorporated into organic binding in the same way as stable iodine. The amount of iodine which is collected by the thyroid gland at a certain time after its administration is called iodine uptake. Thyroid uptake is one expression of the gland's capacity to manufacture its hormone. The thyroid uptake is controlled at least in part by the anterior pituitary (44,45). One should bear in mind, however, that the I-131 uptake is of value on a comparative basis only when all other factors such as the amount of iodine intake in food, age and environmental temperature remain the same.

After the maximum uptake is reached, which usually depends on the species of animal, I-131 concentration in the thyroid declines exponentially. The decrease in radioactivity of the gland is due to the release of iodine labeled hormone. This interpretation is justified by the findings *in vitro* (46,47) and also by the fact that almost all of the organically bound iodine which is delivered to the blood circulation is thyroxine and triiodothyronine (48,49). The decrease in I-131 in the thyroid may, therefore, be considered to reflect secretion of I-131 labeled hormone (50). The conversion of monoiodotyrosine or diiodotyrosine I-131 to thyroxine I-131 and triiodothyronine I-131 would not influence the results obtained by external counting. One factor that could be of importance is the loss of inorganic I-131 from the gland. This is known to occur as a result of exchange with I-127. It must be pointed out, however, that 40 hours after the injection of a tracer dose of radioactive iodine, almost all the thyroid I-131 is in organic (non-exchangeable) combination (49).

The loss of the radioactivity from the thyroid gland can be based on the per cent output per unit of time or on its biological half-life which is the time required for loss of 50 per cent of the radioactivity from the thyroid gland. Corrections must be made for the physical decay of radio-iodine. This method, however, does not give a quantitative estimation of the thyroid secretion rate, and it is of value only on a comparative basis.

Perry (51) in 1951 reported that when rats are given I-131 and different groups are then given graded doses of thyroxine, inhibition of thyroidal I-131 output during a 48-hour period is proportional to the dosage of thyroxine administered. He proposed this as a thyroid assay method. Wolff (50) used a technique similar to Perry's in studying some factors that influence the release of iodine from the thyroid gland.

Reineke and Singh (52) and Henneman _et al_. (53) modified Perry's technique. They gave graded doses of thyroxine successively to the same individual, after the animals had previously been treated with I-131, instead of giving graded doses of thyroxine to different individuals or different groups. This technique has the advantage over the goitrogen method in that the study can be conducted on individual intact animals.

OBJECTIVES AND EXPERIMENTAL APPROACHES

Little work had been done on inheritance in regard to the endocrine system and particularly that of the thyroid gland. Mixner and Upp (38) found strain differences in the thyroid secretion rate of several strains of chickens and Hurst _et al_. (35) reported that three strains of mice showed differences in the thyroid secretion rate.

Several pure inbred strains of mice developed by brother x sister matings for more than twenty generations were selected. These mice were genetically homogeneous (Heston, 54), and, therefore, it was safe to suggest that any differences which might exist in thyroidal activity, among these strains, would be due to heritable factors, particular for each strain.

Thyroid I-131 uptake, output rate constant and thyroid hormone secretion rate by the method of I-131 administration and the use of exogenous thyroxine (Perry, 51, and Reineke and Singh, 52) were used as criteria for thyroid activity. The mice used were A/Jax, BALB/c, C57BR and C57BL and the hybrids CAF_1 and BBF_1.

A large number of heterozygous mice were primarily used to establish a method for determining the thyroid hormone secretion rate.

Materials and Methods

Heterozygous mice, pure inbred strains and their F_1 hybrids were used in this study. Heterozygous mice were obtained from Carworth Farms and from the Michigan State Health Laboratory. Inbred mice of strains A/Jax, BALB/c, C57BR/cd, C57BL/6 and the F_1 hybrids, CAF_1(BALB/c♀ x A/Jax♂) and BBF_1(C57BL/6♀ x C57BR/cd♂) were obtained from the Jackson Memorial Laboratory. The parental strains are well established inbred lines maintained by brother-sister matings for many generations (54). The inbred mice within each strain or their F_1 hybrids are theoretically identical in genetic background.

The animals were fed with Hoppert's ration consisting of yellow corn meal 140 parts, ground whole wheat 100 parts, alfalfa leaf meal 24 parts, whole milk powder 80 parts, Brewer's yeast 12 parts and iodized salt 4 parts. Animals were kept in an air-conditioned room with a temperature of approximately 74°F all through the year. Constant lighting was maintained from 8:00 A. M. until 10:00 P. M. Mice used were of both sexes, with ages ranging from a few weeks to a few months. Their body weights ranged from 17 to 30 gm., varying with age and sex.

Mice were allotted to groups, ranging from five to ten animals in each group. The mice from the Carworth Farms were studied in two or three trials. In the first experiment, the I-131 uptake and output rate constant were determined. The same mice were again used to establish the thyroid hormone secretion rate by the method of I-131 administration and the use of exogenous thyroxine. The time elapsed between the first and the second experiment on the same group was at least three weeks. The mice were given doses of carrier-free I-131 ranging from 5 uc to 20 uc, with an average of 10 uc per mouse. There was no special reason for the level of I-131 given, except that a higher dose was more appropriate to obtain a higher counting rate. It was assumed, however, that this range of I-131 was below any toxic dosage of radiation.

The subsequent experiments were somewhat modified, when it was found that the retreatment of mice with relatively high amounts of I-131 damaged the epithelial cells of the thyroid follicles.

Mice from the Michigan State Health Laboratory were selected. The amount of I-131 administered to these groups did not exceed 1 uc per mouse. In order to obtain higher counts, the geometry of the apparatus was changed.

A much thinner lead shield with only 1.2 mm. of thickness was used, whereas in the earlier experiments a shield with 4.8 cm thickness was applied. It was thus possible to increase the counting efficiency four to five times and to some extent compensate for the lower level of I-131 dose used. Furthermore, in order to avoid any damage to the thyroid gland, each group of mice was used only once, unless otherwise stated. It did not seem probable, however, that such a low dose would do any harm to the thyroid follicles. On these mice, only I-131 uptake and thyroid hormone secretion, by Perry's method, was determined.

Inbred mice from Jackson Memorial Laboratory were used to determine the radio-iodine uptake curve, output rate constant and the thyroid hormone secretion rate. To measure the uptake and output curves of each strain, two mice from each sex of each strain were selected. Five uc of carrier-free I-131 was injected intraperitoneally into each mouse. Counts were taken at frequent intervals from 7-180 hours after I-131 injection, with shorter intervals at the beginning and longer intervals at the end. The percentage of the injected dose was calculated for each count. The results are plotted as the log of per cent of injected dose against time in hours.

To measure I-131 uptake and thyroid hormone secretion rate the mice were divided into two groups with 12 mice of each strain in each group. Five uc of I-131 was injected as before.

Two counts were taken on each mouse at 48 and 96 hour intervals after I-131 injection. Thyroid hormone secretion rate was determined by the method of Reineke and Singh. This method requires more days of counting--at least ten days after I-131 injection--and, in order to have reliable counts, it was not possible to use less than 5 uc I-131. In Perry's method only two counts are sufficient, with four days' interval between them.

Measurement of I-131 Uptake, Output Rate Constant and Thyroid Hormone Secretion Rate

I. <u>I-131 Uptake</u>: Either 24 or 48 hours after radiodiodine injection, external thyroid counts were obtained. I-131 uptake was calculated on the basis of the per cent of the injected dose detected in the thyroid gland.

II. <u>Measurement of the Output Rate Constant</u>: Sufficient time was given, after I-131 injection, for the thyroid gland to take up the radioiodine and to let the excess iodide be excreted. After 24 or 48 hours uptake, counts were obtained. The log of the per cent of injected dose was then calculated and plotted against time in days. A straight line was fitted to the points. The slope of the line gives the exponential rate of loss of I-131 from the thyroid gland, which can be expressed either as the per cent output per day or the biological half life. This exponential loss of I-131 can be expressed with the equation: $\frac{A_t}{A_o} = e^{-Bt}$, where A_t and A_o are the activities at time t and at zero time sugsequently, and -B is the turnover rate constant of I-131.

III. Determination of the Thyroid Hormone Secretion Rate by Injection of I-131 and Exogenous Thyroxine:

a. Perry's Method: Seven groups of ten mice each were given 0.4 uc I-131 per mouse. Each group except the controls received graded doses of l-thyroxine twenty-four hours after injection of I-131. The thyroxine treatment was continued for four days. A second count was then taken on the fifth day. Each mouse was counted at the same time of the day in both counts, so that the time between the first and second counts was approximately the same for all the mice. The counts were corrected back to zero time and the per cent of the initial count was calculated for each group. The per cent of previous count was plotted against the thyroxine dosage in ug. per hundred grams of body weight on coordinate paper. A regression line was fitted to the ascending portion of the curve by the method of least squares with the prediction equation $Y = a + bX$. If the per cent of previous count (Y) is taken as 100 or 1.00 (Figures 2-21, Appendix), the thyroid secretion rate (X) is then estimated as ug/100 gm. body weight. In some cases the per cent of previous count leveled off before it reached 100 per cent (Figure 4, Appendix). In such cases the (Y) was determined at the point where the line leveled off.

b. Measurement of Thyroid Hormone Secretion Rate by Method of Reineke and Singh: This technique is a modification of Perry's technique, the thyroxine dose being progressively increased on the same group of mice. This method allows one to observe the degree of I-131 output inhibition by various amounts of thyroxine on each individual mouse. This method, however, takes a longer time and requires at least three to four counts on the ascending portion of the curve in order to obtain reliable values.

Counting Method: A Scintillation Counter* was used. External thyroid counts were taken by use of a heavy lead counting table similar to that described by Albert (55). The voltage used was 1100. The count rate meter was always calibrated before counting was done. A cobalt-60 standard was used to check the machine. The mice were restrained in a long taper conical 50 ml. centrifuge tube with a nose opening at the end. The thyroid region was centered over the tapered opening in the counting setup. The length of time required for counting each mouse varied between 3 and 5 minutes, depending on the activity of the gland. With a high count, the count rate meter quickly adjusted to a stable reading, whereas with low counts it was necessary to wait for a few minutes before a reliable count could be recorded. The sensitivity was always set at the 10 second time interval.

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*Nuclear Instrument and Chemical Corporation, Model DS-1.

Preparation of Standard: Standards were made at the time of radio-iodine injection. Ten per cent of the injected dose was usually put within a 5 mm. circle drawn in the center of a small porcelain dish with a wax pencil. The standards were left for a few hours in order to dry and then counted. This count was considered the zero time standard. It was found that the loss of counts of the standards on the days subsequent to their preparation were higher than the loss which could be accounted for by physical decay, even when they were prepared in casein. This loss was exponential, with a half life of approximately six days, as shown in Fig. 1 of the Appendix. In order to avoid systematic errors, all the calculations which required the use of a standard were referred back to the activity of the standard at zero time. Standards were used only to obtain the uptake values.

Body background was taken over the epigastric region. The thyroid counts were corrected by one-half the body background plus the general background as suggested by Wolff (50). This method of measuring the background could have given too high values because of the collection and excretion of the radioiodine from the bladder. It was safe to assume that all the extra radioiodine which was not collected by the thyroid gland had already been excreted through the urine by the time the 48 hour count was taken.

For the measurement of the radioiodine uptake curves of the inbred mice, where counts were taken every few hours after I-131 injection, Wolff's correction for the body-background did not seem adequate, since considerable amounts of radioiodine were still present in the extrathyroidal tissues and particularly in kidney and bladder. Since we were primarily interested in results on a comparative basis, possible errors in calculating the per cent of injected dose and also in correcting the background did not affect the results to an appreciable extent.

Preparation of Thyroxine Solutions: The L-thyroxine used was supplied by Glaxo Laboratories, Greenford, Middlesex, England. L-thyroxine crystals were purified by Dr. E. P. Reineke and kept in a desiccator in the refrigerator. Ten mg of crystallized thyroxine was weighed on an analytical balance, dissolved in distilled water in a 50 cc. beaker and made slightly alkaline with NaOH. Enough acid (HCl) was added to make the solution slightly cloudy; at this point the monosodium salt of thyroxine is formed. This solution was then placed in a 100 cc. volumetric flask and distilled water was added up to the mark. This solution contained a concentration 100 ug. of l-thyroxine per ml. of solution. The stock solution was stored in the refrigerator.

RESULTS

Heterozygous Mice

Radioiodine Uptake Values

Groups of mice, each group consisting of ten individuals, were used. The amount of carrier-free I-131 given was between 10 and 20 uc per mouse and is indicated in appropriate tables (Tables I-VI, Appendix). The averages of 48-hour radioiodine uptake with their standard errors are summarized in Table I. The 48-hour uptake for each individual mouse of each group appears in Appendix (Tables I-VI). Table II shows the averages for the 24-hour uptake values for the mice from the Michigan State Health Laboratory. Forty-eight-hour uptake was not measured in the mice from the Michigan State Health Laboratory, because the mice were treated with thyroxine 24 hours after the I-131 injection for the measurement of thyroid secretion rate by Perry's method. The averages of the 48-hour uptake values are between 5 and 11 per cent of the injected dose, as shown in Table I. The results obtained for the 48-hour uptake on mice from Carworth Farms in the second experiment did not substantiate the observations of the first experiment which showed a slightly higher 48-hour uptake for the males.

TABLE I

AVERAGE PER CENT 48-HOUR UPTAKE AND THEIR STANDARD ERRORS IN MICE* FROM CARWORTH FARMS

Sex	Trial No.	Average % 48-Hour Uptake	
		First Experiment	Second Experiment
♀	1	7.77±1.27	11.30±1.28
♀	2	6.11±0.40	7.25±0.70
♀	3	5.08±0.36	9.96±1.26
♂	4	8.39±0.95	7.67±0.70
♂	5	10.64±2.00	8.90±0.77
♂	6	8.45±1.10	6.90±0.28

*Values obtained for each individual mouse and the number of mice in each group appear in the Appendix.

TABLE II

24-HOUR AND 48-HOUR UPTAKE OF MICE FROM MICHIGAN STATE HEALTH LABORATORY*

Group No.	First Experiment 1/17/56		Second Experiment 2/1/56		Third Experiment 3/9/56	
	No. of mice in each group	Average 24 hrs. uptake	No. of mice in each group	Average 24 hrs. uptake	No. of mice in each group	Average 48 hrs. uptake
1	10	15.40	10	13.84	10	16.10
2	10	13.60	9	13.08	10	19.90
3	9	12.66	10	10.86	10	14.44
4	10	12.89	10	11.00	10	17.90
5	10	14.12	10	12.30	10	13.40
6	10	11.10	10	11.40	10	15.54
Average						

*The mice in the Second Experiment are the same mice as those used in the First Experiment. Both times they received .4 uc I-131. Mice of the Third Experiment received 1.0 uc I-131. The mice were approximately 5 weeks old at the time of the First Experiment.

The mice from Michigan State Health Laboratory seemed to have a higher uptake than the mice from Carworth Farms. Unfortunately, the comparison cannot be made between these mice and those from Carworth Farms, because of the differences in time when uptakes are measured and also because of the wide differences in the radioiodine dosage.

A frequency distribution of 48-hour I-131 uptake for the mice from Carworth Farms, regardless of sex, is also presented (Figure 1). It is noted that almost 50 per cent of the uptake values fall within 4 to 6 per cent of the injected dose. In more than 80 per cent of these uptake values, the range is between 3 and 7 per cent of the injected dose. The uptake exceeded 7 per cent of the injected dose in only 20 per cent of the uptake values.

Determination of Per Cent Output per Day and Biological Half-Life (T½)

These two express the output rate or turn-over rate of the thyroid hormone. Per cent output per day and biological half-life (T½) were calculated. The averages of the per cent output per day and the standard errors appear in Table II. The values obtained for per cent output per day and also the biological half-life for each individual mouse of each group is observed in Appendix Tables I to VI. The average per cent output per day were as follows: female mice: 13.72 ± 1.08; 17.00 ± 1.1; 7.10 ± 0.44 for the first run, and 18.02±1.46; 14.00±1.62 and 18.89±0.83 for the second run, and for males: 13.12±1.12; 14.10±1.10; 14.80±0.83 for the first run, and 12.67±1.50; 19.42±3.40 and 16.10±1.70 for the second run. There do not seem to be any significant differences between males and females. A low value of 7.1 per cent per day for the females of trial 3 on the first run cannot be explained. This low value is apparently due to experimental error, since on the second run an average value of approximately 19 per cent per day was obtained. The results for per cent output per day were not consistent in regard to sex differences. Moreover, the output rate values were relatively close for both the first and the second experiment, as is seen from Table III. This might suggest that the effect of radiation which damaged some of the thyroid follicles, as the histological studies showed, did not affect thyroid function appreciably.

A frequency distribution of T½ is also given for both sexes of mice from Carworth Farms (Figure 2). These include the values of the first and the second experiment. Seventy-three per cent of the T½ values were within 3-6 days, with a majority distributing within 4 to 6 days.

An attempt was made to find whether there was any correlation between the per cent uptake and output rate constant in the heterozygous mice. Forty-eight-hour uptake was plotted against the biological half-life for every

TABLE III

AVERAGE PER CENT OUTPUT PER DAY AND THEIR STANDARD ERRORS IN MICE* FROM CARWORTH FARMS

Sex	Trial No.	Average % Output per Day	
		First Experiment	Second Experiment
♀	1	13.72±1.08	18.02±1.46
♀	2	17.0 ±1.1	14.0 ±1.62
♀	3	7.1 ±0.44	18.89±1.23
♂	4	13.12±1.12	12.67±1.50
♂	5	14.10±1.10	19.42±3.40
♂	6	14.80±0.83	16.10±1.70

*Values obtained for each individual mouse and the number of mice in each group appear in the Appendix.

mouse of each sex. There was no significant correlation between these two values (Figure 3). There was a trend, however, towards a longer T½ with a lower uptake. The non-linearity between these two values was indicated by the absence of any correlation.

Measurement of Thyroid Secretion Rate of Heterozygous Mice

The method of Reineke and Singh and that of Perry were used to measure the thyroid secretion rate. Figures 4 to 11 of the Appendix show the extrapolated results for the thyroid secretion rate of these mice. The equation of the line, standard errors of the mean and the correlation r_{xy} between Y, the per cent of previous activity, and X the thyroid secretion rate are indicated in appropriate figures. The correlations between X and Y were all highly significant. The values of X measured by Perry's method are as follows: 3.08, 2.26, 2.09 and 2.26 ug./100 gm. body weight. The values of secretion rate measured by the method of Reineke and Singh are 1.76, 1.31, 1.26, 2.18 and 3.09 ug. These mice were obtained from Carworth Farms, whereas the mice used to measure thyroid secretion rate by the method of Perry were provided from Michigan State Health Laboratory.

Mice of Inbred Strains and Their F_1 Hybrids

Radioiodine Uptake

Tables IV and V show the radioiodine uptake in different strains of mice 7-180 after I-131 injection, and the average percentage of 48-hour uptake. Tables VII-IX of the Appendix show the results of analysis of variance of strain differences. The values show that C57BL/6 and their BBF_1 hybrid had values consistently lower than albinos of A/Jax and BALB/c strains and their CAF_1 hybrid. Figure 4 shows the comparative values for the 48-hour uptake.

Measurement of Output Rate Constant

Table V and Figures 4 and 5 show the results obtained.

The uptake of I-131 a few hours after I-131 injection was very irregular but was stabilized after 24 hours. This might have been due to incomplete I-131 uptake by the thyroid gland and that the excess of I-131 had not yet been excreted. The body background at early hours was very high and in some cases even higher than the thyroid counts. It was shown that the thyroid was still taking up iodine 21 hours after I-131 injection. It was observed that the C57BL/6 strain had the highest output rate. The BBF_1 mice were second and C57BR/cd third in order. From the analysis

TABLE IV

THE AVERAGE I-131 UPTAKE IN TERMS OF PER CENT OF INJECTED DOSE IN TWO MALES AND TWO FEMALES IN EACH GENOTYPIC GROUP AT DIFFERENT TIMES AFTER I-131 INJECTION

Hrs. after I-131 Injection	A/Jax		CAF_1		BALB/c		C57BR/cd		BBF_1		C57BL/6	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
7	23.8	18.6	25.8	28.8	17.8	22.6	6.6	14.4	-	17.5	4.1	10.2
12	15.2	16.4	20.4	22.9	14.5	18.9	12.2	12.8	10.0	15.0	3.8	7.6
21	17.6	11.0	18.0	26.0	15.1	18.4	11.1	10.6	10.2	14.0	12.9	6.5
29	16.9	11.1	17.3	25.0	13.8	17.8	10.5	8.8	9.4	12.8	9.0	5.1
46	7.1	8.6	16.8	22.7	12.5	16.1	9.0	7.5	6.6	11.3	6.2	3.8
70	12.9	8.2	12.6	20.4	8.7	13.1	7.5	5.8	4.7	6.1	4.5	2.5
84	12.2	5.3	9.7	7.4	10.4	12.3	6.0	4.2	3.7	5.4	3.6	2.1
134	7.4	4.5	8.9	12.7	6.9	8.0	3.9	2.8	2.1	3.3	2.4	1.2
180	5.4	3.7	6.9	9.1	5.0	6.6	2.6	1.9	1.3	2.1	1.5	0.4

TABLE V

AVERAGE PERCENTAGE OF BOTH I-131 UPTAKE AND OUTPUT RATE CONSTANT IN EACH GENOTYPIC GROUP OF MICE

	No. Mice in Each Group	A/Jax Mean±s.e.	CAF_1 Mean±s.e.	BALB/c Mean±s.e.	C57BR/cd Mean±s.e.	BBF_1 Mean±s.e.	C57BL/6 Mean±s.e.	Average Mean±s.e.
% Uptake	10 ♂'s	16.0±1.8	20.3±1.0	15.9±1.3	12.1±1.2	11.5±1.4	8.6±0.7	16.1±1.3
	10 ♀'s	16.1±0.7	18.5±1.0	18.8±2.2	8.7±1.1	9.2±1.5	7.1±1.4	13.1±1.3
	20	16.1±1.3	19.4±1.0	17.4±1.8	10.4±1.2	10.4±1.5	7.9±1.1	14.6±1.3
Output Rate Constant	10 ♂'s	15.4±1.1	13.0±1.2	16.9±1.8	27.1±1.9	30.2±2.00	31.8±3.4	22.4±1.9
	10 ♀'s	15.2±0.6	14.5±0.8	19.0±1.8	24.8±1.7	34.4±3.4	36.2±3.4	24.0±1.9
	20	15.3±0.9	13.8±1.0	18.0±1.8	26.0±1.8	32.3±3.7	34.1±3.4	23.2±1.9

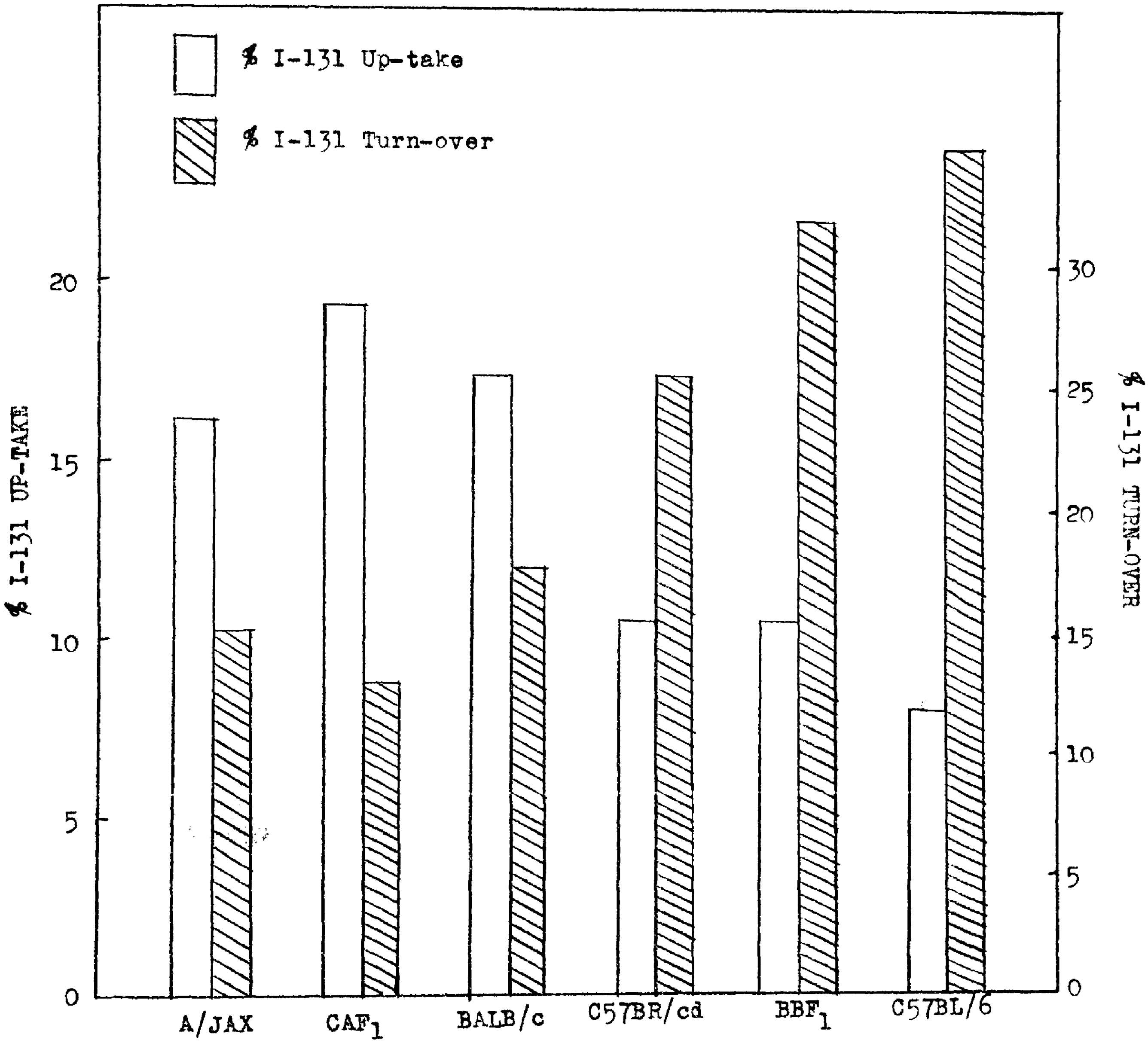

Fig. 4. Average percentage of I-131 uptake and rate of turn-over in six genetically different strains of mice

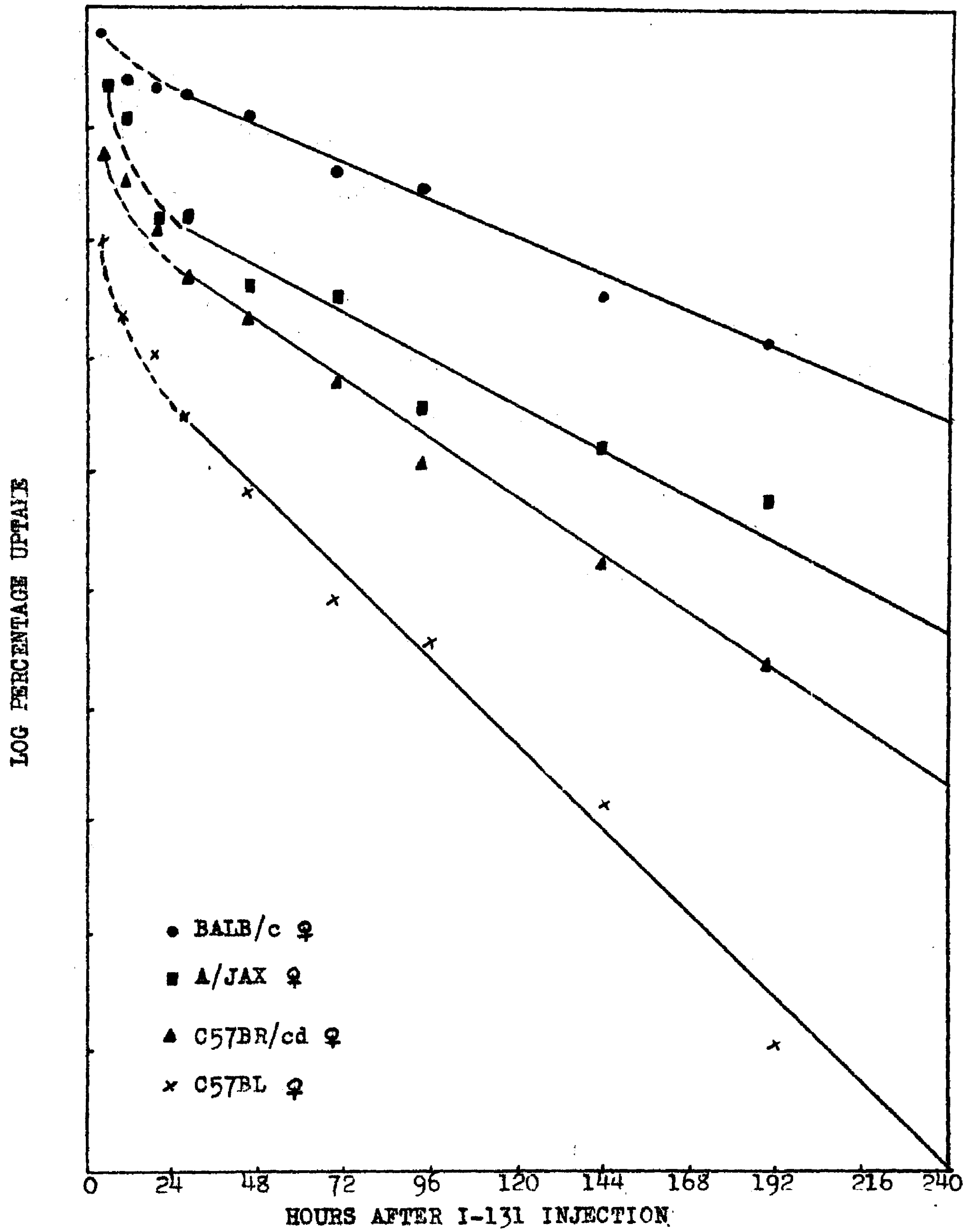

Fig. 5. Log percentage of I-131 uptake with respect to time following injection in mice from four different inbred strains

of variance it was found that both variances attributed to genotype and genotype x sex were highly significant (Tables VII and VIII of Appendix). The level of significance at $P_{.05}$ between any two group means was obtained as 4.02 per cent. The differences between the means for C57BL and that for any other group except BBF_1 were significant. No significant difference between the means for A/Jax and BALB/c was shown, but the difference between that of CAF_1 mice and their maternal parent, A/Jax, was significant.

Measurement of Thyroid Secretion Rate in Inbred Mice and Their F_1 Hybrid

The method used was that of Reineke and Singh. The results are plotted as the per cent of previous activity, against the thyroid secretion rate in ug/100 gm. of body weight. The equation of the line, correlation between X and Y and the standard errors of the means appear in the Figures 10-21 of the Appendix. When Y, the fraction of previous activity, is equal to 1.00, X is the thyroid secretion rate.

In order to be able to test for differences in the thyroid secretion rate among different strains, the thyroid secretion rate of each mouse of each strain was measured and treated by the method of least squares. The values obtained for each mouse and the average in each strain are seen in Table VI.

TABLE VI

THYROID SECRETION RATE OF INBRED AND F_1 HYBRID MICE

Mouse #	Thyroid Secretion Rate* in ug/100 gm. of Body Weight											
	A/Jax		CAF_1		BALB/c		C57BR/cd		BBF_1		C57BL/6	
1	2.62	2.43	2.05	1.30	2.21	1.74	2.58	2.24	2.69	2.15	4.07	2.35
2	2.28	2.14	1.87	1.80	1.03	2.13	3.13	2.00	2.40	2.05	3.83	3.37
3	2.13	1.99	1.57	2.01	2.62	1.61	3.74	2.36	3.06	1.61	4.92	1.97
4	1.76	2.03	2.14	1.50	2.01	2.40	2.46	2.92	2.15	1.74	3.80	2.84
5	1.92	1.98	1.83	1.75	2.52	2.45	4.40	2.29	2.36	2.71	5.44	4.28
6	2.02	1.57	3.30	1.05	1.69	2.08	3.67	2.44	6.63	2.39	2.78	2.36
7	2.25	1.64	2.83	1.89	2.29	1.10	5.87	2.70	5.98	2.71	3.68	4.81
8	2.84	1.34	2.38	2.51	3.18	1.86	4.23	2.61	2.61	4.17	8.14	3.30
9	1.96	1.48	2.72	1.53	2.92	1.31	3.78	3.09	6.13	2.60	8.18	3.85
10	1.62	1.85		2.01	3.11	1.51		2.19	8.63	3.15	3.17	5.26
11									2.46	4.32		
12	2.09	1.77	2.56	1.67	2.07	1.65	3.67	3.04	3.07	2.85	3.37	3.04
Average	2.13± .118	1.84± .105	2.35± .185	1.84± .106	2.44± .210	1.93± .118	3.35± .345	2.45± .108	3.79± .644	2.74± .268	4.19± .643	3.33± .341

*Secretion rate is measured in each individual mouse of each strain by the method of least squares.

The values obtained are as follows for males and females of each strain: A/Jax 2.13±0.118 and 1.84±0.108; BALB/c 2.44±0.210 and 1.93±0.118; C57BR/cd 3.35±0.345 and 2.45±0.108; C57BL/6 4.19±0.643 and 3.33±0.341 ug/100 gm. of body weight. The secretion rate for the hybrids CAF_1 and BBF_1 males and females, respectively, were: 2.34±0.185; 1.84±0.106 and 3.79±0.644 and 2.74±0.268 ug./100 gm. Figure 6 is a graphical representation of the thyroid secretion rate in each sex of each strain. The order of magnitude in the secretion rate of different strains of mice and their F_1 hybrids were C57BL/6, BBF_1, C57BR/cd, BALB/c, CAF_1 and A/Jax. Analysis of variance showed (Table IX of Appendix) a significant difference between the different strains of mice as follows: C57BL had a significantly higher secretion rate than all other strains, except BBF_1. BBF_1 was significantly higher than all other strains except C57BL. C57BR/cd had a significantly higher secretion rate than the strains A/Jax, BALB/c and the hybrid CAF_1. There was no significant difference in the thyroid secretion rate among strains A/Jax, BALB/c and their CAF_1 hybrid.

When all the data on each sex of each strain were plotted and treated by the method of least squares, slightly different values from that when the values of each was treated separately were obtained. Figures 10-21 of the Appendix represent the results. The values of the secretion

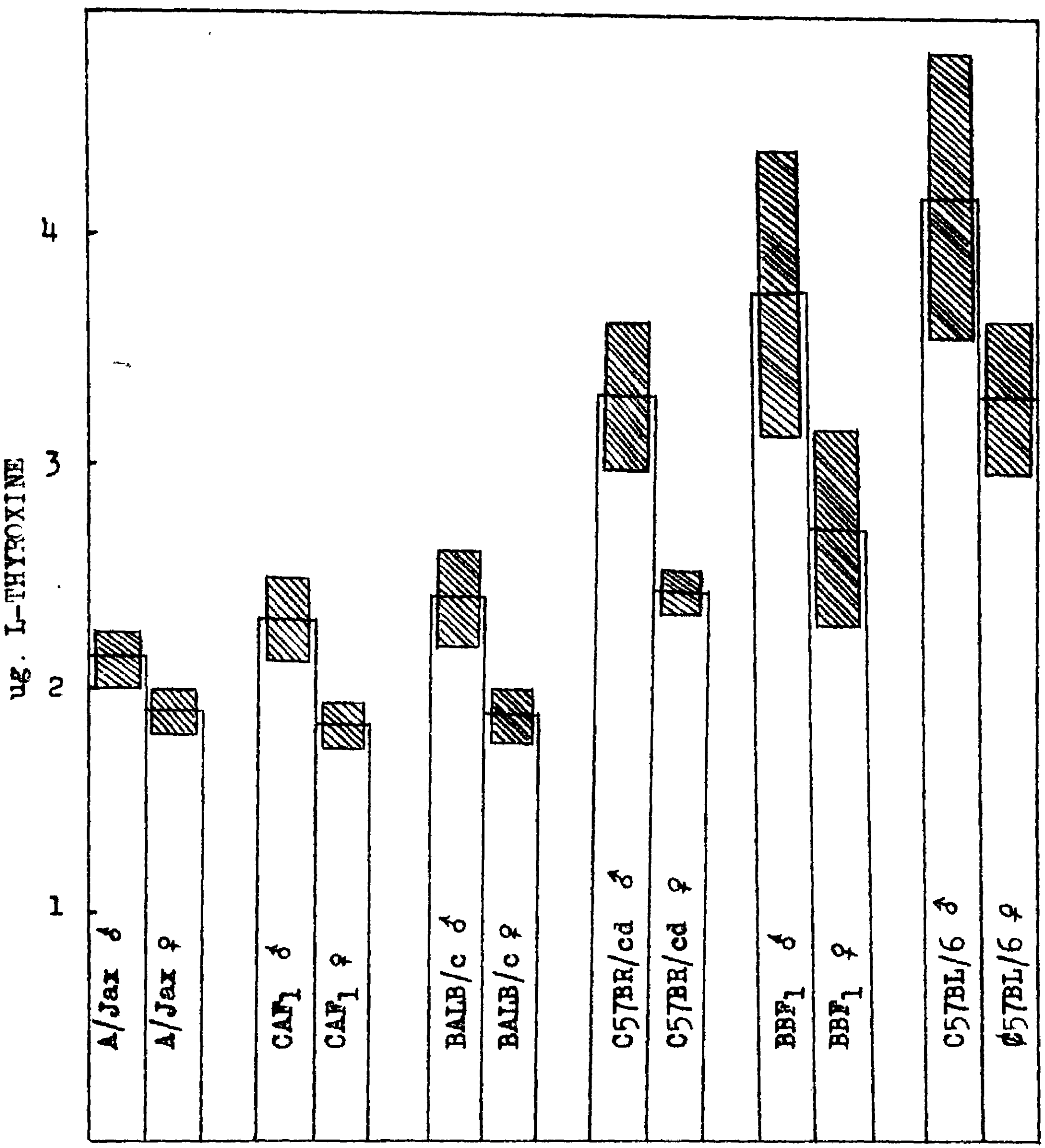

Fig.6. Thyroid secretion rate of inbred and the F_1 hybrid mice. The secretion rate is expressed as ug./100 gm. of body weight. The striped bars represent the standard error of each sex of each strain.

rate for males and females of each strain and their F_1 hybrids are as follows: A/Jax, 2.09 and 1.77; CAF_1, 2.56 and 1.67; BALB/c, 2.07 and 1.65; C57BR/cd, 3.67 and 3.04; BBF_1, 3.97 and 2.85; C57BL, 3.37 and 3.04. Highly significant correlation was observed between the X and Y values as follows: 0.818, 0.845, 0.732, 0.758, 0.938, 0.783, 0.900, 0.786, 0.822, 0.862 and 0.814 for the males and females of A/Jax, CAF_1, BALB/c, C57BR/cd, BBF_1 and C57BL/6, respectively.

It is of interest to note that males had a consistently higher secretion rate than females, although the statistical analysis did not show the differences to be significant, except in the C57BR strain, when the data were treated for individual mice. The values obtained for the CAF_1 and BBF_1 hybrids were intermediate between their parent strains.

DISCUSSION

The results obtained from the heterozygous mice are influenced by a few variable factors, such as the I-131 dose range, age, season and their source. These heterozygous mice, however, showed some uniformity in their 48-hour uptake and their output rate constant (Tables I and II). The I-131 dose range did not seem to affect the iodine uptake. Although I-131 injection damaged the thyroid gland of the mice from Carworth Farms, when they were treated with I-131 for the second or third time, the I-131 uptake still remained approximately the same, nor did the output rate constant change to any appreciable extent. The possible explanation is that the undamaged follicles of the thyroid had a compensatory increase in action, and the thyroid was still apparently able to function normally. In one case when 100 uc I-131 was injected into a group of mice, a very rapid release of the I-131 from the thyroid indicated the complete disruption of the thyroid follicles.

The thyroid secretion rate, measured both by the method of Reineke and Singh and the method of Perry, gave values between approximately 1.00 and 3.00 ug./100 gm. body weight. It is not possible to draw any conclusion about the wide difference in the thyroid secretion rate. It might be due to the way the l-thyroxine solutions were prepared. The high values obtained might have been due to racemization of l-thyroxine, which reduces the thyroxine activity considerably (56,57). In the case where the thyroid secretion rate of mice was found to be 3.00 ug. (Figure 3 of Appendix), which seemed to be too high, the melting point of the thyroxine solution used was measured, as a test for racemization. No definite answer could be obtained. The small amount of the crystallized thyroxine did not allow measurement of the optical rotation as a test for l-thyroxine. The fact that these mice were run at different times of the year might have contributed considerably to these differences in the thyroid secretion rate.

These sources of error, namely, dosage range, thyroxine preparations, age and season, were eliminated for the inbred and their F_1 hybrid mice. The same stock solution of thyroxine was used for all these mice. The mice were all approximately at the same age at the time of experiment. The I-131 dosage was constant, 5 uc per mouse.

The results obtained on the inbred mice are interesting in that significant differences among different strains are observed. C57BL had the highest output rate constant, as measured by the per cent of I-131 loss per day, and the highest thyroid secretion rate. The values on BBF_1 hybrid, both in output rate constant and thyroid secretion rate were intermediate between its parent strains.

The CAF_1 hybrid had values for thyroid secretion rate intermediate between its parent strains, but showed a higher uptake and lower output rate than its parent strains.

Female mice had in general a higher output rate constant. This was not consistent for all the strains. The values obtained for thyroid secretion showed a higher rate for males than for females, which was consistent for all the strains. Since both the output rate constant and measurement of thyroid secretion rate are actually an expression of the same factor, namely, release of organically bound iodine, their difference in the same mice may be due to possible experimental error rather than actual differences. These differences, however, were statistically insignificant and cannot be taken as conclusive evidence.

A reciprocal relationship exists between the I-131 uptake on one hand and the output rate constant and thyroid secretion rate on the other hand which was explained previously. A low uptake in strain C57BL, which has the highest secretion rate, might prove that a high per cent uptake of I-131 does not indicate a high thyroidal activity by itself. It is interesting to note that these strains of mice and their F_1 hybrids show different behaviour as far as the thyroidal activity is concerned. These finding are in agreement with other experiments of a completely different nature, which appears in the following.

The C57 strain is probably the most widely used in various fields of biological research. Some of their biological properties have been found quite distinct from those of other strains. For instance, it has been shown that the C57BL mice are more aggressive in competition for food than the BALB/c, highly resistant to cold, superior to BALB/c in fighting (58), and more vigorous and rugged by observation than most other inbred mice. The present findings agree quite well with these traits in that the thyroid activity of the C57BL is the highest among those which we studied.

Histological studies have shown (59) that the follicles of the thyroid gland of the C57 strain have a larger cell height than C_3H strain which is similar to the A/Jax and BALB/c strains.

In connection with cancer research, C57BL mice are in general less susceptible to different types of tumors, except sarcomas, but evidence indicates that these mice are highly susceptible to pituitary tumors after thyroidectomy (60,61). It was also found that all pituitary tumors which were assayed were thyrotropic and of the dependent type (62).

From the genetic standpoint the differences between the means of the F_1 hybrids and that of their respective parents, with the exception of CAF_1, which showed different values, in both iodine uptake and output rate constant are of interest. The F_1 hybrids have radioiodine uptake and thyroid secretion rate values intermediate between their parent strains. Although these animals are not significantly different from their parents, they may indicate that thyroidal iodine uptake and its output rate are two genetically separate phases of thyroid function and that the intermediate values for uptake and output rate constant are a result of hybridization, which requires multiple factors with dominant genes. The hypothesis suggested from the results of uptake and output rate constant along with the thyroid secretion rate is that both thyroid iodine space and the release of the hormone from the thyroid gland are controlled by separate genetic factors.

Although our results are not exactly in agreement with the findings of Mixner and Upp (38), who found a much higher thyroidal activity in hybrid chicks, as compared to single cross chicks, they both suggest a genetic transmission of thyroidal activity. In the experiments reported in this paper, the thyroid activity of the hybrid mice did not exceed both paternal and maternal parents, but was intermediate between them. This does not exclude, however, the possibility of heterosis, suggested by Mixner and Upp.

Strain differences have been observed in the thyroid uptake of the strains A, C57 and I by Lyon (63). Lyon measured the thyroid uptake of these strains of mice at intervals from 2 to 48 hours after radioactive sodium iodide. He found a 1½ to 2 times higher uptake in A mice than the I strain. The values for the C57 were found to be between those of the other two strains.

Although the higher thyroid uptake alone does not indicate a higher thyroidal activity, it does suggest, however, a strain specificity. Lyon, furthermore, found that after the administration of one IU of TSH, one hour before the iodide injection, the increase in I-131 uptake was four times greater in the A strain than in the C57 mice.

Aside from the genetical differences in thyroid activity, numerous studies have been conducted on physiological and biochemical aspects which indicate strain specificity (64,65,66,67). Of special interest are the findings of strain differences in mice and rats given alloxan (68). Based on the studies on alloxan-treated mice and rats, Beach _et al_.(68) suggested the existence of radically different endocrine endowment in different strains.

Further investigations are required to draw definite conclusions as to the heritable factors involved in the thyroid and other endocrine systems.

SUMMARY AND CONCLUSIONS

Inheritance of thyroid activity was investigated. Pure inbred mice and their F_1 hybrid were used. These mice were A/Jax, BALB/c, C57BR/cd, C57BL/c and their F_1 hybrid, CAF_1(BALB/c♀ x A/Jax♂) and BBF_1(C57BL/6♀ x C57BR/cd♂). The mice were obtained from the Jackson Memorial Laboratory. These mice were produced by brother x sister matings for more than 20 generations and are, therefore, considered to be quite homogeneous.

I-131 uptake and its turn-over rate was measured. Turn-over rate was detected by: 1) the measurement of the exponential rate of release of injected radioiodine from the thyroid gland of the intact animals, and 2) by the method of I-131 injection and the use of exogenous thyroxine which was required to inhibit the release of radioiodine from the thyroid gland. Heterozygous mice which were obtained from the Carworth Farms and from the Michigan State Health Laboratory were primarily used to establish a method for the thyroid secretion rate.

The thyroid secretion rate was measured by the administration of graded doses of l-thyroxine either into different groups (Perry's method) of mice, or to the same individual mouse (Reineke and Singh). The amount of l-thyroxine which was required to prevent the output of radioiodine was considered to be equal to the amount of thyroid hormone which is normally put out by the thyroid gland of the animal itself.

The results indicate that the strains A/Jax, BALB/c and their CAF_1 hybrid had a high 48-hour uptake and low "turn-over rate," whereas the strains C57BL/6, C57BR/cd and their BBF_1 hybrid showed a low 48-hour uptake and a high turn-over rate. C57BL/6 had the highest turn-over rate and the lowest uptake.

Statistical analysis showed that the C57BL strain was significantly different from all other strains studied except BBF_1. C57BR was also significantly different from the strains A/Jax, BALB/c and their CAF_1 hybrid. There were no significant differences among the strains A/Jax, BALB/c and the CAF_1 hybrid.

A reciprocal relationship was observed between the 48-hour I-131 uptake and its turn-over rate in the inbred mice and their F_1 hybrids, but not in the heterozygous mice. The values obtained for the thyroid secretion rate on the inbred mice and their F_1 hybrids show values for the CAF_1 and BBF_1 in both cases intermediate between their parent strains.

The results support the view that the thyroid mechanism is under genetic control. Furthermore, it appears that thyroidal iodine "space," as indicated by the uptake data, and the mechanism controlling thyroid output are regulated by separate genetic factors.

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APPENDIX

TABLE I. TRIAL 1

THE VALUES OBTAINED FOR THE PER CENT OF INJECTED DOSE, THE PER CENT OUTPUT PER DAY AND THE BIOLOGICAL HALF-LIFE FOR EACH INDIVIDUAL MOUSE OF THE CARWORTH STRAIN

Mouse No.	First Experiment* 3/21/55			Second Experiment* 7/1/55		
	Injected with 10 uc I-131			Injected with 20 uc I-131		
	% Uptake at 48 hrs.	% Output per Day	T½ in Days	% Uptake at 48 hrs.	% Output per Days	T½ in Days
1	11.45	12.00	5.75	5.62	21.70	3.19
2	5.69	13.26	5.22	16.78	21.30	3.25
3	5.32	16.00	4.31	-	-	-
4	4.74	13.44	5.15	8.01	16.50	4.20
5	16.13	14.43	4.80	14.98	22.90	2.67
6	8.48	8.61	8.48	13.04	10.30	6.72
7	7.59	13.10	3.29	11.23	15.80	4.39
8	-	-	-	-	-	-
9	5.52	17.80	3.89	11.20	19.70	3.51
10	5.06	14.90	4.65	9.55	16.00	4.33
Average	7.77±1.27	13.72±1.08		11.30±1.28	18.02±1.46	

*The same mice were used in both the First and the Second Experiment.

TABLE II. TRIAL 2

THE VALUES OBTAINED FOR THE PER CENT OF INJECTED DOSE, THE PER CENT OUTPUT PER DAY AND THE BIOLOGICAL HALF-LIFE FOR EACH INDIVIDUAL MOUSE (CARWORTH)

Mouse No.	First Experiment* 3/21/55			Second Experiment* 7/15/55		
	Injected with 10 uc I-131			Injected with 15 uc I-131		
	% Uptake at 48 hrs.	% Output per day	T½ in days	% Uptake at 48 hrs.	% Output per day	T½ in days
11	5.12	13.90	4.98	9.36	9.70	7.14
12	6.21	17.60	3.93	6.85	19.80	3.50
13	5.36	10.00	6.93	7.71	8.10	8.55
14	5.85	21.40	3.23	-	-	-
15	6.43	17.80	3.89	8.48	15.60	4.44
16	6.79	20.30	3.41	-	-	-
17	5.08	17.90	3.87	4.13	15.40	4.50
18	-	-	-	-	-	-
19	4.74	17.70	3.91	7.83	15.50	4.47
20	6.71	16.60	4.17	6.40	13.90	4.98
	6.11±.40	17.0±1.1		7.25±.70	14.0±1.62	

*The same mice were used in the First and the Second Experiment.

TABLE III. TRIAL 3

THE VALUES OBTAINED FOR THE PER CENT OF INJECTED DOSE, THE PER CENT OUTPUT PER DAY AND THE BIOLOGICAL HALF-LIFE FOR EACH INDIVIDUAL MOUSE (CARWORTH)

Mouse No.	First Experiment* 3/3/55			Second Experiment* 7/1/55		
	Injected with 10 uc I-131			Injected with 15 uc I-131		
	% Uptake at 48 hrs.	% Output per day	T½ in days	% Uptake at 48 hrs.	% Output per day	T½ in days
21	5.79	5.90	11.47	10.26	17.50	3.96
22	5.85	8.90	7.78	8.02	18.18	3.68
23	3.33	7.60	9.11	7.37	19.00	3.64
24	4.89	7.10	9.76	10.17	-	-
25	3.40	5.60	12.37	7.37	17.50	3.96
26	-	-	-	-	-	-
27	4.10	4.90	14.14	12.54	18.20	3.80
28	5.92	7.30	9.74	9.47	14.10	4.91
29	5.92	8.10	8.55	8.55	20.70	3.34
30	4.35	8.30	8.34	15.93	25.90	2.67
	5.08±.36	7.1±.44		9.96±1.26	18.89±1.23	

*The same mice were used in both the First and the Second Experiment.

TABLE IV. TRIAL 4

THE VALUES OBTAINED FOR THE PER CENT OF INJECTED DOSE, THE PER CENT OUTPUT PER DAY AND THE BIOLOGICAL HALF-LIFE FOR EACH INDIVIDUAL MOUSE (CARWORTH)

Mouse No.	First Experiment* 5/12/55			Second Experiment* 8/8/55		
	Injected with 10 uc I-131			Injected with 15 uc I-131		
	% Uptake at 48 hrs.	% Output per day	T½ in days	% Uptake at 48 hrs.	% Output per day	T½ in days
1	13.01	13.00	5.33	10.78	11.70	5.92
2	9.34	17.90	3.84	4.80	19.60	3.53
3	4.61	8.60	8.05	6.45	8.50	8.15
4	7.58	9.00	7.70	8.04	8.10	8.55
5	6.55	13.30	5.21	-	-	-
6	7.33	11.80	5.87	8.04	12.50	5.54
7	5.40	12.90	5.38	8.04	12.50	5.54
8	9.66	13.10	5.29	7.58	15.80	12.65
9	12.05	18.50	3.74	-	-	-
10	-	-	-	-	-	-
	8.39±.95	13.12±1.12		7.67±.70	12.67±1.5	

*The same mice were used in both the First and the Second Experiment.

TABLE V. TRIAL 5

THE VALUES OBTAINED FOR THE PER CENT OF INJECTED DOSE, THE PER CENT OUTPUT PER DAY AND THE BIOLOGICAL HALF-LIFE FOR EACH INDIVIDUAL MOUSE (CARWORTH)

Mouse No.	First Experiment* 5/12/55			Second Experiment* 8/8/55		
	Injected with 10 uc I-131			Injected with 15 uc I-131		
	% Uptake at 48 hrs.	% Output per day	T½ in days	% Uptake at 48 hrs.	% Output per day	T½ in days
11	16.16	13.70	5.05	6.86	17.60	3.93
12	6.86	14.60	4.74	7.36	18.80	3.68
13	5.36	17.60	3.93	7.73	41.20	1.68
14	13.72	8.40	8.25	11.42	8.10	8.43
15	12.80	18.80	3.80	7.72	19.00	3.64
16	4.80	15.60	4.44	7.72	18.00	3.80
17	8.28	15.10	4.85	9.42	12.70	5.45
18	-	-	-	-	-	-
19	21.23	14.40	4.81	12.88	20.00	3.46
20	6.33	8.70	7.96	-	-	-
	10.64±2.00	14.10±1.10		8.90±.77	19.42±3.40	

*The same mice were used in both the First and the Second Experiment.

TABLE VI. TRIAL 6

THE VALUES OBTAINED FOR THE PER CENT OF THE INJECTED DOSE, THE PER CENT OUTPUT PER DAY AND THE BIOLOGICAL HALF-LIFE FOR EACH INDIVIDUAL MOUSE (CARWORTH)

Mouse No.	First Experiment* 5/23/55			Second Experiment* 7/25/55		
	Injected with 10 uc I-131			Injected with 15 uc I-131		
	% Uptake at 48 hrs.	% Output per day	T½ in days	% Uptake at 48 hrs.	% Output per day	T½ in days
21	5.60	12.70	5.45	7.30	17.20	4.02
22	7.75	14.40	4.81	7.00	9.40	7.37
23	4.82	14.30	4.84	6.47	13.00	5.33
24	6.10	11.80	5.87	-	-	-
25	6.03	15.00	4.62	-	-	-
26	5.36	15.10	4.58	6.08	12.90	5.37
27	9.90	13.90	4.98	6.63	14.50	4.77
28	15.62	22.10	3.00	8.80	26.70	2.59
29	9.50	14.80	4.68	6.56	17.60	3.93
30	13.84	14.50	4.77	6.47	17.60	3.93
	8.45±1.10	14.80±.83		6.90±.28	16.10±1.70	

*The same mice were used in both the First and the Second Experiment.

TABLE VII

RESULTS OF ANALYSIS OF VARIANCE OF I-131 UPTAKE AMONG GENETICALLY DIFFERENT GROUPS OF MICE AT 48 HOURS AFTER INJECTION

Source of Variance	Degree of Freedom	Mean Squares	F
Total	119		
Genotypes	5	1547.05	39.69
Sex	1	77.28	1.98
Genotype x Sex	5	1576.35	40.45
Error	108	38.97	

$P < .01$

Level of significance between means of two groups at t .05 and 38 degrees of freedom - 2.60. It was computed as follows (Snedecor, 1946):

$$\sigma_{m_1-m_2} = \sqrt{\frac{2s^2}{n}} = 1.28$$

$n = 20$, the number of individuals in each group

$s^2 = 16.43$, the variance within each group; here the mean square in the error term was used.

$$P\ .05(38 \text{ degrees of freedom}) = 2.03 = \frac{(m_1-m_2) - 0}{m_1-m_2}$$

$$m_1-m_2 = 2.598 \doteq 2.60$$

TABLE VIII

RESULTS OF ANALYSIS OF VARIANCE OF I-131 OUTPUT RATE CONSTANT AMONG GENETICALLY DIFFERENT GROUPS OF MICE

Source of Variance	Degree of Freedom	Mean Squares	F
Total	119		
Genotype	5	429.64	26.15
Sex	1	28.72	1.74
Genotype x sex	5	460.62	28.04
Error	108	16.43	

Level of significance between means of two groups at P .05 and 38 degrees of freedom = 4.02.

TABLE IX

RESULTS OF ANALYSIS OF VARIANCE OF THYROID SECRETION RATE AMONG GENETICALLY DIFFERENT GROUPS OF MICE

Sources of Variance	Degree of Freedom	Sum of Squares	Mean Squares	F
Total	120	241.81		
Genotypes	9	139.85	15.54	16.925
Error	111	101.97	.918	

$$K. = \frac{1}{11}\left[\sum n_i - \frac{n^2}{n_i}\right] = \frac{1}{11}\left[121 - \frac{122}{121}\right] = \frac{1}{11}\left[11.9\right] = 10$$

$$\sigma_{\bar{x}} = \sqrt{\frac{.918}{10}} = .302$$

A + P.05 significant differences among different strains of mice is as follows:

C57BL/6 $>$ all other strains but BBF_1

BBF_1 $>$ all other strains but C57BL/6 ♂ and ♀

C57BR/cd ♂ $>$ C57BR/cd ♀, A/Jax, BALB/c and CAF_1

No difference between A/Jax, BALB/c and CAF_1

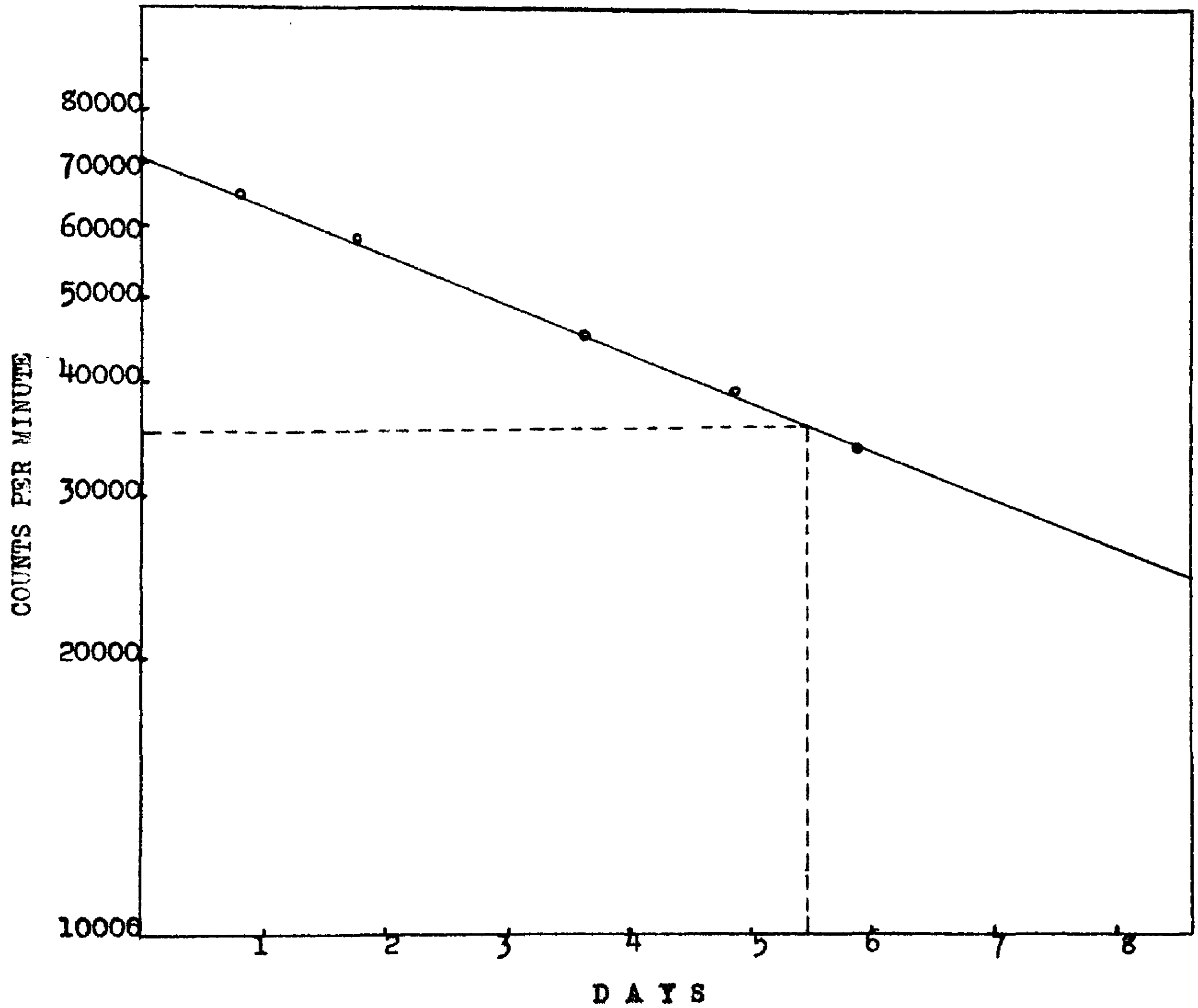

Fig. 1. Loss of the activity of carrier-free I-131 standard in counts per minute, drawn on semi-log scale against time in days(see text).

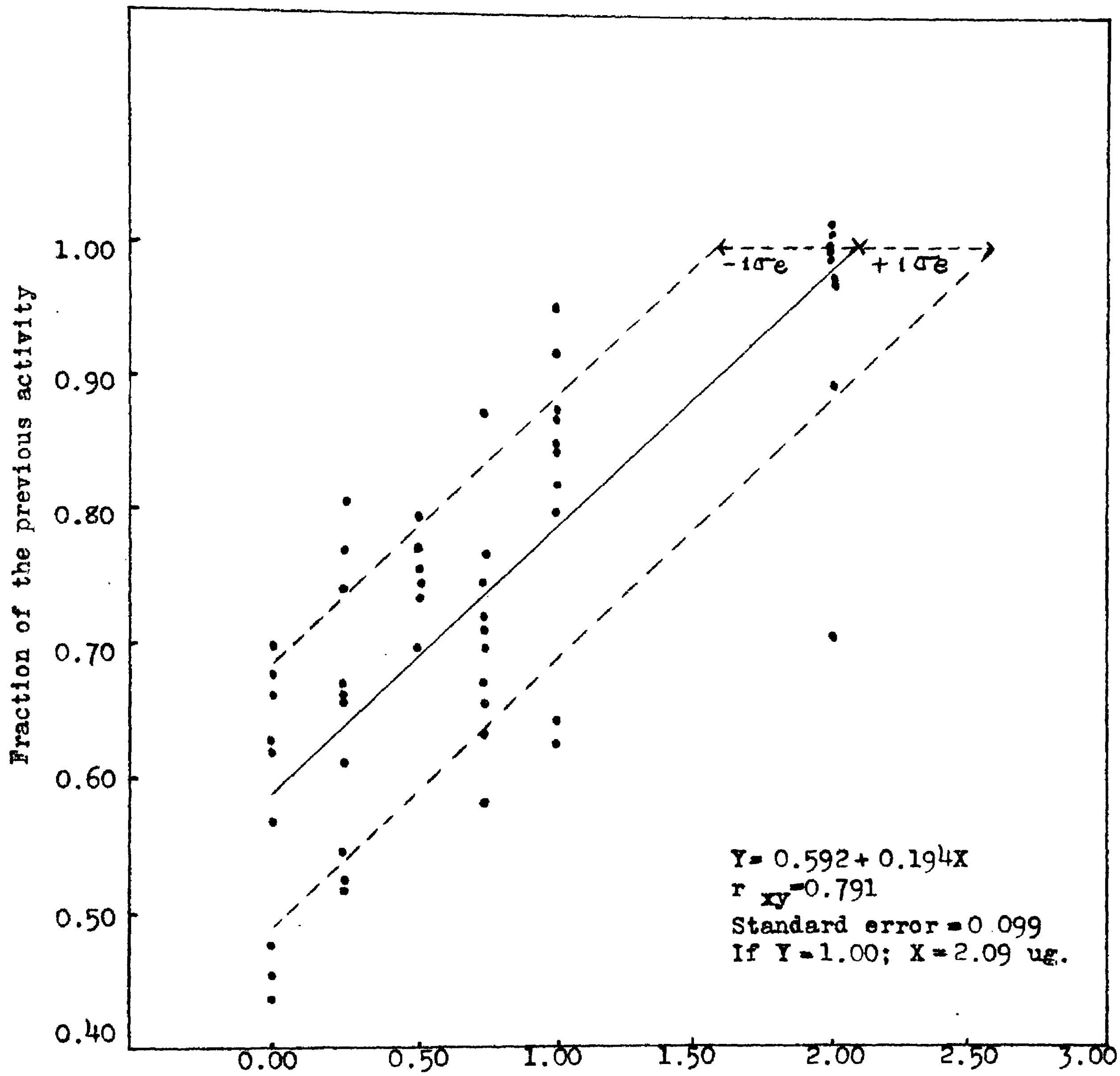

Fig. 2. Predicting daily thyroid secretion rate from per cent of previous count on a group of ordinary mice.

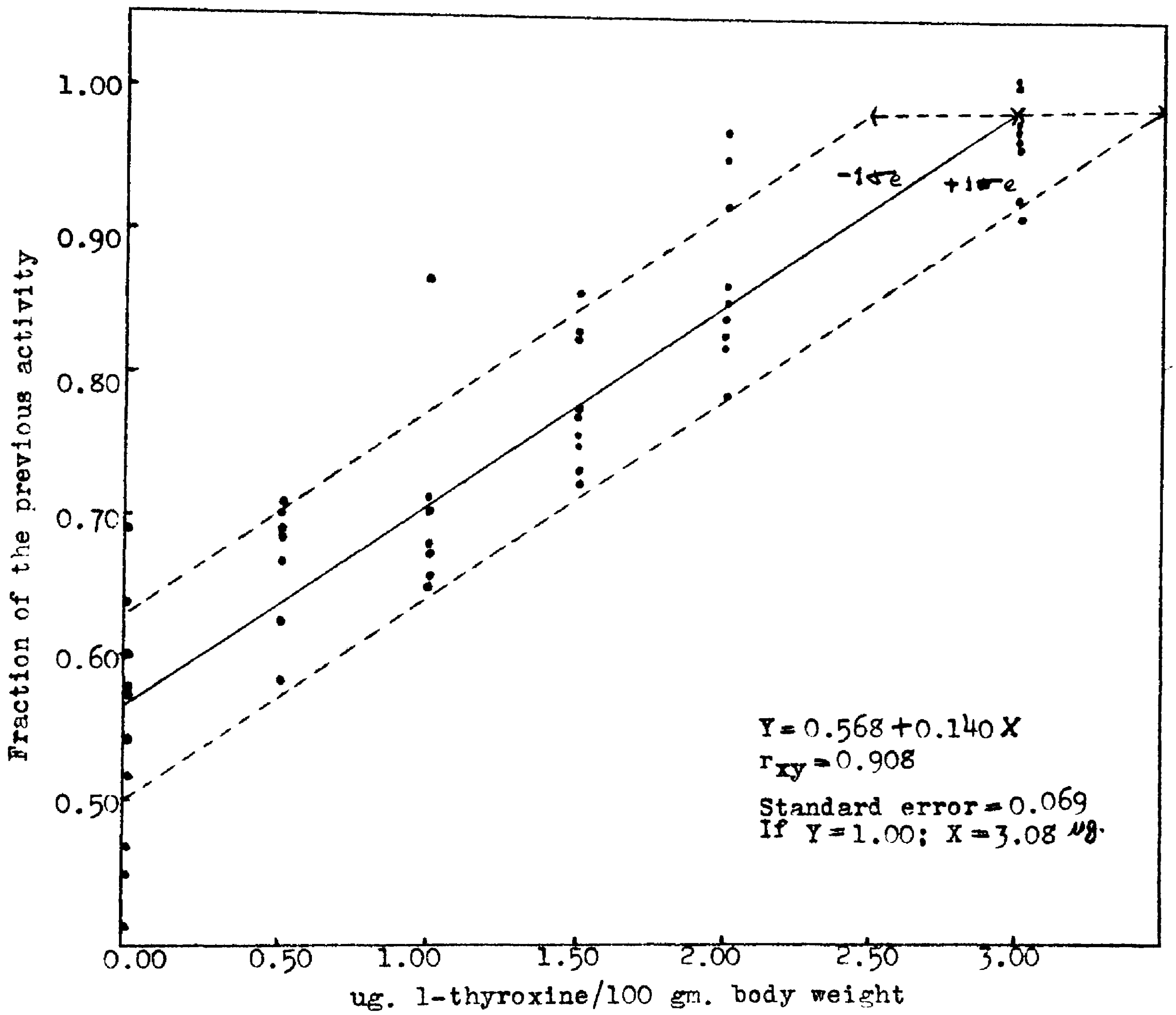

Fig. 3. Predicting daily thyroid secretion rate from per cent of previous count on a group of ordinary mice.

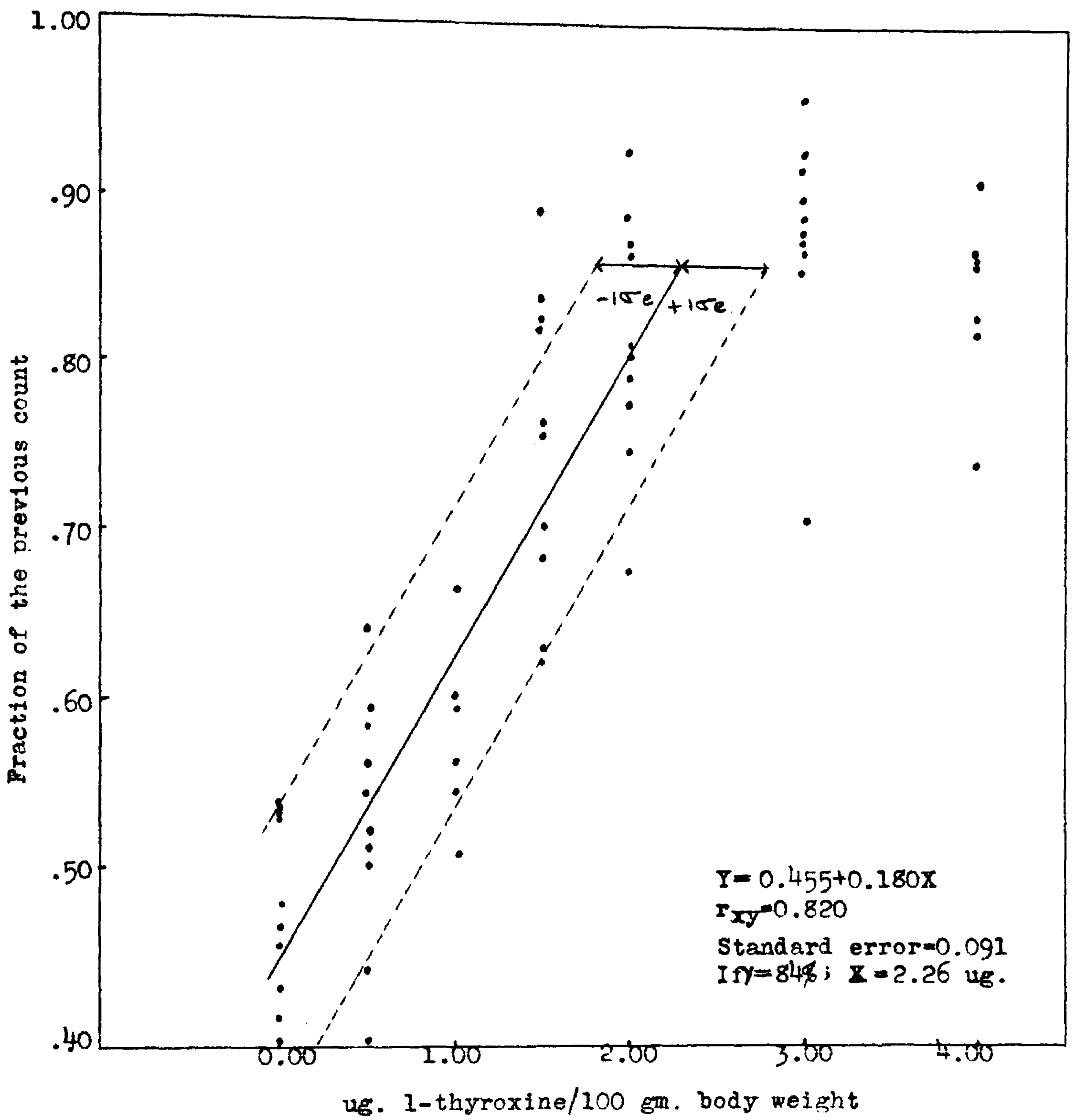

Fig. 4. Predicting daily thyroid secretion rate from per cent of previous count on a group of ordinary mice.

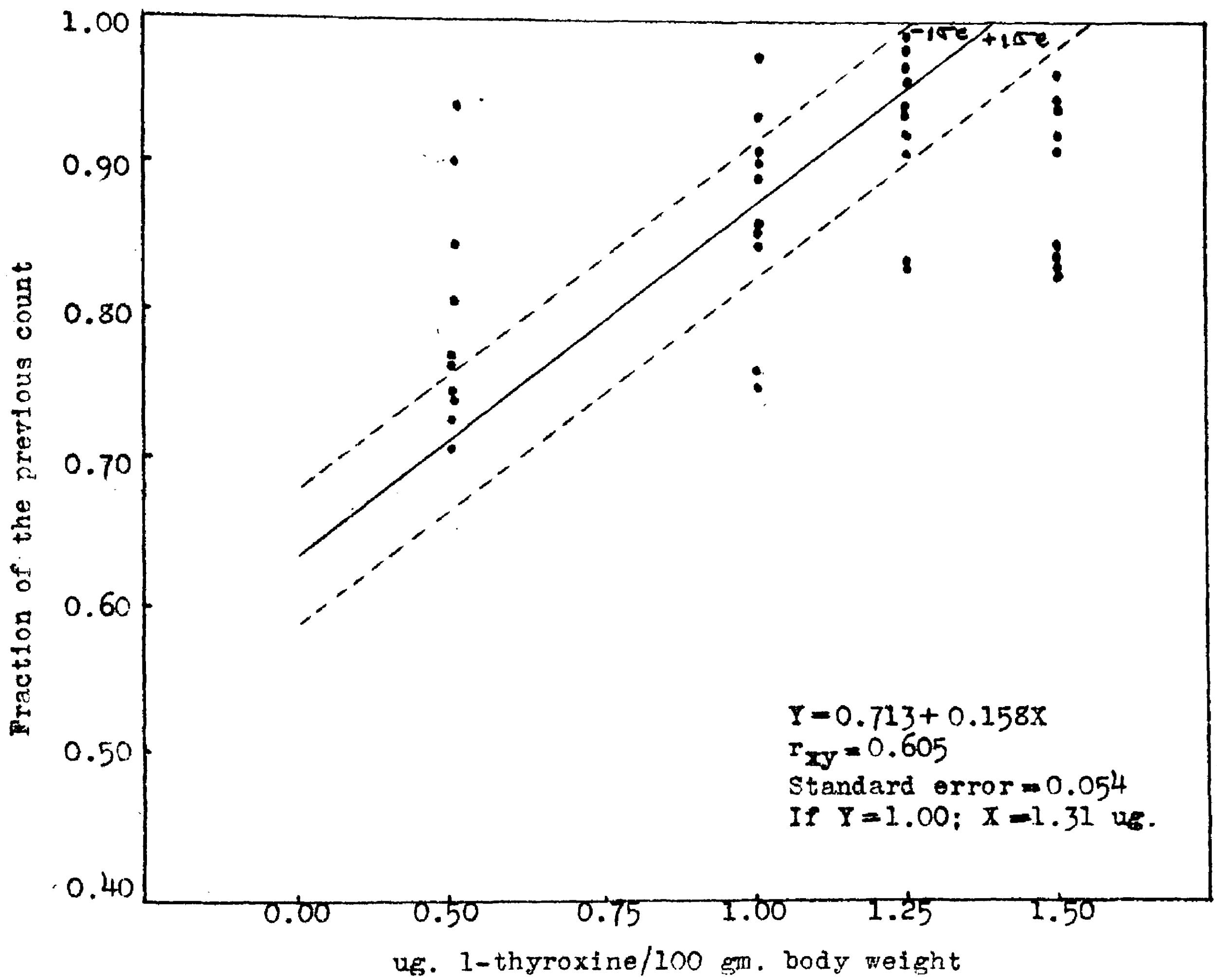

Fig. 5. Predicting daily thyroid secretion rate from per cent of previous count on a group of ordinary mice.

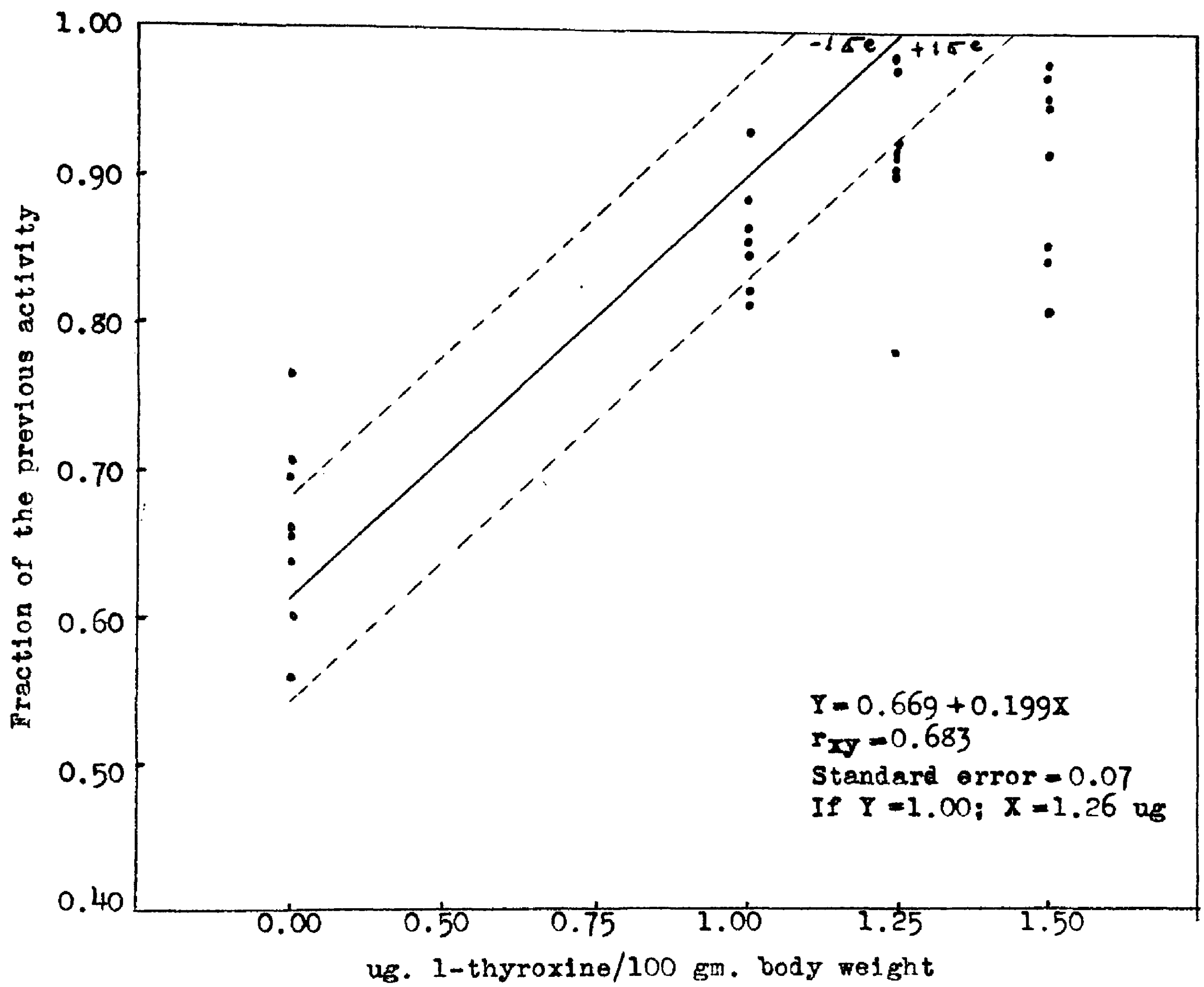

Fig. 6. Predicting daily thyroid secretion rate from per cent of previous count on a group of ordinary mice.

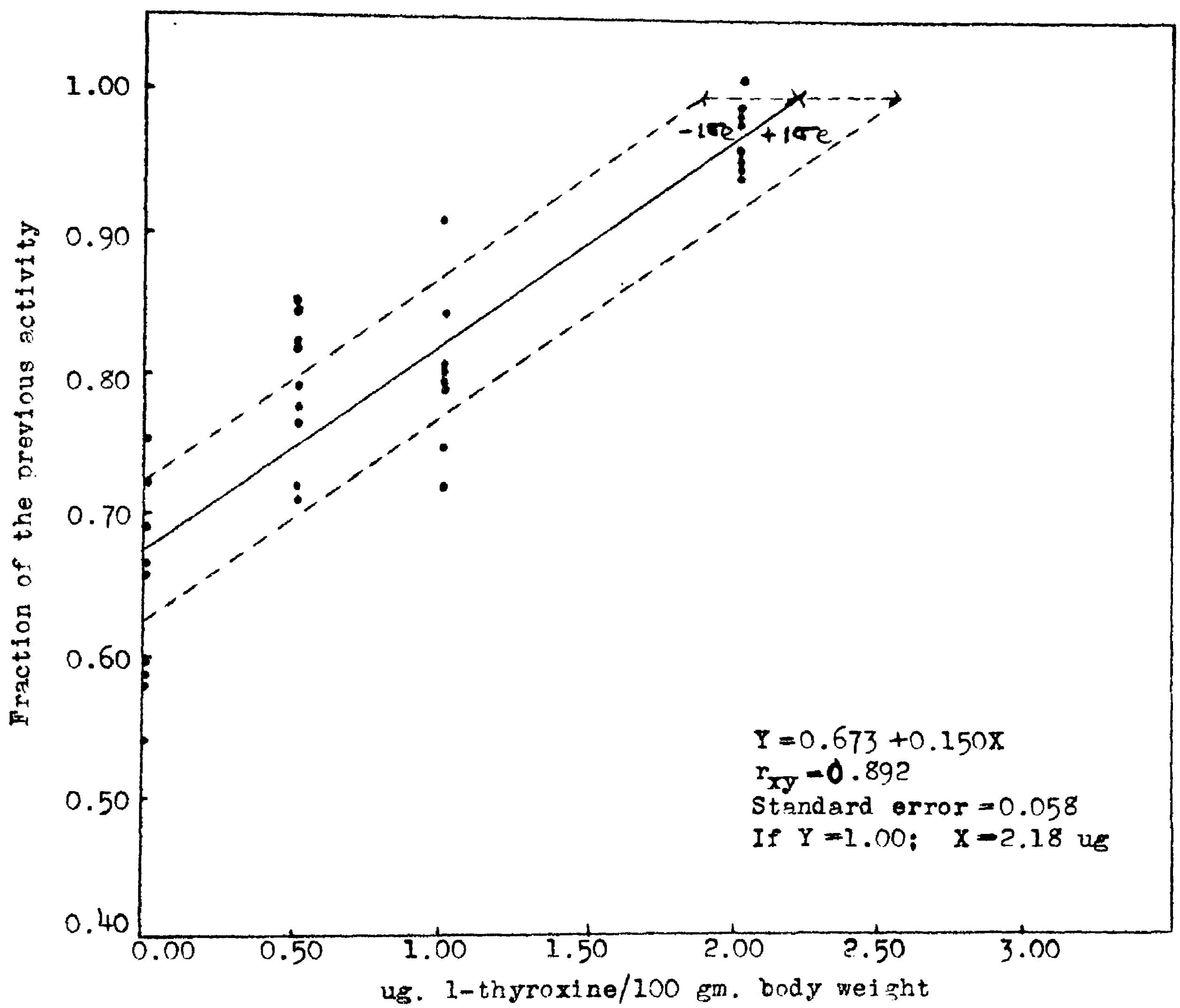

Fig. 7. Predicting daily thyroid secretion rate from per cent of previous count on a group of ordinary mice.

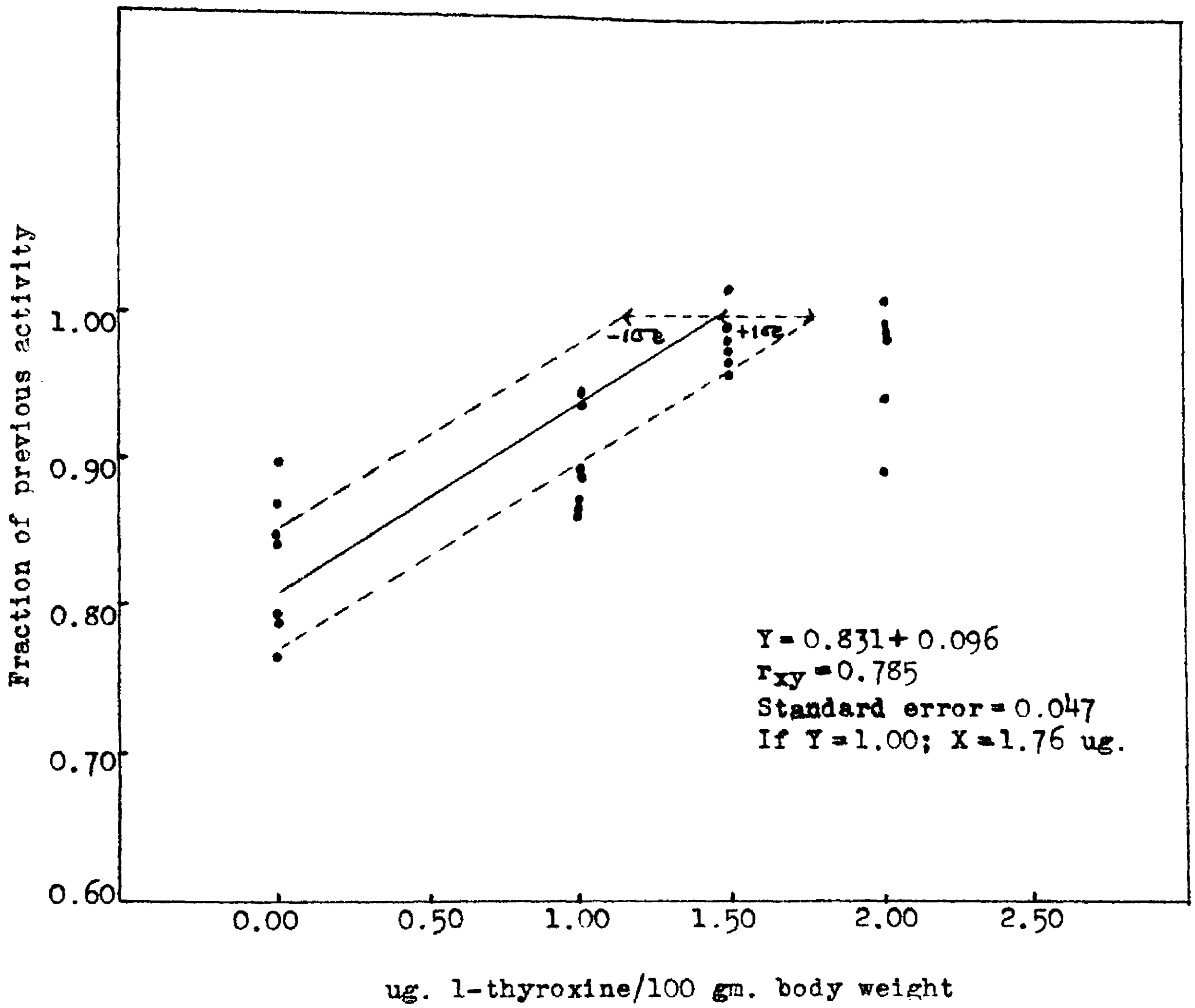

Fig. 9. Predicting daily thyroid secretion rate from per cent of previous count on a group of ordinary mice.

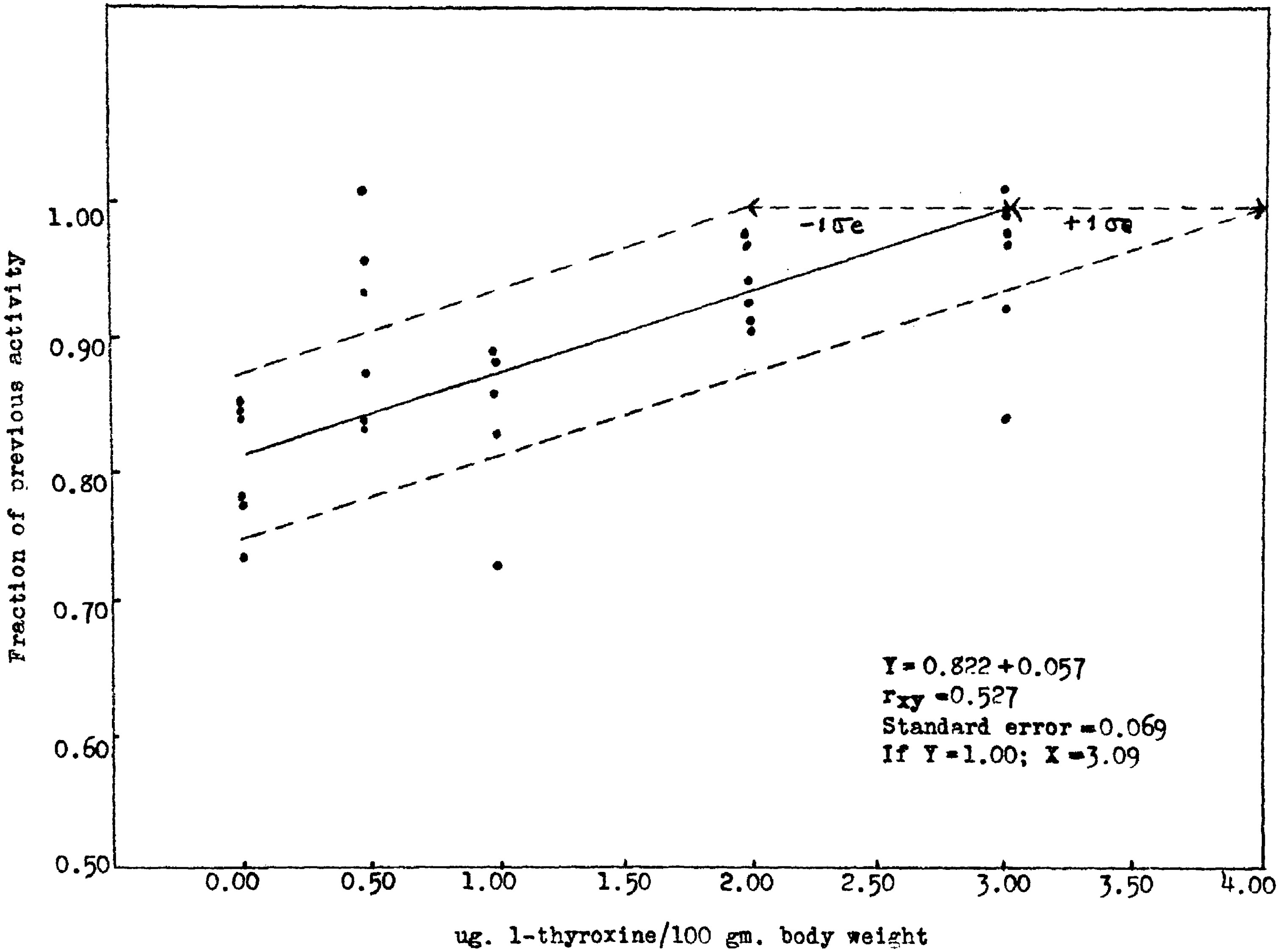

Fig. 9. Predicting daily thyroid secretion rate from per cent of previous count on a group of ordinary mice.

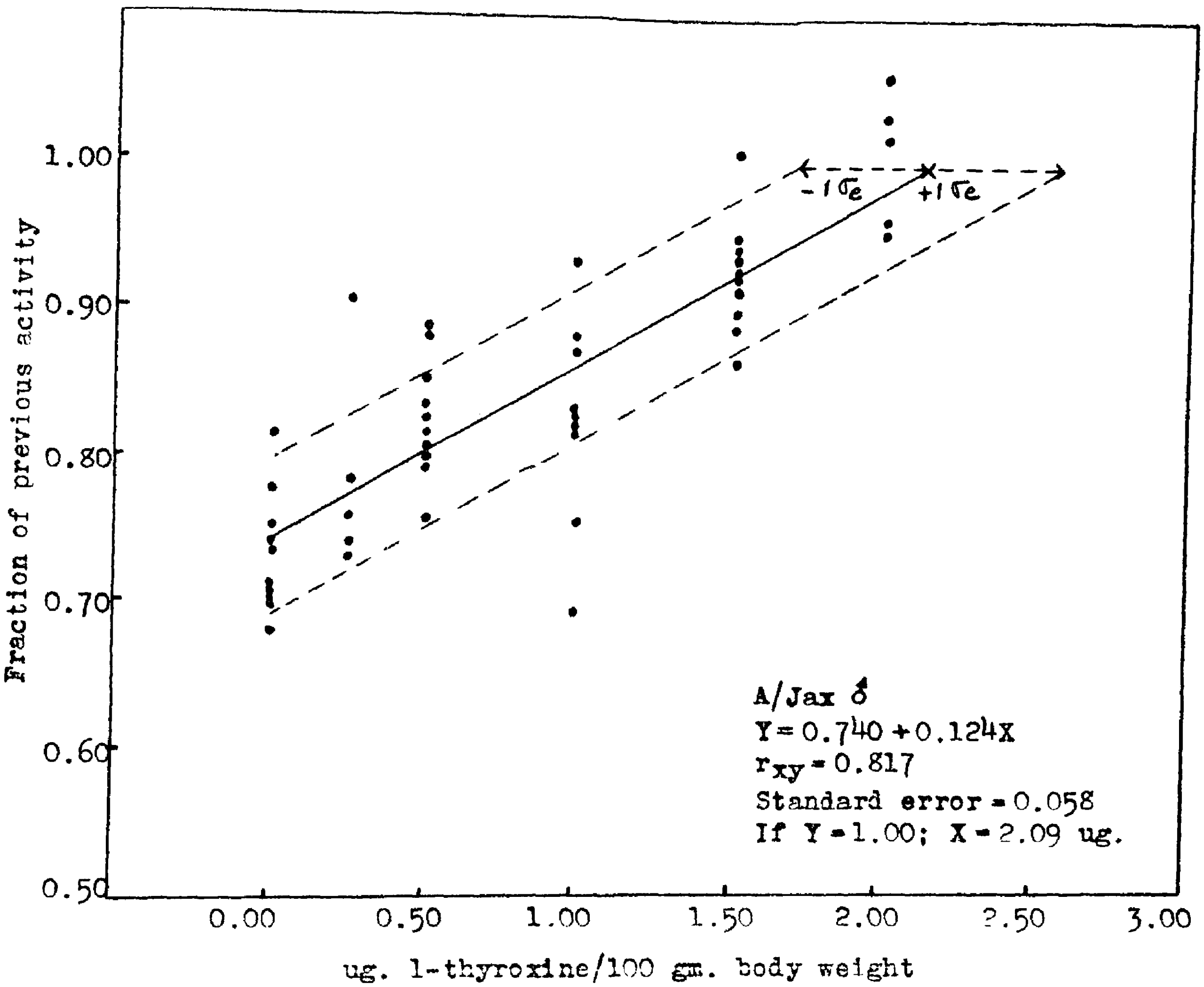

Fig. 10. Predicting daily thyroid secretion rate from per cent of previous count on strain A/Jax

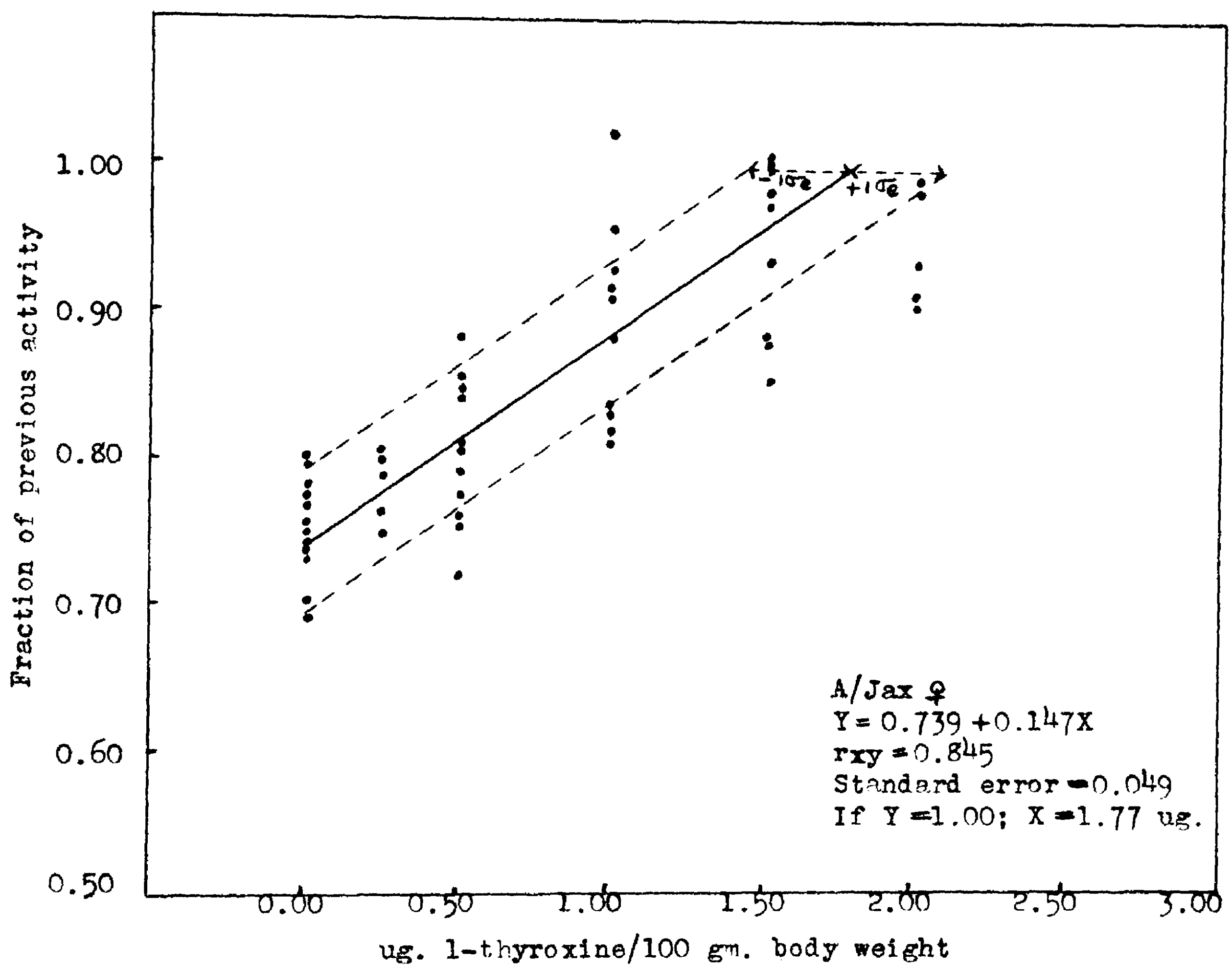

Fig. 11. Predicting daily thyroid secretion rate from per cent of previous count on strain A/Jax

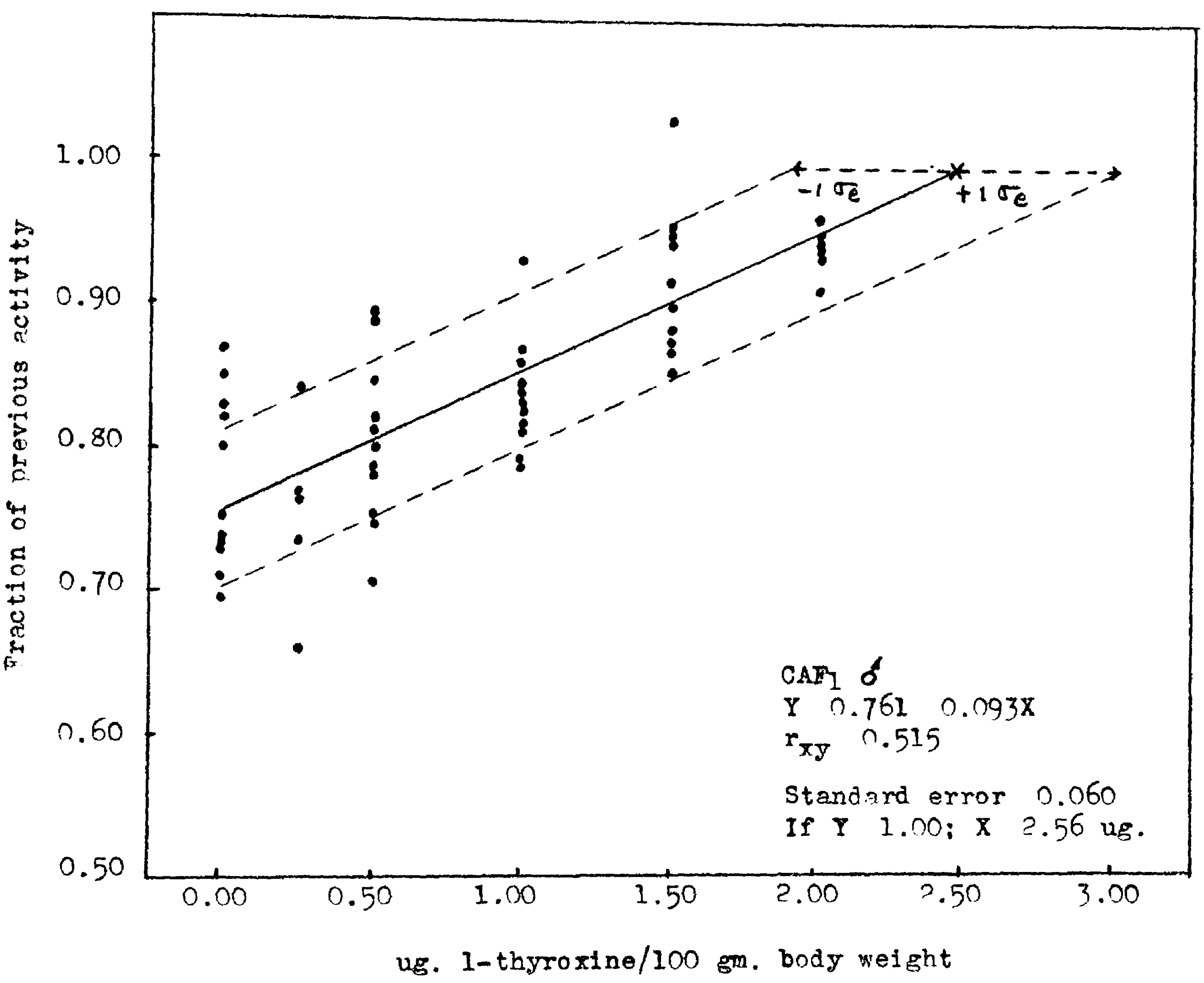

Fig. 12. Predicting daily thyroid secretion rate from per cent of previous count on strain CAF_1

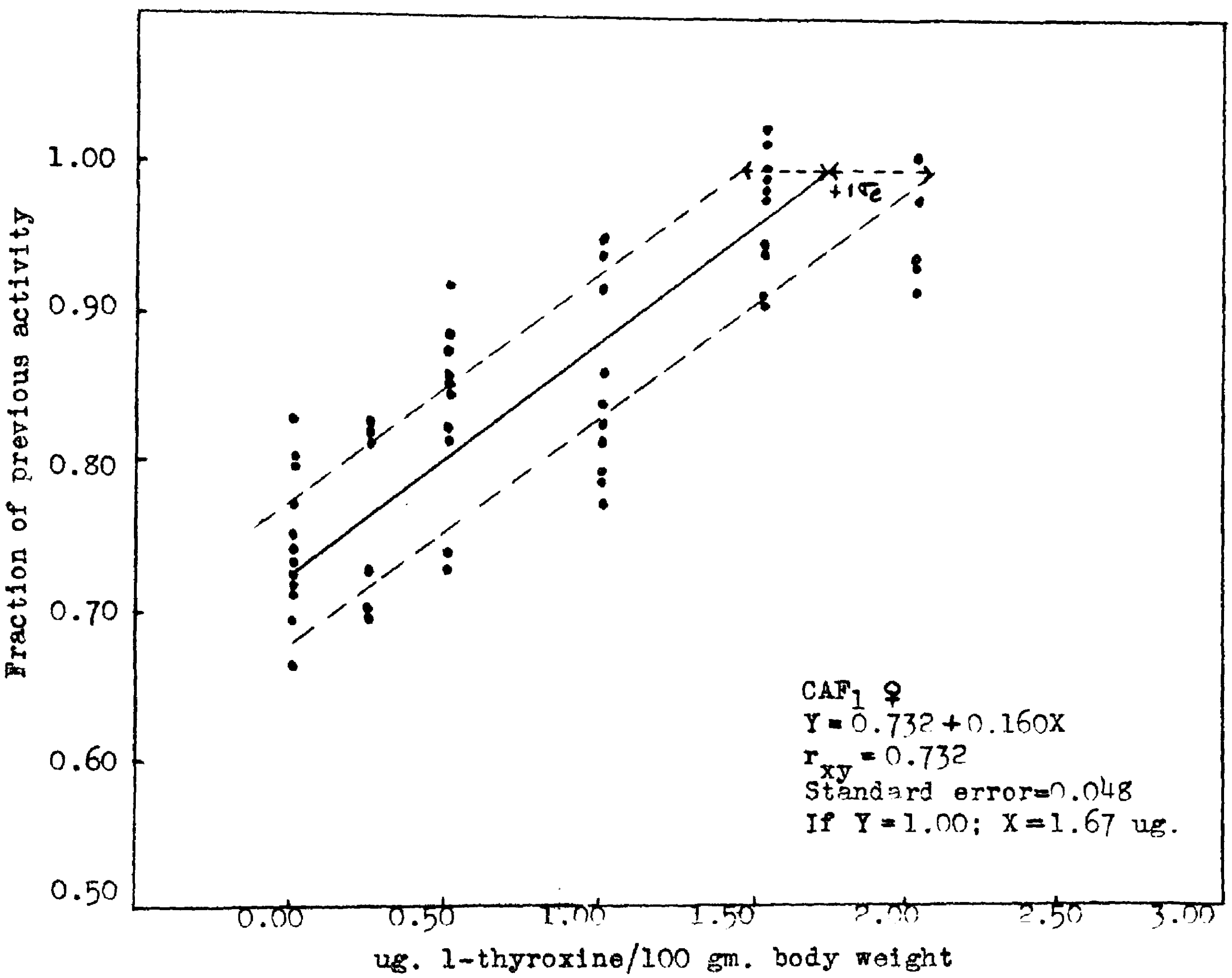

Fig. 13. Predicting daily thyroid secretion rate from per cent of previous count on strain CAF_1

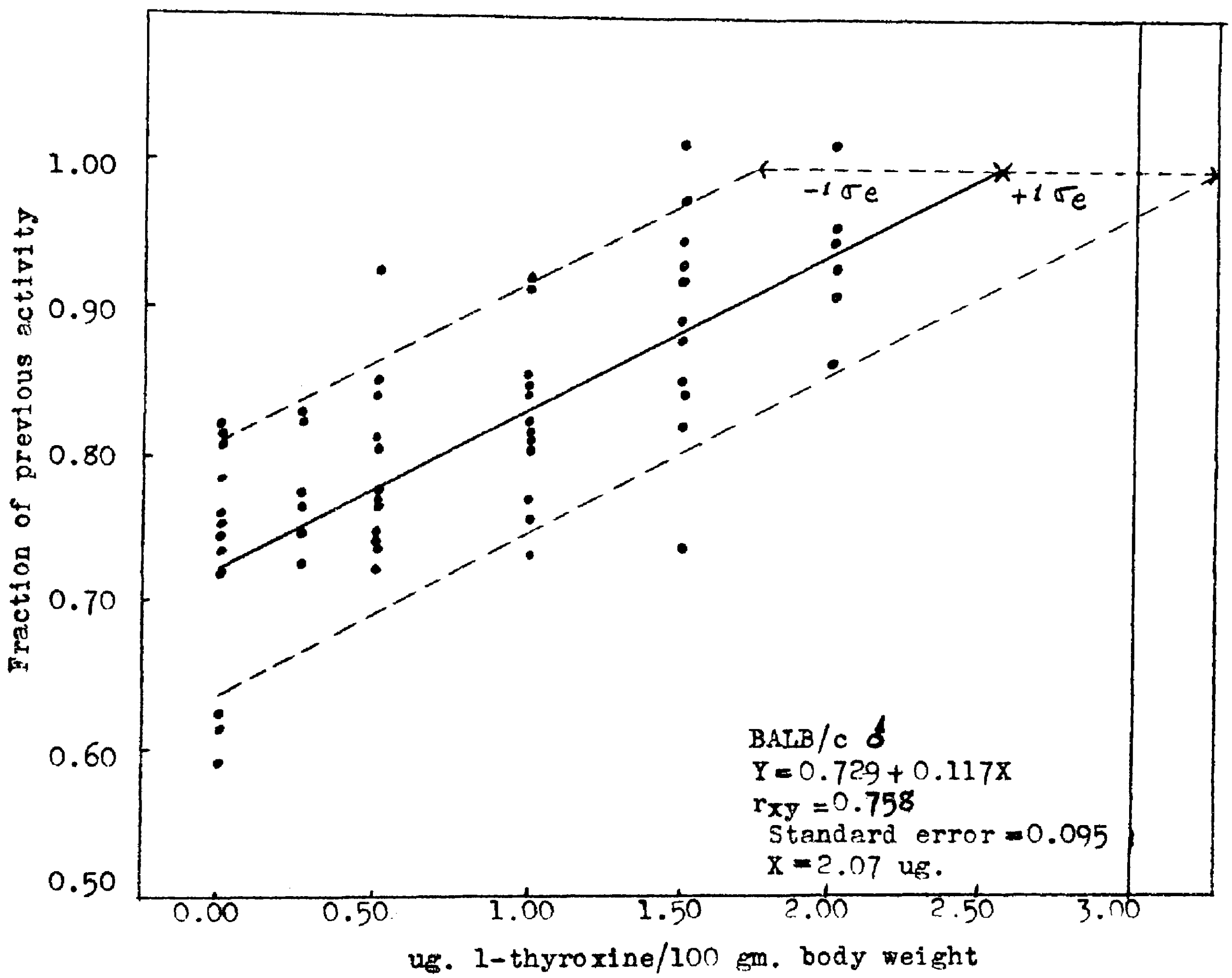

Fig. 14. Predicting daily thyroid secretion rate from per cent of previous count on strain BALB/c

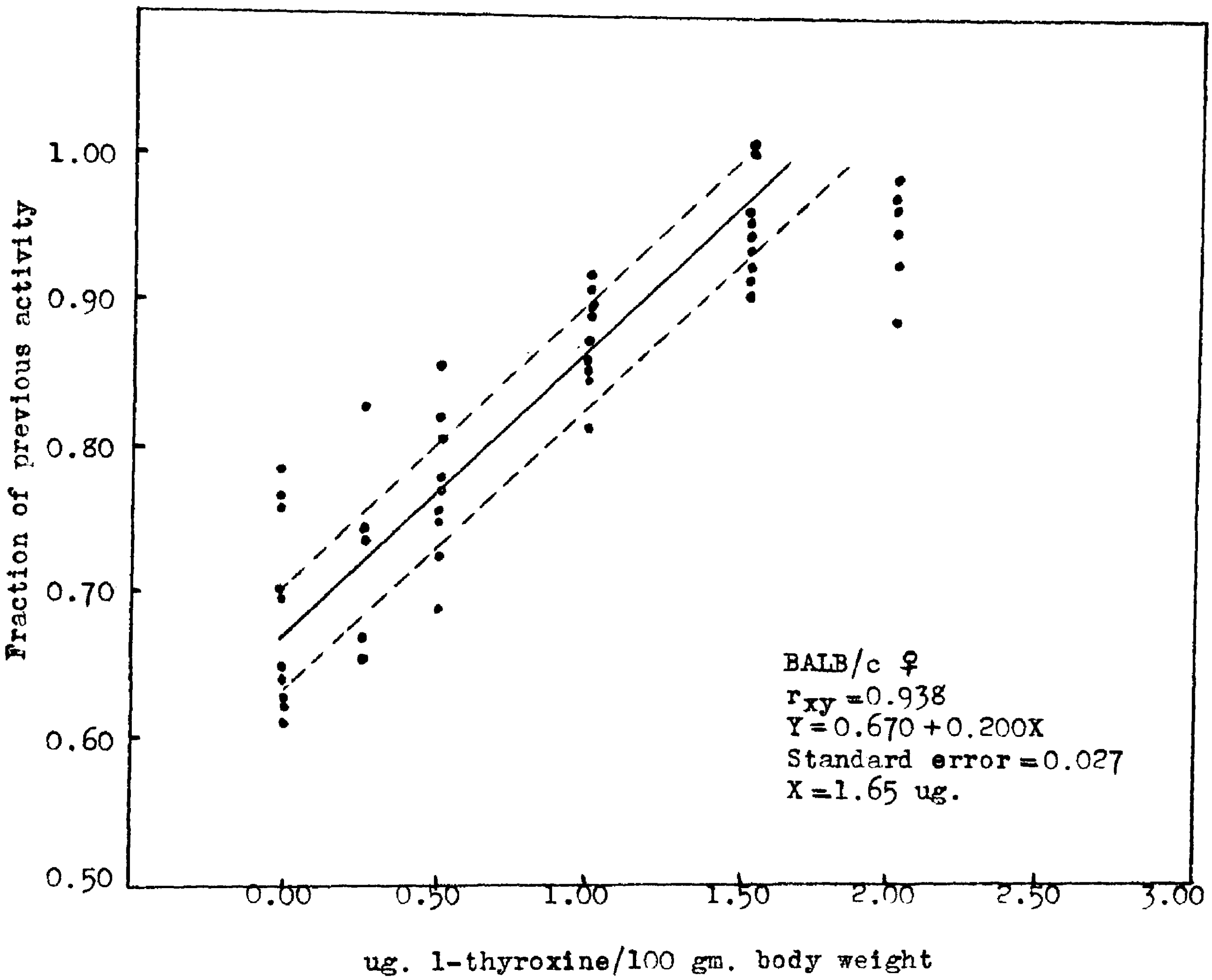

Fig. 15. Predicting daily thyroid secretion rate from per cent of previous count on strain BALB/c

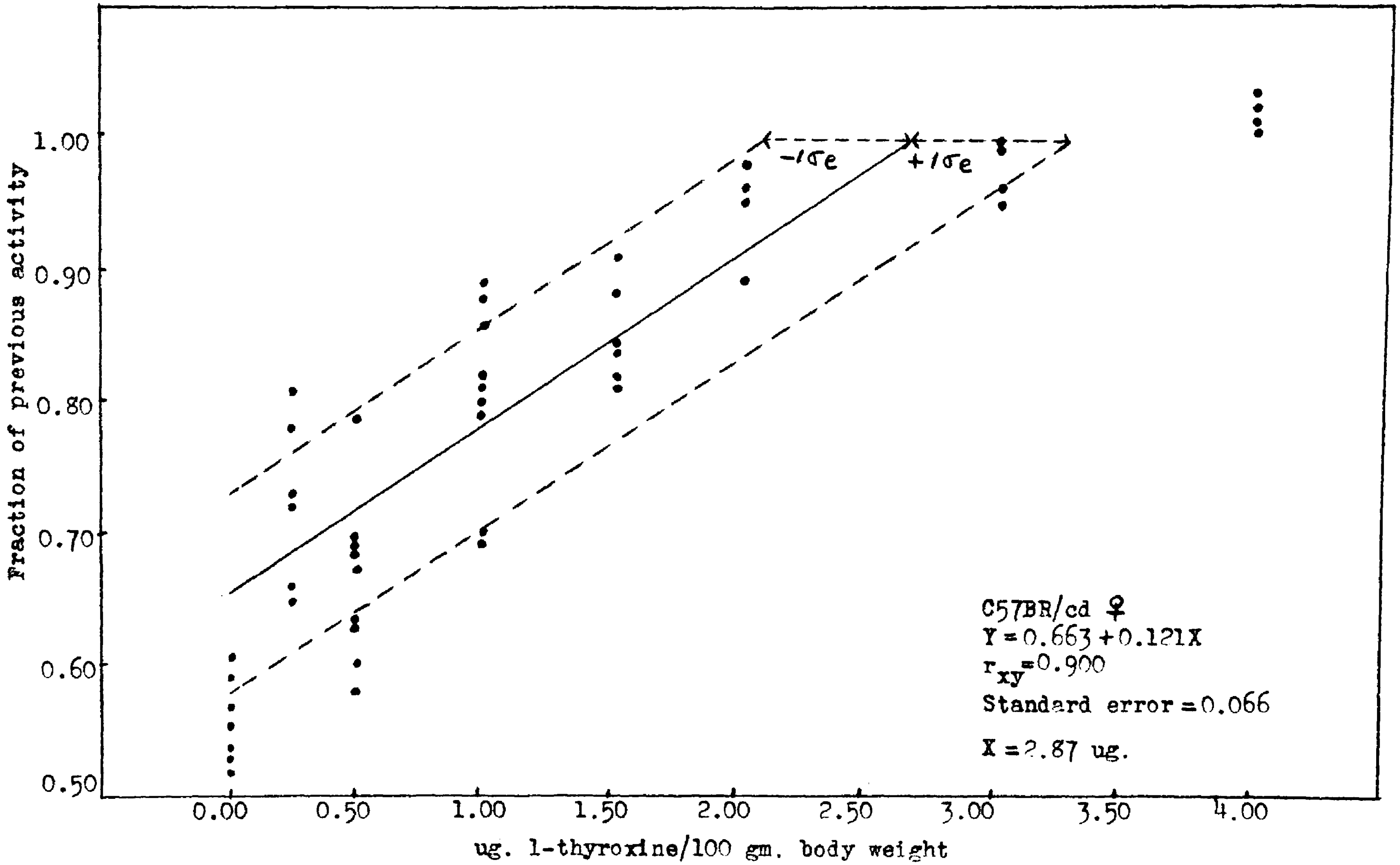

Fig. 16. Predicting daily thyroid secretion rate from per cent of previous count on strain C57BR/cd

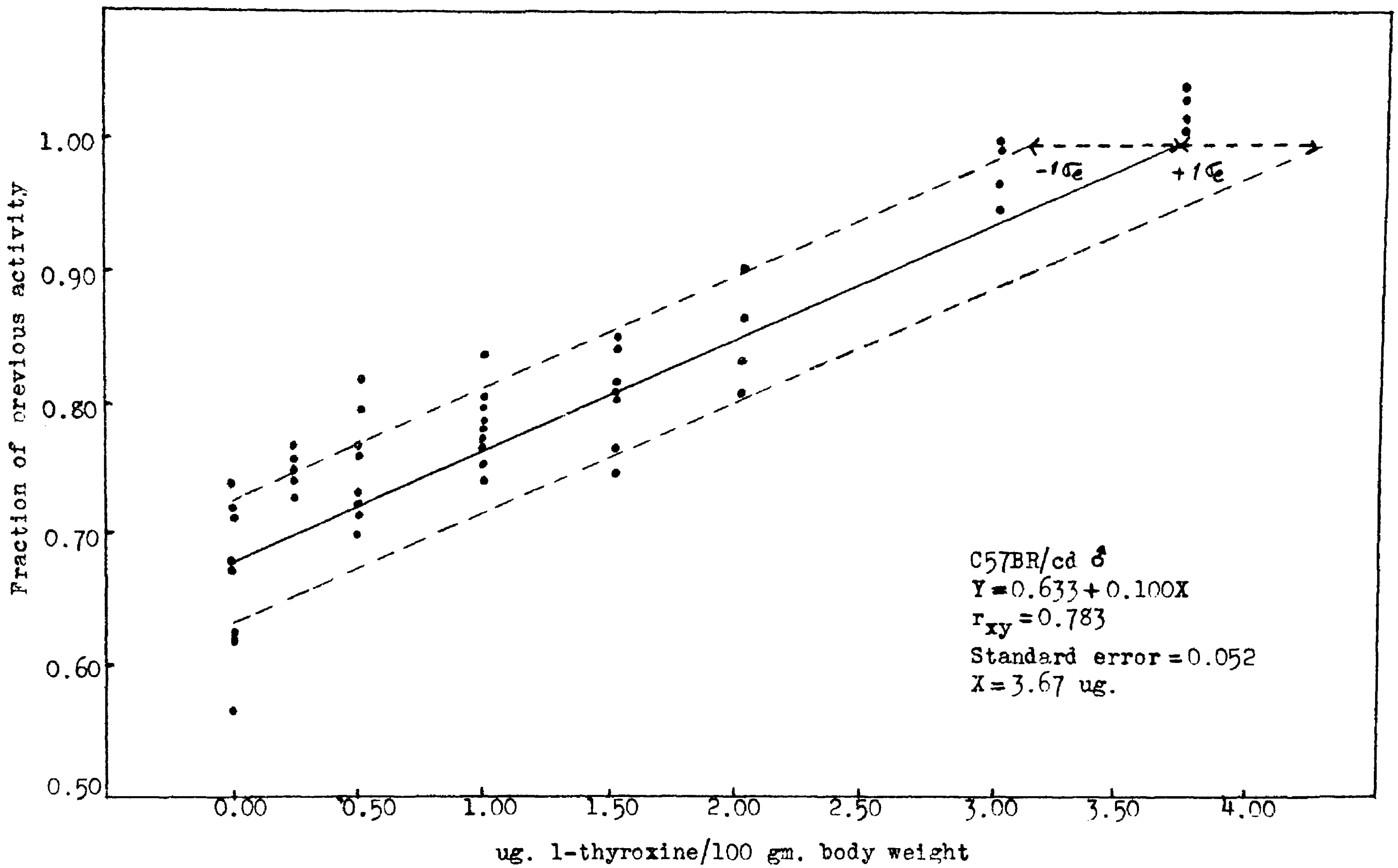

Fig. 17. Predicting daily thyroid secretion rate from per cent of previous count on strain C57BR/cd

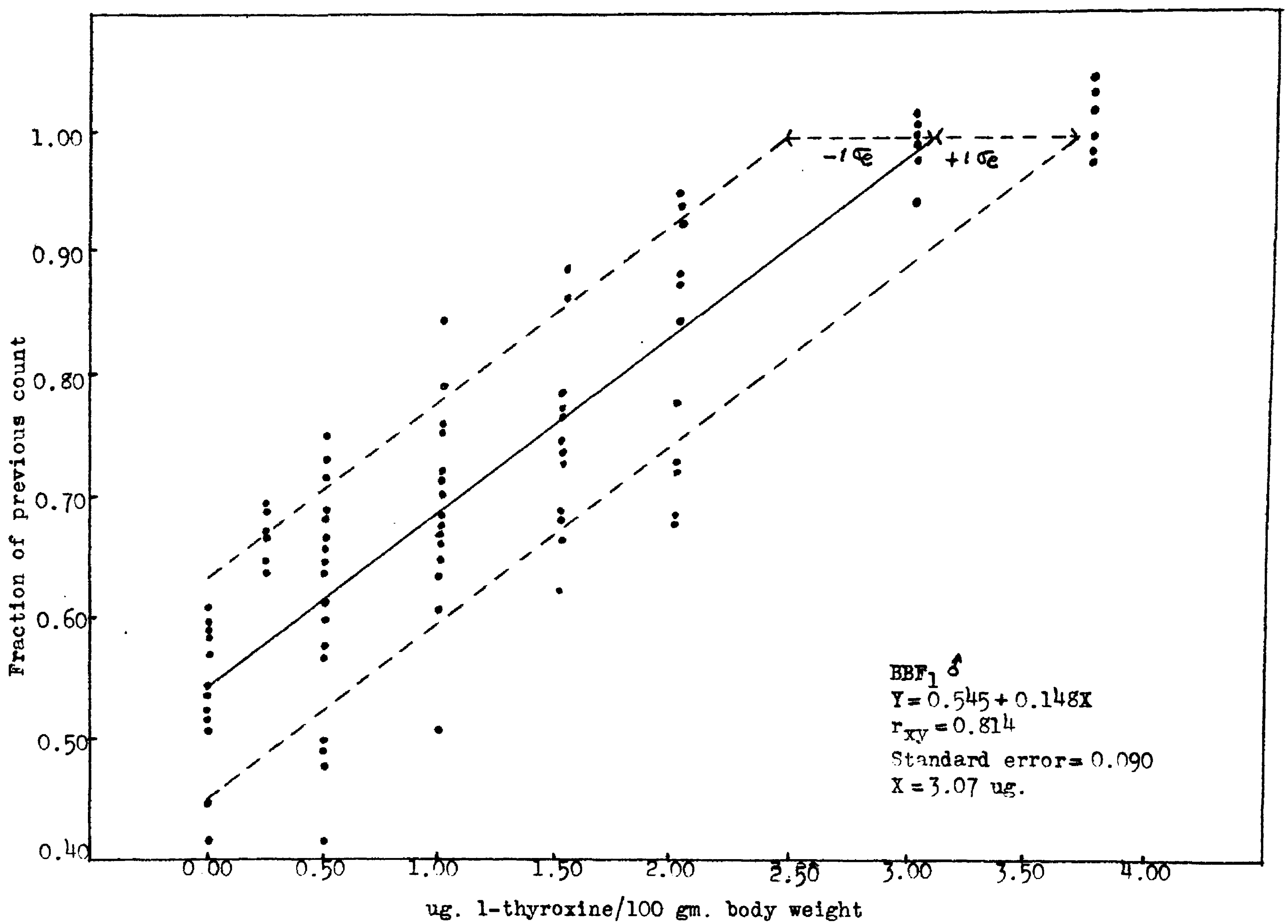

Fig. 18. Predicting daily thyroid secretion rate from per cent of previous count on strain BBF$_1$

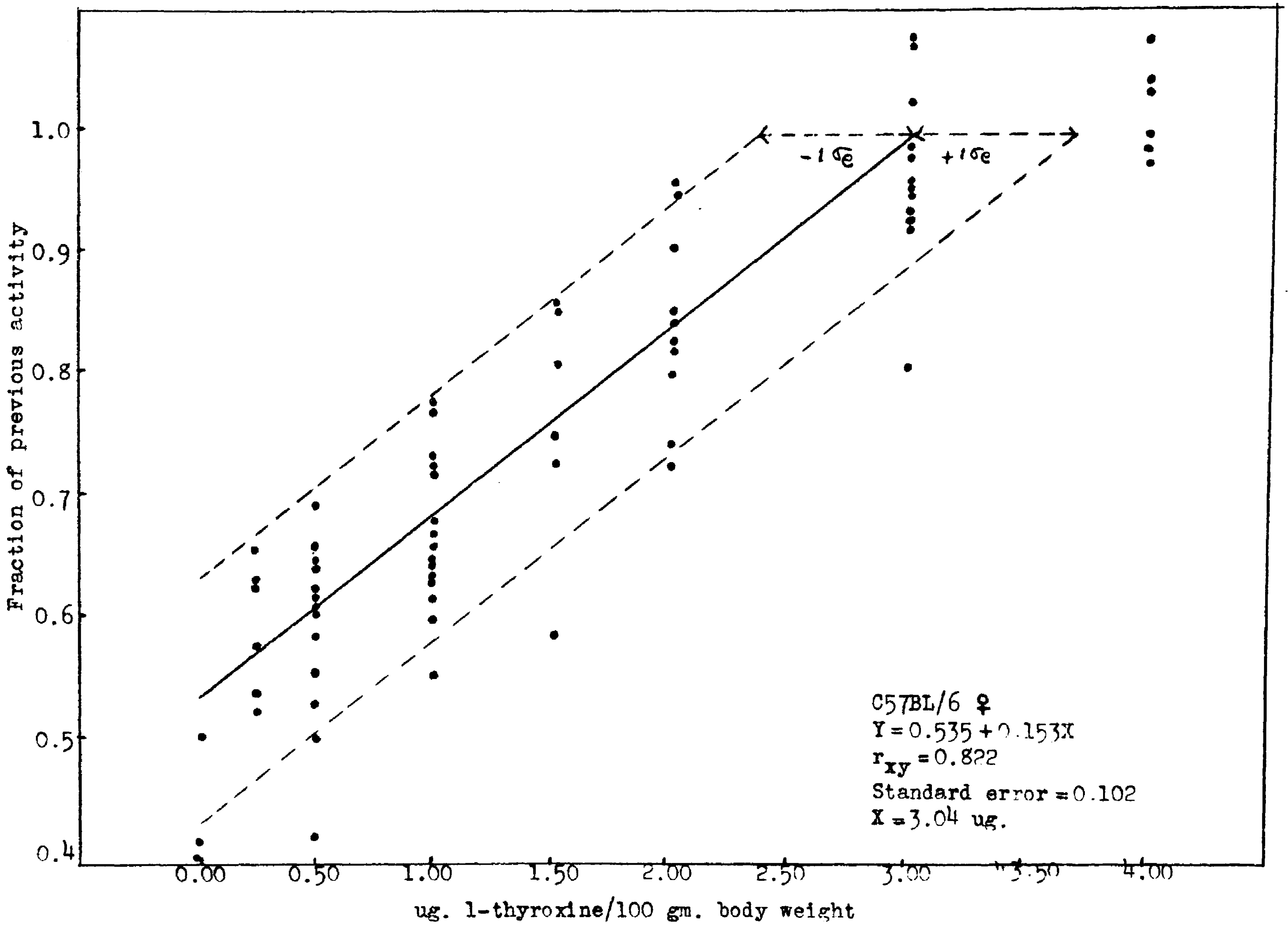

Fig. 1?. Predicting daily thyroid secretion rate from per cent of previous count on strain C57BL/6

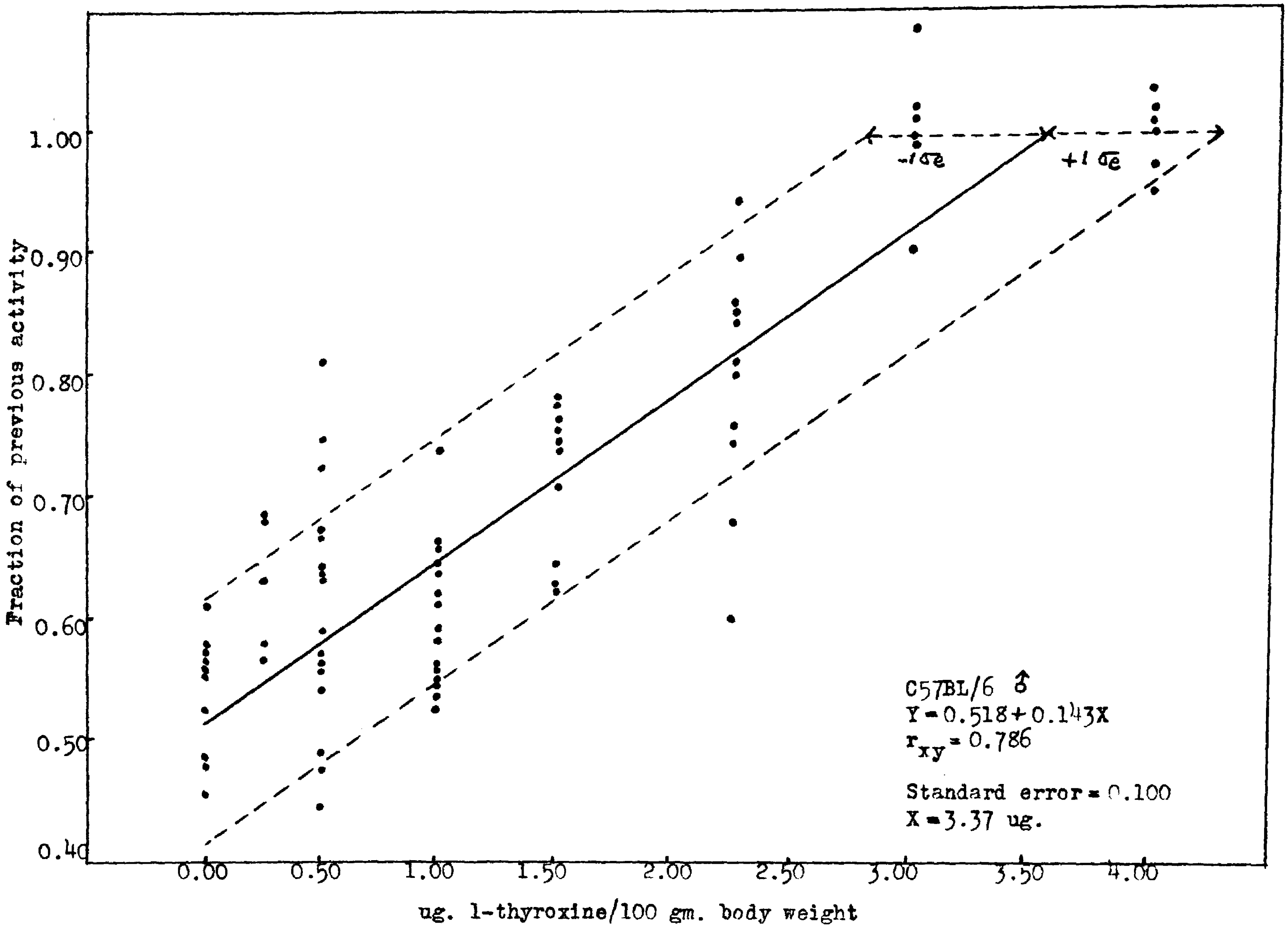

Fig.20. Predicting daily thyroid secretion rate from per cent of previous count on strain C57BL/6

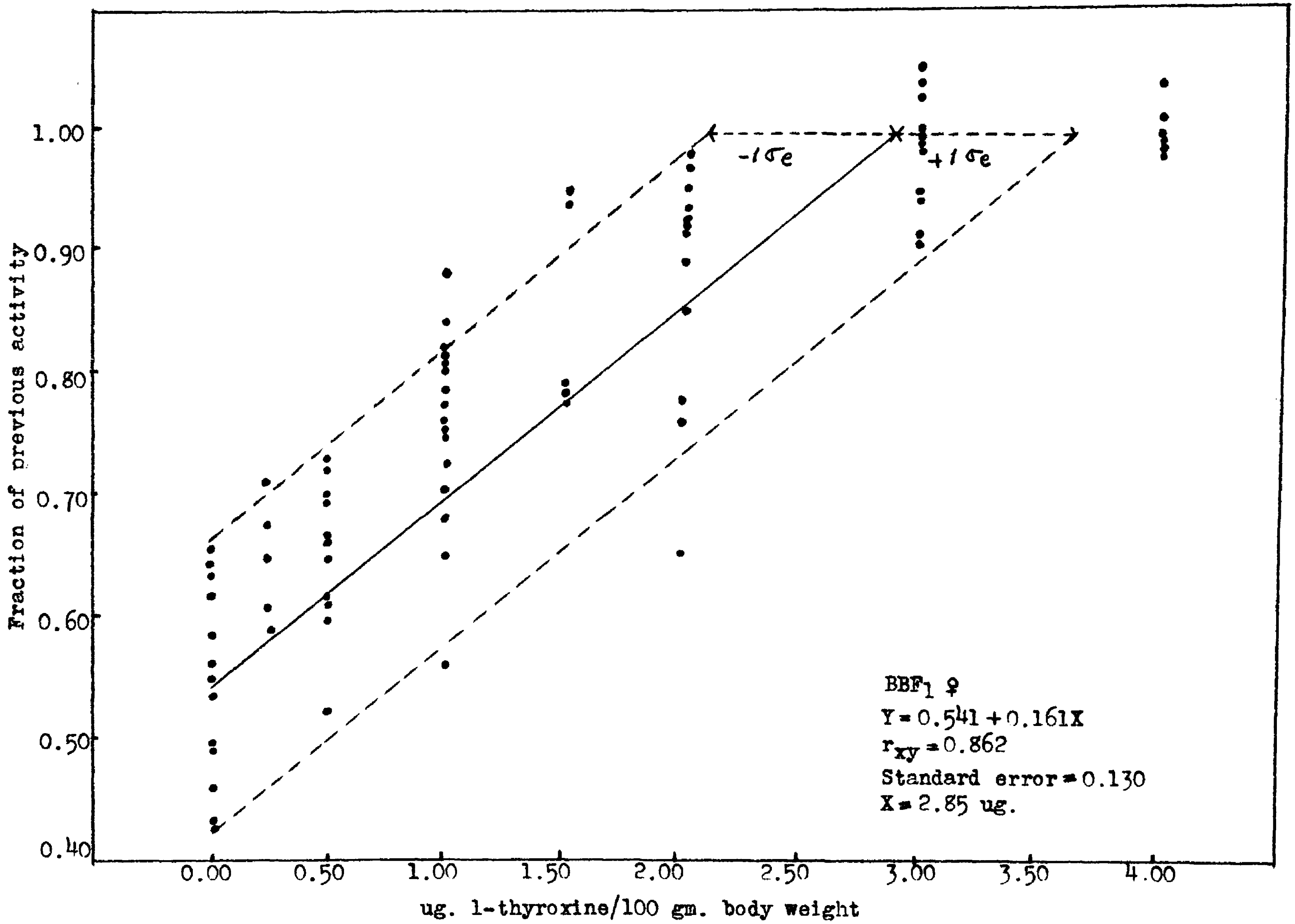

Fig. 21. Predicting daily thyroid secretion rate from per cent of previous count on strain BBF$_1$