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The Effect of Prolonged Thyroxine Treatment on the Ageing Male Rat*

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The role of the thyroid gland in the process of ageing has not been properly evaluated. *Korenchevsky* and associates (1948, 1950) showed that the natural involution of many organs in the ageing rat is reversed during treatment with thyroid hormone. The possibility that ageing could be due to hypofunction of the thyroid gland is further suggested by the similarities between the syndromes of hypothyroidism and ageing (*Horsley*, 1884; *Lorand*, 1904; *Hogg*, 1942; *Goldzieher*, 1946; *Basylewycz*, 1949). Evidence of reduced secretion of thyroxine in the old rat has been given by *Grad and Hoffman* (1955), *Verzár and Freydberg* (1956) and *Wilansky* et al. (1957).

On the other hand, *Brailsford Robertson* (1928) found that continuous treatment of the mouse with desiccated thyroid, in quantities that stimulated growth, shortened the life duration.

The purpose of this investigation is to determine the effect of prolonged thyroxine treatment in middle age, the stage in life which precedes the main degenerative phase, on the course of ageing in various body functions in the male rat.

Materials and Methods

One hundred and ten male rats of a non-inbred Wistar strain were housed in 11 cages in a small air-conditioned room (temperature $25 \pm 1°$ C, relative humidity $55 \pm 10\%$) and fed a pelleted diet as described previously (*Everitt*, 1958a).

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Rats were weighed on an Ohaus sliding weight animal balance, at weekly intervals from weaning until natural death. Animals were grouped on the basis of their relative weight increment between the ages of 300 and 400 days, $\frac{W_{400}-W_{300}}{W_{400}}$, which is directly correlated with life duration in these rats (*Everitt and Webb*, 1957). The 10 rats with the lowest weight increments were discarded, as they were sick and therefore had a short life expectancy. Of the remaining 100 rats, 50 were used for studying the effects of small (physiological) doses of thyroxine on the course of ageing, and 50 for studying the effects of moderately large doses.

In the small dosage experiment 25 rats were treated with thyroxine and 25 control rats with saline. Rats were carefully matched on the basis of their relative weight increments before assigning at random to these two treatment groups. Thyroxine was injected subcutaneously once daily for 6 days per week, on alternate weeks, between the ages of 400 and 600 days. From the data of *Grad and Hoffman* (1955) it was calculated that the middle aged male rat secretes about 10 μg of L-thyroxine per day. Therefore the daily dose of thyroxine was raised from 5 μg in the first week, to 10 μg in the third week and 20 μg in the fifth week, during which time careful studies of body weight changes were made. Injections were continued at the dosage level of 20 μg for the remainder of the treatment period. Each rat received a total of about 2 mg of L-thyroxine in 90 divided doses.

Thyroxine solutions were prepared twice weekly and stored at $-10°$ C when not in use. The required weight of L-thyroxine sodium (*L. Light & Co.*) was dissolved in the minimum volume of 0.1N NaOH and then diluted with 0.9% NaCl. Control rats received subcutaneous injections of 0.5 ml of 0.9% NaCl of pH 9 at the same times as the thyroxine treated rats. Solutions were warmed to 37° C before injection.

In the large dosage experiment 25 rats were originally assigned at random to thyroxine treatment and 25 to saline treatment. Due to the accidental death of 2 thyroxine treated rats and the necessary rejection of their matched controls, the group size was reduced to 23. Thyroxine was administered at the dosage level of 100 μg per day for 6 days per week, on alternate weeks (to avoid intoxication), during middle age from 400 to 600 days of age. Each rat received a total of about 9 mg of L-thyroxine sodium in 90 divided doses during the period of treatment.

Using methods described in greater detail elsewhere (*Everitt*, 1958a, b, c and d; *Everitt and Webb*, 1958) metabolic, excretory, haematological and electrocardiographic studies were performed on these rats in young adult life before treatment was commenced, in middle age during treatment, and in old age about 150 days after the discontinuation of treatment.

In the metabolic and excretory studies rats were placed individually in metabolism cages, and after allowing two days for adaptation, the consumption of food and water and the production of faeces and urine were determined over a 24-hour period. Urinary creatinine was determined by the alkaline picrate method of *Folin* (1914), uric acid by the arsenophosphotungstate method of *Benedict and Franke* (1922), protein by the trichloracetic acid turbidimetric method (*Henry* et al., 1956), non-protein nitrogen by the hypobromite titrimetric method of *Rappaport and Eichhorn* (1947), chloride by the titrimetric silver nitrate-thiocyanate method of *Harvey* (1910) and phosphate by the method of *Fiske and Subbarow* (1925) using 1:2:4 amino-naphthol sulphonic acid as reducing agent.

Blood was collected from the tail of the rat for the estimation of haemoglobin by the oxyhaemoglobin technique (*Sunderman* et al., 1953), the red cell count by a turbidimetric method (*Everitt and Webb*, 1958), and haemocytometer counts of eosinophils using the diluting fluid of *Manners* (1951), and of white cells using 0.5% acetic acid solution coloured with methyl violet as diluent.

The electrocardiogram of the rat in the supine position was recorded under light

pentobarbital anaesthesia (dosage level 40 mg per kg in young adults decreasing to 25 mg per kg in old rats) on a *Cardiotrace* direct writing electrocardiograph (*Philip*'s Electrical Industries, Sydney). Measurements were made of heart rate and of the magnitude (*Goldberger*, 1953) and direction (*Graybiel*, 1950) of the mean QRS vector in the frontal plane.

Statistical analysis of the data was performed using the methods described by *Snedecor* (1953). The significance of the over-all effects of thyroxine on the course of ageing was determined from the analysis of variance. In some cases the analysis of covariance was used to make allowance for initial differences between control and thyroxine groups.

Results

Growth Cessation

The process of growth in the male rat ceases in middle age (*Everitt*, 1955). In a preliminary study (fig. 1) it was shown that small doses of thyroxine (5 μg to 20 μg) were unable to stimulate the growth of the middle aged rat, but on the contrary produced small reductions in body weight in proportion to the dosage level. Therefore growth cessation in the middle aged male rat cannot be due to a deficiency of thyroxine. The growth stimulating action of thyroxine is only observed in hypothyroid animals as in thyroidectomized (*Evans* et al., 1939; *Scow* et al., 1949; *Eartly and Leblond*, 1954) or hypophysectomized rats (*Laqueur* et al., 1941; *Asling* et al., 1954).

Large doses (100 μg) of thyroxine actually terminated growth very soon after the commencement of treatment at 400 days

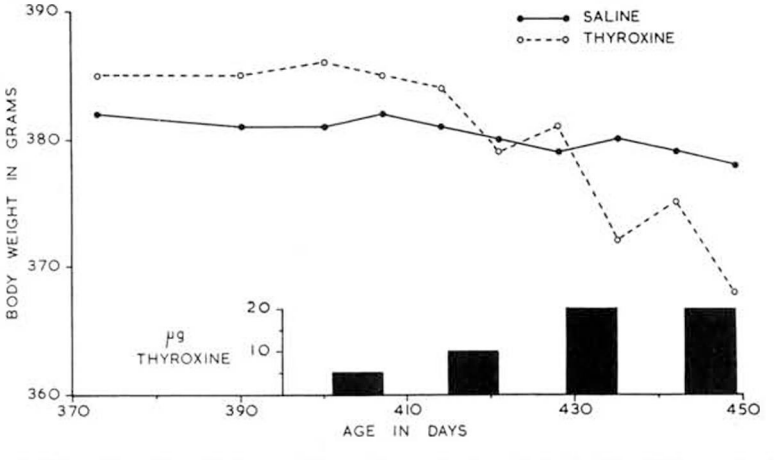

Fig. 1. The effect of small doses of thyroxine on body weight in 25 middle aged rats. Control data were obtained from 25 rats of the same age, injected with 0.9% NaCl.

Table I

The Effect of the Daily Administration of Thyroxine at 2 Dosage Levels, During the Period from 400 to 600 Days of Age, on the Mean Body Weight of Male Rats at Various Ages

Ex-periment	Group	Body weight (grams) ± S. E. at 300 days	400 days	500 days	600 days	700 days	death	Maximun weight (grams)	Age at maximum weight (days)
Small dose	Control (25)*	369 ± 7.1	381 ± 7.3	366 ± 8.5	325 ±12.7	299 ±13.1	246 ± 9.0	404 ± 7.0	461 ±16
	Thyroxine (25)	374 ± 7.2	386 ± 7.7	359 ±13.5	321 ±14.5	305 ±14.5	245 ± 7.8	402 ± 8.1	445 ±16
	Difference	+ 5	+ 5	− 7	− 4	+ 6	− 1	− 2	−16
Large dose	Control (23)	404 ± 4.5	421 ± 4.0	413 ± 8.0	382 ±13.1	345 ±16.1	269 ±10.0	440 ± 4.2	483 ±20
	Thyroxine (23)	391 ± 5.3	408 ± 6.2	363 ± 9.8	330 ±12.1	310 ±13.0	245 ± 7.3	415 ± 6.2	403 ±12
	Difference	−13	−13	−50	−52	−35	−24	−25	−80

* Number of rats.

(table I). Rats treated with large doses of thyroxine attained their maximum weight at 403 days, compared with 483 days for the controls. This difference of 80 days in the duration of growth was statistically significant ($t = 3.40$, $P < 0.01$). Small doses of thyroxine had no significant effect on growth cessation (table I).

Body Weight

In table I and fig. 2 the mean body weights of thyroxine treated rats before treatment (at 300 and 400 days), during treatment (at 500 and 600 days) and after treatment at 700 days are compared with the corresponding weights of control animals. In order to overcome errors due to the death of animals in long term population studies (*Davies*, 1954), the mean body weights of the whole group (including the cumulative weight of dead rats) have been determined at these ages (*Templeton and Patras*, 1938; *Everitt*, 1955).

Treatment with small doses of L-thyroxine (20 μg per day on alternate weeks) had no statistically significant effect on body weight during the period of treatment (table I). However, large doses of L-thyroxine (100 μg per day on alternate weeks) produced a mean over-all loss in body weight of about 40 grams. This difference was shown to be significant ($F_{1,43} = 5.65$, $P < 0.05$) by the

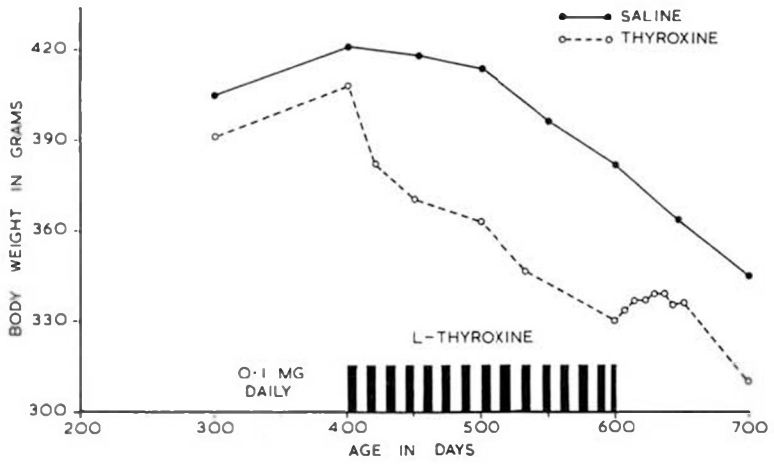

Fig. 2. The effect of long term treatment with large doses of thyroxine on the age change in the body weight of 23 male rats. The control curve was plotted from data obtained on 23 rats of the same age, injected with 0.9% NaCl.

method of covariance analysis, which allows for initial differences in the weight of the two groups.

When thyroxine treatment was discontinued in the large dosage experiment at 600 days, the mean body weight of the 15 surviving animals increased by 21 grams over the following 4 weeks. The 19 surviving control animals, however, lost 12 grams in this period (fig. 2).

Treatment with thyroxine had no significant effect on the mean body weight at death (table I).

Life Duration

The life duration (L) of the male rat of this local Wistar strain is directly correlated with the relative weight increment ($\triangle W$) from 300 to 400 days of age (*Everitt and Webb*, 1957). An equation developed from this relationship, $L = 6698 + 2140 \triangle W$, was used in the forecast of life duration in this study (table II). There was good agreement between the predicted and actual life durations of the thyroxine and control groups in the small dosage experiment, indicating that treatment with small doses of thyroxine did not affect the life duration (table II). However, when the life durations of individual rats (table III) were examined, animals treated with small doses of thyroxine showed an increased mortality rate during

Table II

The Effect of the Daily Administration of Thyroxine, at 2 Dosage Levels, During the Period from 400 to 600 Days of Age, on the Life Duration of Male Rats. The Actual Life Duration is Compared with the Life Duration Predicted from the Relative Weight Increment from 300 to 400 Days

Experiment	Group	Number of rats	Relative weight increment	Predicted life duration (days)	Actual life duration (days)
Small dose	Control	25	0.032 ± 0.006*	737	742 ± 31
	Thyroxine	25	0.031 ± 0.005	736	745 ± 41
Large dose	Control	23	0.042 ± 0.006	758	772 ± 39
	Thyroxine	23	0.042 ± 0.006	759	722 ± 35

* S.E. $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

the period of treatment (8 thyroxine treated rats and 5 controls died), and a reduced mortality rate in the post-treatment period (mean life duration of surviving thyroxine treated rats 854 days, controls 793 days).

Table III

The Effect of the Daily Administration of Thyroxine at 2 Dosage Levels, During Middle Age from 400 to 600 Days of Age, on the Life Duration in Days of Individual Rats

Small dose				Large dose			
Controls		Thyroxine		Controls		Thyroxine	
504	750	457	715	412	752	453	738
504	763	459	814	507	863	521	760
539	779	466	867	548	877	524	803
543	789	467	911	551	890	531	809
596	789	540	928	637	910	541	892
627	834	561	940	637	968	549	897
634	846	585	962	649	989	551	901
665	900	586	974	662	993	580	903
670	902	628	998	681	1010	672	909
671	958	644	1012	686	1025	682	913
729	972	652	1017	729	1040	693	1060
732	1101	666	1089	748		726	
741		700					

The mean life duration of rats treated with large doses of thyroxine was 50 days less than that of the controls. This difference was not statistically significant in either the t test ($t = 0.96$, $P > 0.05$), or in the analysis of covariance ($F_{1,43} = 2.00$, $P > 0.05$). The mortality rate was increased during treatment (8 thyroxine treated rats and 4 controls died), but was not changed in the post-treatment period (mean life duration of surviving thyroxine treated rats 824 days, controls 829 days).

Although thyroxine, in both small and large doses increased the mortality rate during the period of treatment, the observed increases were not as marked as those reported by workers using toxic doses of thyroid hormone (*Abderhalden und Wertheimer*, 1929; *Ershoff*, 1947; *Gemmill*, 1957).

Consumption of Food and Water, and Production of Faeces and Urine

In order to study the effect of thyroxine on the natural age changes in body function in these rats, an analysis of variance was carried out on each set of data as shown for food consumption in table IV. Values given in table V for the variance ratio (F) between ages, show that the consumption of food and water and the production of faeces changed significantly with age. Since the F between treatment values were also significant for these 3 variables, large doses of thyroxine had a significant effect on the consumption of food and water and on the production of faeces. In all 3 cases the interaction between ages and treatments was significant, thus showing that these age changes were affected by treatment with thyroxine, and also that the effect of thyroxine on these variables

Table IV

Analysis of Variance of Food Consumption in Relation to Age and to Treatment with Thyroxine

Source of variation	Sum of squares	df	Variance	F	P
Between ages	477.4	2	238.7	26.6	<0.01
Between treatments	49.6	1	49.6	5.53	<0.05
Interaction ages × treatments	167.0	2	83.5	9.3	<0.01
Error	538.4	60	8.97		
Total	1232.4	65			

depended on age. Further analysis showed that middle aged rats treated with large doses of thyroxine had significantly higher values for food consumption ($t = 5.08$, $P<0.01$), faeces production ($t = 5.13$, $P<0.01$) and water consumption ($t = 5.57$, $P<0.01$) than the controls. The effect of thyroxine in increasing urine production was only of borderline significance ($t = 2.08$, $P = 0.05$).

In rats treated with small doses of thyroxine the changes in food and water consumption and in faeces production were less marked, but were still statistically significant. Once again urine production exhibited the smallest change, but in this case the change was more significant ($t = 2.32$, $P<0.05$).

None of the changes produced by thyroxine in middle age, were present in old age about 150 days after the discontinuation of treatment.

Excretion of Various Urinary Constituents

The F between ages values given in table VI show that the excretion of protein and uric acid increased significantly with age, while the excretion of creatinine and chloride decreased significantly.

Table V

The Effect of the Daily Administration of 100 μg of Thyroxine on Alternate Weeks for 200 Days During Middle Age, on the Age Changes in Food and Water Consumption and in Faeces and Urine Production. The Age Changes were Studied in 11 Thyroxine Treated Rats and 11 Control Rats which all Survived until Old Age

Quantity	Units	Control Youth	Control Middle age	Control Old age	Thyroxine Youth	Thyroxine Middle age	Thyroxine Old age	F between ages	F between treatments
Age	days ± S.E.	312 ± 8.6	498 ± 8.5	749 ± 2.4	311 ± 7.9	511 ± 6.1	741 ± 2.7		
Body weight	grams ± S.E.	404 ± 6.9	427 ± 6.8	383 ±14.3	392 ± 6.4	369 ±11.6	337 ±13.4		
Food consumption	g/day ± S.E.	19.7 ± 1.1	19.4 ± 1.0	16.3 ± 1.0	19.4 ± 0.8	25.6 ± 0.6	15.6 ± 0.7	26.6**	5.53*
Faeces production	g/day ± S.E.	11.4 ± 0.5	10.0 ± 0.8	9.8 ± 0.9	11.0 ± 0.7	15.7 ± 0.7	8.7 ± 0.6	14.4**	6.20*
Water consumption	ml/day ± S.E.	26.9 ± 0.8	24.9 ± 1.5	29.6 ± 0.9	27.1 ± 1.0	35.7 ± 1.2	25.6 ± 1.8	3.87*	5.13*
Urine production	ml/day ± S.E.	10.5 ± 0.6	9.8 ± 0.9	12.5 ± 0.9	10.7 ± 0.6	12.3 ± 0.8	13.4 ± 1.6	3.10	2.29

* Significant at 5% level.
** Significant at 1% level.

Treatment with large doses of thyroxine in middle age led to significant increases in the excretion of protein ($t = 3.12$, $P < 0.01$), non-protein nitrogen ($t = 2.44$, $P < 0.05$), uric acid ($t = 2.59$, $P < 0.05$), phosphate ($t = 3.77$, $P < 0.01$) and chloride ($t = 2.84$, $P = 0.01$) compared with the controls. Similar, but less pronounced, changes were seen in rats treated with small doses of thyroxine. However, at neither dosage level of thyroxine, did any of these differences persist after the discontinuation of treatment.

Blood Picture

The F between ages values given in table VII show that the blood haemoglobin level, the red cell count and the eosinophil count changed significantly with age.

During the period of treatment, large doses of thyroxine increased the white cell count significantly ($t = 2.67$, $P < 0.05$), but had no significant effect on the haemoglobin level, the red cell

Table VI

The Effect of the Daily Administration of 100 μg of Thyroxine on Alternate Weeks for 200 Days During Middle Age, on the Age Changes in the Excretion of Various Urinary Constituents. These Age Changes were Studied in 11 Thyroxine Treated Rats and 11 Control Rats which all Survived until Old Age

Quantity	Units	Control			Thyroxine			F	F
		Youth	Middle age	Old age	Youth	Middle age	Old age	between ages	between treatments
Age	days	312	498	749	311	511	741		
	± S.E.	± 8.6	± 8.5	± 2.4	± 7.9	± 6.1	± 2.7		
Body weight	grams	404	427	383	392	369	337		
	± S.E.	± 6.9	± 6.8	±14.3	± 6.4	±11.6	±13.4		
Protein excretion	mg N/day	3.9	5.9	10.7	3.6	12.0	19.5	9.8**	3.17
	± S.E.	± 0.8	± 1.5	± 2.7	± 0.7	± 1.2	± 7.4		
Non-protein nitrogen excretion	mg N/day	426	406	427	420	481	427	< 1	2.64
	± S.E.	±11	±28	±19	± 8	±11	±16		
Uric acid excretion	mg/day	3.9	3.9	4.4	3.6	4.6	3.6	3.15*	< 1
	± S.E.	± 0.21	± 0.23	± 0.16	± 0.20	± 0.15	± 0.22		
Creatinine excretion	mg/day	22.3	19.9	17.4	20.9	21.5	17.0	11.2**	< 1
	± S.E.	± 0.6	± 1.0	± 1.1	± 0.7	± 0.9	± 1.3		
Phosphate excretion	mg P/day	16.1	15.3	16.1	17.0	21.1	17.5	1.04	7.16**
	± S.E.	± 1.1	± 1.0	± 1.0	± 1.1	± 1.1	± 1.9		
Chloride excretion	mg NaCl/day	177	155	146	181	205	134	6.58*	1.89
	± S.E.	±14	±12	±14	±11	±13	± 9		

* Significant at 5% level.
** Significant at 1% level.

count or the eosinophil count. Small doses of thyroxine had no significant effect on any of these variables.

In the post-treatment period in old age, the haemoglobin level was significantly higher in rats treated with large doses of thyroxine than in the controls ($t = 4.80$, $P < 0.01$). This change was not seen in rats treated with small doses of thyroxine.

Electrocardiogram

The F between ages values given in table VIII show that the heart rate decreased with age and that the mean QRS vector in the frontal plane rotated to the left.

Treatment with large doses of thyroxine in middle age led to significant increases in heart rate ($t = 6.23$, $P < 0.01$) and in the voltage of the mean QRS vector ($t = 2.38$, $P < 0.05$). Small doses of thyroxine increased the heart rate significantly ($t = 2.58$, $P < 0.05$). These differences, however, were not present after the discontinuation of treatment.

Pathological Changes

The mean postmortem weights of the lungs, heart ventricles,

Table VII

The Effect of the Daily Administration of 100 μg of Thyroxine on Alternate Weeks for 200 Days During Middle Age, on Age Changes in the Blood Picture. These Age Changes were Studied in 10 Thyroxine Treated Rats and 10 Control Rats which all Survived until Old Age

Quantity	Units	Control			Thyroxine			F	F
		Youth	Middle age	Old age	Youth	Middle age	Old age	between ages	between treatments
Age	days ± S.E.	327 ± 7.2	539 ± 0.1	761 ± 3.1	317 ± 9.2	543 ± 0.6	749 ± 2.9		
Body weight	grams ± S.E.	410 ± 7.0	424 ± 6.5	386 ±14.1	398 ± 6.1	365 ±12.2	334 ±13.7		
Haemoglobin	g/100 ml ± S.E.	16.4 ± 0.26	16.4 ± 0.24	17.2 ± 0.22	16.4 ± 0.15	16.8 ± 0.26	18.8 ± 0.27	25.4**	12.6**
Red cell count	10^6 cells/mm^3 ± S.E.	8.6 ± 0.20	8.1 ± 0.17	8.8 ± 0.23	8.4 ± 0.18	8.5 ± 0.11	9.2 ± 0.13	7.44**	2.68
White cell count	10^3 cells/mm^3 ± S.E.	12.0 ± 1.0	12.2 ± 0.5	13.6 ± 1.4	13.2 ± 0.6	17.8 ± 2.0	13.5 ± 0.8	1.99	5.19*
Eosinophil count	cells/mm^3 ± S.E.	540 ±68	344 ±93	254 ±39	508 ±105	375 ±52	279 ±63	9.38**	< 1

* Significant at 5% level.
** Significant at 1% level.

Table IX

The Effect of the Daily Administration of 100 μg of Thyroxine on Alternate Weeks for 200 Days During Middle Age, on the Weights of Various Organs at Autopsy

Group	Number of rats	Body (g)	Weight ± S.E. of Lungs combined (g)	Heart ventricles (g)	Liver (g)	Kidneys combined (g)	Testes combined (g)	Adrenals combined (mg)
Control	23	269 ±10.0	8.1 ± 0.88	1.16 ± 0.05	10.5 ± 0.74	3.11 ± 0.10	1.25 ± 0.15	76 ± 4.5
Thyroxine	23	245 ± 7.3	9.2 ± 0.98	1.20 ± 0.07	9.5 ± 0.61	2.87 ± 0.11	1.27 ± 0.16	72 ± 3.9
t*		1.94	0.83	0.46	1.08	1.58	0.06	0.76

* t = 2.02 at the 5% level of significance.

liver, kidney, testes and adrenals in the 23 rats which received large doses of thyroxine during middle age, were not significantly different from those in the 23 controls (table IX).

The frequency and severity of lung disease at autopsy appeared to be greater in rats treated with large doses of thyroxine than in the controls (table X). The severity of lung disease was assessed macroscopically by estimating the area of the external lung surface

Table VIII

The Effect of the Daily Administration of 100 μg of Thyroxine on Alternate Weeks for 200 Days During Middle Age, on the Age Changes in Heart Rate and the Mean QRS Vector in the Frontal Plane. These Age Changes were Studied in 12 Thyroxine Treated Rats and 12 Control Rats which all Survived until Old Age

Quantity	Units	Control Youth	Control Middle age	Control Old age	Thyroxine Youth	Thyroxine Middle age	Thyroxine Old age	F between ages	F between treatments
Age	days ± S.E.	314 ± 3.8	550 ± 0.9	721 ± 3.3	317 ± 4.6	543 ± 2.0	712 ± 3.8		
Body weight	grams ± S.E.	424 ± 7.6	439 ± 8.5	407 ±14.6	412 ± 7.2	374 ± 9.5	347 ±16.7		
Heart rate	beats/min ± S.E.	392 ± 8.1	364 ±11.0	354 ±12.3	403 ± 7.1	446 ± 7.2	326 ± 8.1	29.8**	7.95**
QRS vector magnitude	μV ± S.E.	292 ±19	289 ±23	332 ±48	307 ±19	382 ±31	333 ±52	< 1	1.14
QRS vector direction	degrees ± S.E.	55 ± 4	46 ± 5	20 ± 8	48 ± 3	36 ± 8	33 ±10	6.51**	< 1

** Significant at 1% level.

covered with lesions. The following classification was used: mild lung disease 1–25% of lung surface covered with lesions, moderate lung disease 26–50%, and severe lung disease more than 50%. Macroscopically healthy lungs (no lung lesions) were observed in 4 thyroxine treated rats and in 8 controls, out of group totals of 23. Severe lung disease was present in 4 rats treated with thyroxine, but in only one control rat.

Gross examination at autopsy showed the presence of only one tumour in rats treated with large doses of thyroxine (30 g skin tumour), compared with 4 tumours in the control group (10.9 g adrenal; 6.9 g testis; 28.3 g kidney; 15 g skin tumour). Other workers have observed a reduced incidence of tumours in rats treated with thyroxine. The literature on this subject has been reviewed by *Pelner* (1957).

Table X

The Effect of the Daily Administration of 100 μg of Thyroxine on Alternate Weeks for 200 Days During Middle Age, on the Incidence of Several Diseases

Group	Time of death	Number of rats	Tumours	Lung disease			Ventricular hypertrophy	Severe nephrosis
			absent	mild	moderate	severe		
Saline	During treatment	4	1	1	2	1	–	–	–
	After treatment	19	3	7	6	5	1	3	2
Thyroxine	During treatment	8	–	3	3	1	1	2	–
	After treatment	15	1	1	7	4	3	3	4

Ventricular hypertrophy, as indicated by ventricular weight exceeding 1.30 gram, was present in 5 thyroxine treated rats (1.47, 1.51, 1.54, 2.00, 2.10 grams) and in 3 control rats (1.51, 1.57, 1.75 grams). Two of the thyroxine treated rats which developed ventricular hypertrophy died during treatment (table X).

Severe nephrosis, as indicated by the urinary protein nitrogen excretion exceeding 20 mg per day in old age, was present in 4 thyroxine treated rats (23.3, 23.9, 26.4, 88.4 mg) and in 2 control rats (26.4, 28.6 mg).

In the small dosage experiment, postmortem data on thyroxine treated rats were very similar to those on the corresponding controls. In a similar study in which male rats were fed 0.18 mg of "Proloid" thyroid extract daily from approximately 400 to 700 days and then

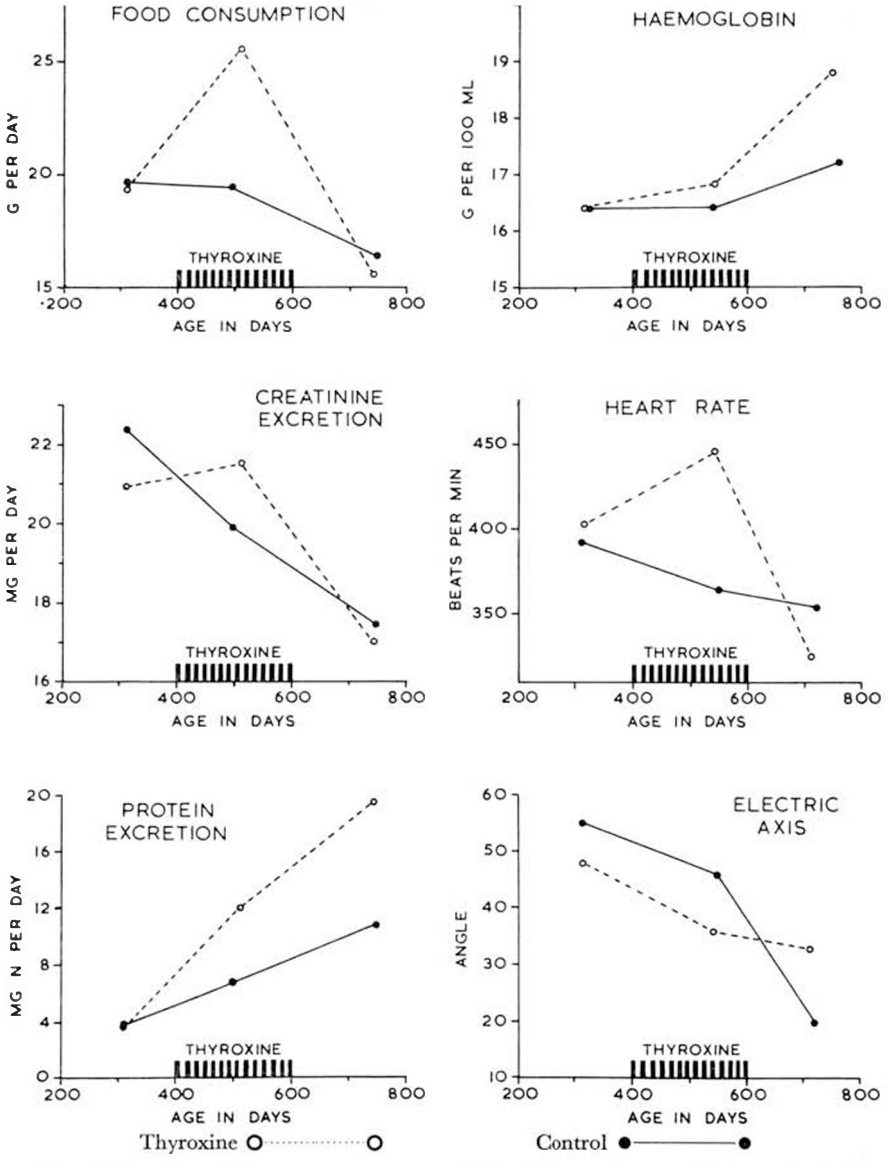

Fig. 3. The effect of long term treatment of 11 middle aged rats with large doses of thyroxine on six indices of ageing. Control data were obtained from 11 rats of the same age, injected with 0.9% NaCl.

sacrificed, *McArthur* et al. (1957) could find no significant histological or anatomical changes.

Discussion

The principal action of thyroxine is to increase tha rate at which metabolic processes occur. In the present study, the treatment of middle aged male rats with thyroxine produced statistically significant increases in the consumption of food and water, in the production of faeces and urine, in the excretion of protein, non-protein nitrogen, uric acid, phosphate and chloride, and in heart rate. Since there is a natural decrease with age in food consumption, faeces production, chloride excretion and heart rate, treatment with thyroxine reversed these age changes. On the other hand, the excretion of uric acid and protein normally increase with age, and therefore these age changes were accentuated by treatment with thyroxine. However, once treatment was discontinued these processes returned to their normal level of activity (fig. 3, tables V, VI and VII).

According to *Pearl* (1928) a high "rate of living", as measured by the metabolic rate, is associated with a short life duration. In the present study moderately large doses of thyroxine increased the "rate of living" in a group of 23 rats for a period of 200 days during middle age. From the respective increases in the consumption of food (32%) and water (43%), in the production of faeces (57%) and urine (25%) and in heart rate (23%), the average increase in metabolic rate was taken as 36%. On this basis it was calculated that the metabolic work done during the 200-day period of thyroxine treatment would normally have taken 272 days. In effect, these rats did 272 days of metabolic work in a period of 200 days. Assuming the concept of *Rubner* (1908) that each animal species produces a fixed quantity of energy in a lifetime, then the life duration of these thyroxine treated rats should have been reduced by about 72 days. This agrees favourably with the observed reduction in life duration of 50 days (table II).

On the other hand, the observed reduction in life duration of rats treated with large doses of thyroxine could have been due entirely to the increased mortality rate during the period of treatment (table III). The mortality rate was not affected in the post-treatment period.

Thyroxine, however, did affect the course of ageing in the post-treatment period. The haemoglobin level in old age, about 150 days after the discontinuation of treatment, was significantly greater than in the controls (table VII). This rise in haemoglobin level was

probably related to an increased frequency and severity of lung disease in these animals in the post-treatment period (table X).

Although thyroxine is able to reverse many histological (*Korenchevsky*, 1948) and metabolic age changes (tables V, VI and VIII), it increases the mortality rate during treatment, and, when administered in large doses, may produce some changes in the course of pathological ageing.

Summary

1. The subcutaneous administration of small doses of L-thyroxine sodium (a total of 2 mg in 90 divided doses) over a period of 200 days in middle age had no significant effect on the life duration of the male rat. The mortality rate was increased during treatment, but was diminished in the post-treatment period.

2. Small doses of thyroxine failed to stimulate growth in the intact middle aged rat. Large doses of thyroxine inhibited growth and reduced the body weight.

3. The administration of moderately large doses of L-thyroxine sodium (a total of 9 mg in 90 divided doses) over a period of 200 days in middle age, produced an average loss of 40 grams in body weight during treatment. The duration of growth was shortened by 80 days and the life duration reduced by 50 days as the result of this treatment. The mortality rate was increased only during the period of treatment.

4. During treatment with both small and large doses of thyroxine in middle age, statistically significant increases occurred in the consumption of food and water, in the production of faeces and urine, in the excretion of protein, non-protein nitrogen, uric acid, phosphate and chloride, and in heart rate. These values returned to normal when treatment was discontinued. The only significant change observed in the post-treatment period in old age, was an abnormal elevation of the blood haemoglobin level in rats treated with large doses of thyroxine. The raised haemoglobin level was probably related to an increased frequency and severity of lung disease in these animals.

Zusammenfassung

1. Die subkutane Injektion von kleinen Dosen L-Thyroxin-Na innerhalb von 200 Tagen (d.h. 2 mg auf 90 Teildosen verteilt) hat bei erwachsenen männlichen Ratten die

Lebensdauer nicht beeinflußt. Die Sterblichkeit war allerdings während der Behandlung erhöht, aber in der Periode danach vermindert.

2. Kleine Thyroxindosen fördern das Wachstum der normalen Ratte im mittleren Alter nicht. Große Dosen hemmen das Wachstum und vermindern das Körpergewicht.

3. Eine Dosis von 9 mg L-Thyroxin-Na auf 90 einzelne Injektionen innerhalb von 200 Tagen verteilt, führte zu einem mittleren Gewichtsverlust von 40 g während der Behandlung. Das Wachstum wurde um 80 Tage und die Lebensdauer um 50 Tage verkürzt. Die Sterblichkeit war nur während der Behandlung vermehrt.

4. Während der Behandlung mit kleinen und großen Dosen von Thyroxin im mittleren Alter ergibt sich eine statistisch signifikante Zunahme des Nahrungs- und Wasserverbrauches, der Bildung von Faeces und Harn, der Ausscheidung von Eiweiß, Nicht-Eiweiß-Stickstoff, Harnsäure, Phosphate und Chloride und der Pulszahl. Diese Werte kehrten zur Norm zurück, wenn die Behandlung unterbrochen wurde. Die einzige signifikante Veränderung in der Nachbehandlungsperiode war ein abnorm hoher Hämoglobingehalt bei alten Ratten, die mit großen Dosen von Thyroxin behandelt waren. Der hohe Hämoglobingehalt steht wahrscheinlich im Zusammenhang mit einer vermehrten Häufigkeit und Schwere von Lungenerkrankungen dieser Tiere.

Résumé

1° L'administration sous-cutanée de petites doses de L-Thyroxine Na (au total 2 mg répartis en 90 doses) pendant une période de 200 jours, chez des rats mâles d'âge moyen, n'a pas eu d'action nette sur leur durée de vie. Le taux de mortalité a été augmenté pendant le traitement, mais fut diminué par la suite.

2° De petites doses de thyroxine n'ont pas réussi à stimuler la croissance du rat intact d'âge moyen. De fortes doses inhibent au contraire la croissance et réduisent le poids corporel.

3° L'administration de doses modérément fortes de L-Thyroxine Na (au total 9 mg répartis en 90 doses) pendant une période de 200 jours a entrainé, chez des rats d'âge moyen, une perte de poids moyenne de 40 grammes pendant le traitement. La durée de la période de croissance fut raccourcie de 80 jours et la longévité de 50 jours par ce traitement. Le taux de mortalité ne fut augmenté que pendant la période de traitement.

4° Pendant l'administration des fortes et des faibles doses de thyroxine chez les rats d'âge moyen, on a noté une augmentation statistiquement significative de la consommation de nourriture et d'eau, de la production des faeces et de l'urine, de l'excrétion des protéines, de l'azote non protéique, de l'acide urique, des phosphates et des chlorures, ainsi que du rythme cardiaque. Ces valeurs revinrent à la normale après cessation du traitement. La seule modification qui se maintint après cessation des injections chez les rats âgés fut une élévation anormale de l'hémoglobine sanguine chez les animaux ayant reçu de fortes doses de thyroxine. Ce phénomène est probablement en rapport avec l'augmentation de fréquence et de gravité des affections pulmonaires chez ces animaux.

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