AGING-REVERSING PROPERTIES OF THYROTROPIN RELEASING HORMONE (TRH)

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STUDIES INITIATED IN 1989 HAVE SHOWN THAT THE TRIPEPTIDE TRH POSSESSES MOST REMARKABLE AGING-REVERSING PROPERTIES IN THE MOST VARIED EXPERIMENTAL CONDITIONS

Table 3
Administration of thyrotropin releasing hormone (TRH) protects thymus size and structure in prednisolone (PRE)-treated mice. The thymotropic activity of TRH does not seem thyroid-dependent: it is not inhibited by blockade of thyroid hormone biosynthesis with methylthiouracil (MTU) and is not exerted via stimulation of thyroxin (T4) release.

Groups (Treatment)	No of mice	TRH (ug/day)	Thymus weight (per 100 grams body weight)	Thymic inhibition (percent vs untreated)	Thymic recovery (percent vs PRE-treated)	T4 (ug/100 ml serum)
A Untreated	9	-	0.73 ± 0.11[a]	-	-	4.58 ± 0.71[a]
B PRE-treated	10	-	0.29 ± 0.07	-60	-	4.54 ± 0.42 (-1%, B vs A)
C PRE + MTU	11	-	0.33 ± 0.12	-55	-	1.15 ± 0.43 (-75%, C vs A)
D PRE + MTU + TRH	11	100	0.61 ± 0.18	-16	+46 (D vs C)[b]	1.22 ± 0.21 (-73%, D vs A)
E PRE + TRH	6	1	0.40 ± 0.16	-45	+28 (E vs B)[c]	5.54 ± 0.67 (+22%, E vs B)[f]
F PRE + TRH	6	10	0.42 ± 0.06	-42	+31 (F vs B)[d]	5.33 ± 0.47 (+17%, F vs B)[g]
G PRE + TRH	5	100	0.54 ± 0.10	-26	+48 (G vs B)[e]	Not measured

Mice were 3 month-old male C57BL/6. MTU was added to the drinking water at the concentration of 0.2 percent on day 0 (same day of PRE injection). Prednisolone acetate (1 mg suspension in 1o percent ethanol) was injected ip on day 0, at 10 AM. TRH at the given daily dose was injected ip in 0.2 ml isotonic saline at 5 PM. The TRH treatment was prolonged for four days. On the fifth day the mice were exsanguinated from the retroorbital plexus under acute aether anaesthesia between 9 and 10 AM and T4 measured in individual sera by RIA (see text). Also the body and thymus weights of the mice were recorded.

[a] Standard deviation
[b] p<0.001 (Analysis of variance)
[c] p<0.003
[d] p<0.003
[e] p<0.003
[f,g] Not significant (E and F vs B)

Administration of TRH protects thymus size and structure in prednisolone (pre)-treated mice. The thymotropic activity of TRH does not seem Thyroid dependent : It is not inhibited by blockade of Thyroid hormone biosynthesis with methylthouracil (MTU) and is not exerted via stimulation of T_4 release

Group	Treatment	No.of mice	TRH (µg/ day)	Thymus weight (per 100 body weight)	Thymic Inhibition (percent vs. Untreated)	Thymic recovery (percent vs. PRE-treated)	T4 (µg/100 ml serum)
A	Untreated	9	---	0.73±0.11[a]	---	-	4.58±0.71 [a]
B	PRE-treated	10	---	0.29±0.07	-60	-	4.54±0.42(-1%,B vs.A)
C	PRE+MTU	11	---	0.33±0.12	-55	-	1.15±0.43(-75%,C vs. A)
D	PRE+MTU+TRH	11	100	0.61±0.18	-16	+46(D vs.C)[b]	1.22±0.21(-73%,D vs. A)
E	PRE+TRH	6	1	0.40±0.16	-45	+28(E vs. B)[c]	5.54±0.67(+22%,E vs.B)[f]
F	PRE+TRH	6	10	0.42±0.06	-42	+31(F vs. B)[d]	5.33±0.47(+17%,F vs.B)[g]
G	PRE+TRH	5	100	0.54±0.10	-26	+48(G vs. B)[e]	Not measured

TABLE 1. Twenty-day treatment with thyrotropin releasing hormone (TRH) reduces significantly body weight, produces mobilization of triglycerides and lowers levels of cholesterol in the blood of non-obese adult male mice.

Treatment	*Body weight before treatment [g]*	*Body weight after treatment [g]*	*Triglycerides [mmol/L]*	*Cholesterol [mmol/L]*
Controls (N=6)	34.3 ± 0.5	34.9 ± 0.6	1.44 ± 0.12	2.6 ± 0.34
TRH (N=9)	35.6 ± 2.5	32.9 ± 2.7 a	2.22 ± 0.42 **	2.3 ± 0.21*

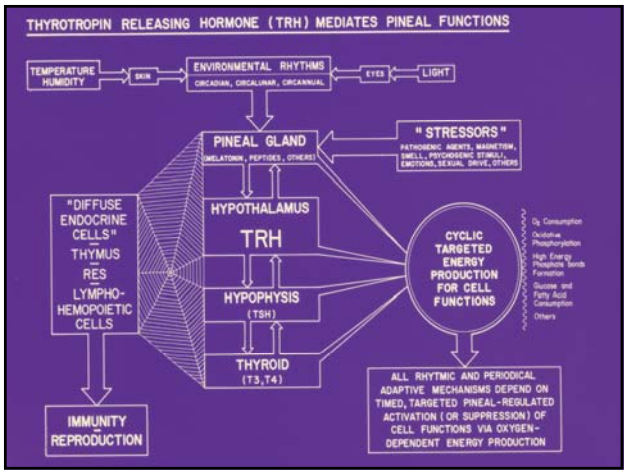
Mean ± SD, a p<0.05 when compared to body weight before treatment ; *p<0.05 when compared to controls;
****p<0.001 when compared to controls (Student's t-Test)**

C57BL/6 male mice aged 9 months, were injected i.p. for 20 days at 6 p.m. (evening) with 0.2 ml volume containing 0.3 mg/kg b.w TRH-tartrate in bidistilled water. Controls were injected with the same amount of diluent. Blood was taken from the retroorbital venous plexus at 12 a.m. under halothane anesthesia.

TABLE 2a. 30-day treatment with thyrotropin releasing hormone (TRH) produces weight loss in 12 month-old C57BL/6 male mice. Thyroid hormones in peripheral blood are significantly decreased.

Day of treatment	TRH (N=8)			CONTROLS (diluent) (N=8)		
	Body weight [g]	TT3 [nmol/L]	TT4 [nmol/L]	Body weight	TT3 [nmol/L]	TT4 [nmol/L]
Before treatment	34.8 ± 1.7	0.96 ± 0.16	51.6 ± 8.8	34.2 ± 2.5	0.96 ± 0.13	51.8 ± 11.7
13 day of treatment	31.2 ± 2.4*	0.68±0.13* a	44.1 ±6.5a	32.4± 2.1	0.78 ± 0.28	51.5 ± 6.4
30 day of treatment	30.9 ± 3.1*	0.74 ± 0.24*	39.4 ± 9.3*	32.6 ± 2.3	0.87 ± 0.28	45.6 ± 9.9

Mean ± SD, *p<0.05 and **p<0.01 when compared with the value before treatment; ap<0.05 when compared to controls (Student's t-Test).
C57BL/6 male mice aged 12 months were injected i.p. for 30 consecutive days at 6 p.m. (evening) with 0.2 ml volume containing 0.3 mg/kg b.w. TRH-tartrate in bidistilled water. Controls were injected with the same amount of diluent. Blood was taken from the retroorbital venous plexus at 9 a.m. under halothane anesthesia.



IN VIEW OF ITS GREAT THERAPEUTIC POTENTIAL, WE CONDUCTED ACUTE AND CHRONIC EXPERIMENTS APT AT VERIFYING IF TRH CAN EXERT IN VIVO VERITABLE ANTI-AGING OR AGING-REVERSING EFFECTS IN THE MOST TYPICAL EXPRESSION OF METABOLIC AGING

THE RESULTS REPORTED HERE DEMONSTRATE BEYOND ANY DOUBT THAT TRH PRODUCES MASSIVE REGRESSION OF AGING-RELATED ALTERATIONS

TABLE 1. ***Acute treatment with TRH lowers blood levels of triglycerides in old mice***

Group	Treatment	Strain	Sex	No.	Age month	Cholesterol (mmol/L)	Triglycerides (mmol/L)	Phospholipids (mmol/L)
A	Saline	BALB/cJ	m	6	21	2.74±0.6	3.23±1.27	ND
B	TRH	BALB/cJ	m	6	21	2.90±0.8	1.98±0.44[a]	ND
C	Saline	C57BL	m	8	19	2.31±0.6	1.28±1.23	ND
D	TRH	C57BL	m	5	19	2.27±0.2	0.87±0.20[b]	ND
E	Saline	C57BL	f	22	18	2.23±0.4	1.07±0.33[c]	2.22±0.44[d]
F	TRH	C57BL	f	32	18	2.10±0.4	0.82±0.16	1.98±0.27
G	Young mice	C57BL	f	10	3	1.81±0.1	0.88±0.16	1.91±0.11

Mean ±SD,[a] p<0.05 A vs. B; [b]p<0.05 C vs. D; [c] p<0.0001 E vs. F; [d] p<0.05 E vs. F.

The mice were injected in the evening (6 p.m.) for 10 consecutive days with TRH-tartrate (10µg/0.2 ml/mouse) or saline(0.2 ml) intraperitoneally

TABLE 2- A. ***Acute treatment with TRH lowers cholesterol and triclycerides in old mice***

Parameters measured	*Young Untreated mice (N=10)*	*Old mice + saline (N=7)*	*Old mice + TRH evening (N=7)*
Aspartate aminotransferase (U/L)	148.4 ± 52.8	134.7 ± 19.5	159.4 ± 37.6
Alanine aminotransferase (U/L)	76.7 ± 27.0	70.3 ± 37.5	55.3 ± 17.1
Alkaline phosphatase (U/L)	132.7 ± 18.8	76.4 ± 14.2	86.3 ± 23.9
Glucose (mmol/L)	9.65 ± 0.94	9.09 ± 1.30	8.34 ± 0.78
Urea (mmol/L)	8.98 ± 0.66	7.90 ± 1.09	8.14 ± 0.64
Total protein (g/L)	54.91 ± 0.95	59.5 ± 1.5	57.6 ± 3.8
Albumin (g/L)	33.74 ± 0.62	31.1 ± 2.0	30.7 ± 2.2
Cholesterol (mmol/L)	1.81 ± 0.12	2.63 ± 0.17	2.24 ± 0.41*
Triglycerides (mmol/L)	0.88 ± 0.16	1.21 ± 0.09	0.96 ± 0.19 *

Mean ± SD*p<0.05 when compared to old controls, **p<0.001 when compared to old controls, (Student's *t*-Test). 15 month-old C57BL female mice were injected in the evening (6p.m) for 15 consecutive days with TRH- tartrate (10µg/0.2 ml/mouse i.p.) or saline (0.2ml/mouse).

TABLE 2-B. Acute treatment with TRH lowers cholesterol and triglycerides in old mice

Parameters measured	*Young Untreated mice (N=10)*	*Old mice + saline (N=7)*	*Oldmice + TRH evening (N=7)*
Phospholipids (mmol/L)	1.91 ± 0.11	2.67 ± 0.27	2.37 ± 0.38
Phosphorus inorganic (mmol/L)	2.33 ± 0.24	2.43 ± 0.19	2.70±0.24*
Calcium (mmol/L)	2.33 ± 0.04	2.40 ± 0.07	2.42 ± 0.10
Creatine enzymatic (µmil/L)	52.1 ± 3.8	57.8 ± 9.6	58.3 ± 9.29
Sodium (mmol/L)	149.5 ± 0.8	152.4 ± 2.7	152.5 ± 1.2
Potassium (mmol/L)	4.74 ± 0.34	5.91 ± 0.37	6.14 ± 0.33
Chloride (mmol/L)	107.6 ± 1.2	108.2 ± 1.7	111.4 ± 0.06*
Albumin / globulin ratio	1.60 ± 0.05	1.10 ± 0.15	1.14 ± 0.06
Globulin (g/L)	21.12 ± 0.58	28.39 ± 2.48	26.37 ± 1.44

Mean ± SD*p<0.05 when compared to old controls, **p<0.001 when compared to old controls, (Student's *t*-Test). 15 month-old C57BL female mice were injected in the evening (6p.m) for 15 consecutive days with TRH- tartrate (10µg/0.2 ml/mouse i.p.) or saline (0.2ml/mouse).

TABLE 3-A. *Acute treatment with TRH improves aging- related plasma chemistry parameters in old mice*

Parameters measured	*Young Untreated mice (N=10)*	*Old mice +saline (N=9)*	*Old mice +TRH morning (N=9)*	*Old mice +TRH evening (N=9)*
Aspartate aminotransferase (U/L)	134.7±19.5	127.1±30.8	157.3±19.5*	155.2±39.2
Alanine aminotransferase (U/L)	76.7±27.0	73.7±24.6	68.9±10.6	55.0±18.9
Alkaline phosphatase (U/L)	132.7±18.8*	172.4±35.0	141.1±18.7*	132.4±29.2*
Glucose (mmol/L)	9.65±0.94**	8.0±0.8	9.8±0.80*	9.47±1.1*
Urea (mmol/L)	8.98±0.66**	7.94±1.05	8.48±0.56	8.01±1.29
Total protein (g/L)	54.91±0.95**	59.6±01.2	58.7±2.2	59.0±1.9
Albumin (g/L)	33.74±0.62	33.6±0.8	33.1±1.04	33.3±0.9
Cholesterol (mmol/L)	1.81±0.12*	2.15±0.28	1.91±0.06*	1.94±0.22
Triglycerides (mmol/L)	0.88±0.16	1.07±0.33	0.80±0.11*	0.73±0.12*

Mean ±SD,*p<0.05 when compared to old controls,**p<0.001 when compared to old controls, (Student's *t*-Test).
18 month-old C57BL female mice were injected in the morning(10°.m) or in the evening(6 p.m) for 15 consecutive days with TRH-tartrate (10µg/0.2 ml/mouse i.p.) or saline (0.2 ml/mouse) intraperitoneally

TABLE 3-B. *Acute treatment with TRH improves aging- related plasma chemistry parameters in old mice*

Parameters measured	*Young Untreated mice (N=10)*	*Old mice +saline (N=9)*	*Old mice +TRH morning (N=9)*	*Old mice +TRH evening (N=9)*
Phospholipids (mmol/L)	1.91±0.11	2.12±0.30	1.87±0.15*	1.90±0.13
Phosphorus inorganic (mmol/L)	2.33±0.24	2.28±0.22	2.18±0.20	2.06±0.27
Calcium (mmol/L)	2.33±0.04	2.32±0.05	2.39±0.04*	2.37±0.06
Creatine enzymatic (µmol/L)	52.1±3.8	79.2±59.8	30.5±22.0*	48.1±6.4
Sodium (mmol/L)	149.5±0.8*	147.6±1.7	156.6±2.4**	147.4±2.3
Potassium (mmol/L)	4.74±0.34**	5.60±0.47	6.04±0.28	5.97±0.22
Chloride (mmol/L)	107.6±1.2**	114.4±1.5	110.8±1.8**	109.2±1.1*
Albumin/globulin ratio	1.60±0.05**	1.31±0.06	1.32±0.09	1.29±0.09
Globulin (g/L)	21.12±0.58**	26.02±1.21	25.6±1.7	25.6±1.3

Mean ±SD,*p<0.05 when compared to old controls,**p<0.001 when compared to old controls, (Student's *t*-Test).

18 month-old C57BL female mice were injected in the morning(10 a.m) or in the evening(6 p.m) for 15 consecutive days with TRH-tartrate (10µg/0.2 ml/mouse i.p.) or saline (0.2 ml/mouse) intraperitoneally

TABLE 4. *2-month chronic oral treatment with TRH corrects to more juvenile values peripheral blood lymphocytes, thyroxin and plasma zinc (Zn) levels and lowers lipid levels in old C57BL male mice*

Parameters measured	*Old control Mice (N=6)*	*Old mice +TRH (N=6)*	*Young Untreated mice (N=5)*
Blood leucocytes (No/mm^{3}x 10^3)	12.3±3.3	15.0±3.5	13.0±3.0
% lymphocytes	68.3±9.1	75.8±5.1	84.0±3.6
Blood lymphocytes (No/mm^{3}x 10^3)	8.2±2.3	12.2±2.5*	11.0±2.4
Triglycerides (mmol/L)	0.97±0.20	0.73±0.11*	0.85±0.15
Cholesterol (mmol/L)	1.42±0.11	1.20±0.11*	1.10±0.9
T4 (mmol/L)	49.7±2.9	54.6±4.3*	60.3±5.6
Zn plasma levels (µg/dL)	48.2±8.1	64.0±8.5*	117.3±4.6

Mean ± SD, *p<0.05 when compared to old controls, (Student's *t* –Test). 20 month-old C57BL/6 male mice were treated permanently (day and night) for 2 months with TRH-tartrate in the drinking water at the concentration of 100ug/ml

TABLE 5. ***Four-month chronic oral treatment with TRH restores to more juvenile levels peripheral blood lymphocytes and thyroid hormones, and lowers triglycerides in old C57BL male mice***

Parameters measured	*Old control Mice (N=6)*	*Old mice +TRH (N=6)*	*Young Untreated mice (N=5)*
Blood leucocytes (No/mm^{3}x 10^3)	7.8±1.2	7.6±1.0	8.0±1.0
%lymphocytes	64.2±11.8	80.6±9.6*	85.0±2.9
Blood lymphocytes (No/mm^3 x 10^3	5.0±1.0	6.2±0.9	6.8±0.6
Triglycerides (mmol/L)	1.10±0.37	0.70±0.20*	0.86±0.16
Cholesterol (mmol/L)	1.20±0.17	1.00±0.19	0.97±0.15
T4(mmol/L)	40.3±7.9	51.8±9.0*	59.3±5.5
T3 (mmol/L)	0.48±0.15	0.92±0.19*	1.20±0.12
Testosterone plasma level (µmol/L)	3.6±1.8	4.8±3.0	320.0±50.0
Glucose (µmol/L)	6.4±0.9	6.0±0.8	7.7±0.2

Mean ±SD,*p<0.05 when compared to old controls, (Student's *t*-Test).

20 month-old C57BL male mice were treated permanently (day and night) for 4 months with TRH-tartrate in the drinking water at the concentration of 100 µg/ml.

TABLE 6. *Chronic oral treatment with TRH restores juvenile organ weight in old C57BL male mice*

Parameters measured	*Old control Mice (N=6)*	*Old mice +TRH (N=6)*
Body weight (BW)(g)	33.3±3.0	29.7±3.3
Thymus weight (TW)(mg)	14.3±4.7	21.3±4.8*
TW/BW ratio	0.43±0.16	0.71±0.14*
Adrenals weight (AW)(mg)	3.6±0.58	2.1±0.27**
AW/BW ratio	0.11±0.02	0.07±0.02*
Testes weight (TeW)(mg)	173.9±12.3	181.9±12.7
TeW/BW ratio	5.2±0.17	5.9±0.19**
Heart weight (HW)(mg)	172.3±14.9	173.8±13.8
HW/BW ratio	5.2±0.55	5.9±0.29*
Kidneys weight (KW)(mg)	457.8±27.9	451.7±48.6
KW/BW ratio	13.5±0.85	15.2±1.15*
Spleen weight (SW)(mg)	94.4±23.9	103.8±20.6
SW/BW ratio	2.82±0.66	3.56±1.11
Liver weight (LW) (g)	1.67±0.10	1.67±0.18
LW/BW ratio	0.05±0.001	0.05±0.01

Mean ±SD,*p<0.05 when compared to old controls, **p<0.01 when compared to old controls (Student's *t*-Test).

20 month-old C57BL male mice were treated permanently (day and night) for 4 months with TRH-tartrate in the drinking water at the concentration of 100 µg/ml.

Figure 1

Female hybrid mice treated day and night with TRH-tartrate in the drinking water (100 ug/ml) from the age of 16 months. No significant prolongation of life span is visible

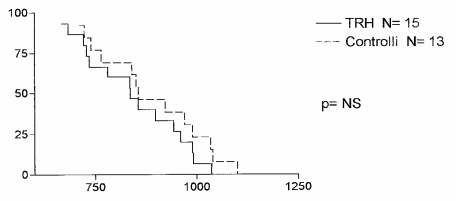


Figure 2

C57/BL6 female mice treated permanently with TRH-tartrate (100ug/ml) in the drinking water starting from 20 months of age. No prolongation of their life span is visible

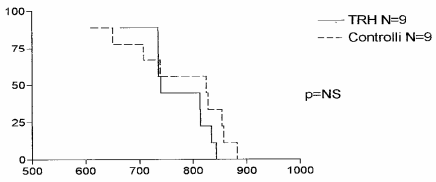


Figure 3 A. Testicle of a 24 month-old C57BL/6. Note atrophy of follicles and abrogation of spermatogenesis. Haematoxylin-eosin, x 200

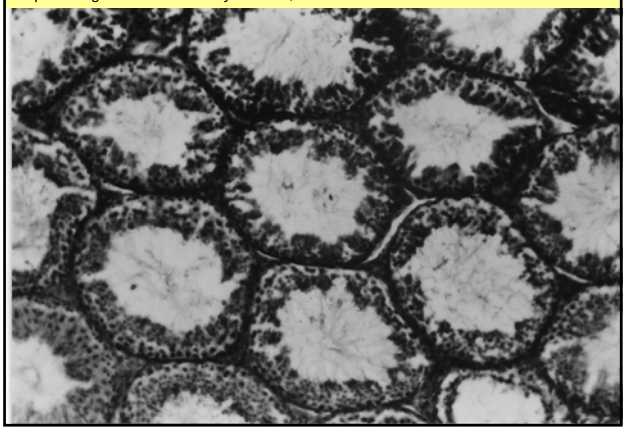


Figure 3 B Testicle of a 24 month-old C57BL/6 mouse, receiving TRH in the drinking water for 4 months at the dosage of 100ug/ml. Note total recovery of spermatogenesis. Haematoxylin-eosin, x 200

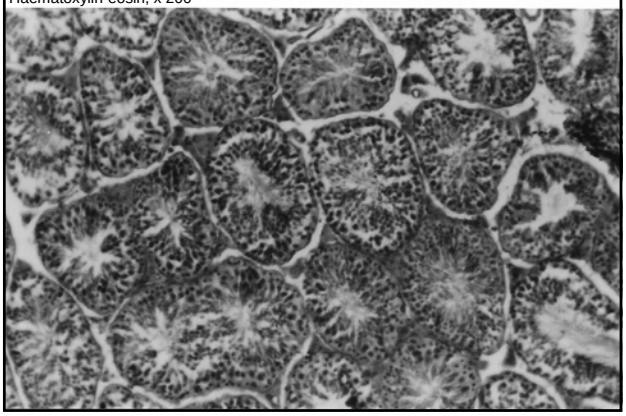


Figure 3 C Testicle of a 24 month-old C57BL/6 mouse. Note atrophy and absence of active spermatogenesis. Haematoxylin-eosin, x 400

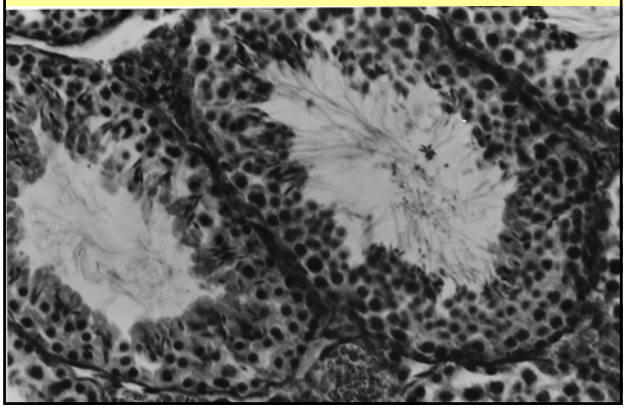
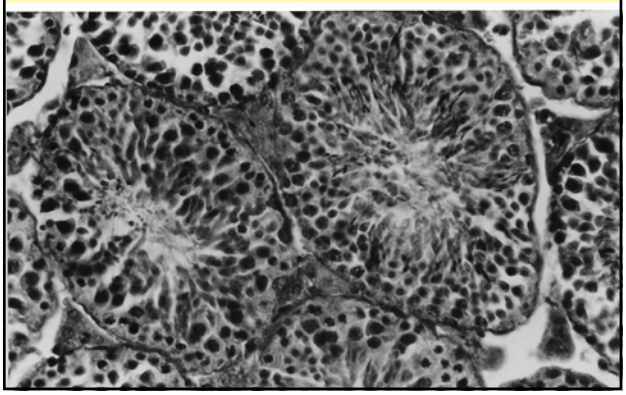


Figure 3 D Testicle of a 24 monht-old C57BL/6 mouse, after four month treatment with TRH in the drinking water (100 ug/ml). Note complete reconstitution of spermatogenesis and maintenance of function. Haematoxylin-eosin, x 400



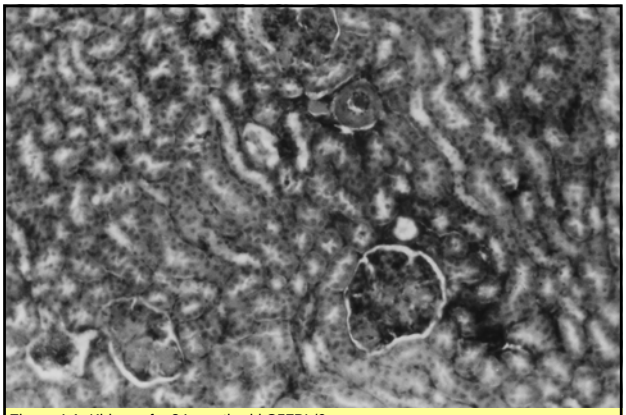


Figure 4 A. Kidney of a 24 month-old C57BL/6 mouse. Note massive hyalin degeneration and glomerular atrophy. Haematoxylin-eosin, x 200

Figure 4 B. Kidney of a 24 month-old C57BL/6 mouse after 4 month treatment with TRH in the drinking water (100 ug/ml). Note the perfect maintenance and/or reconstitution of kidney structure and glomerular cellularity. Haematoxylin-eosin, X 200

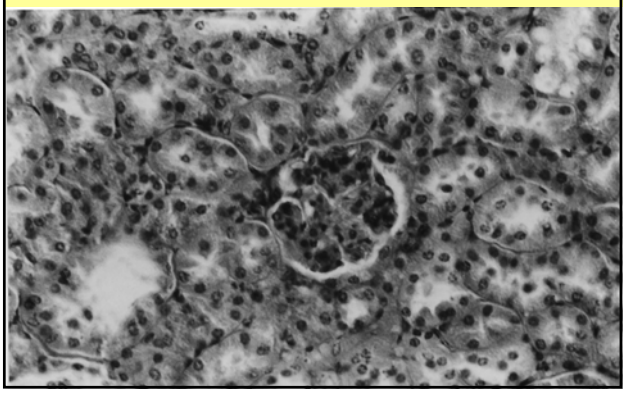


Figure 4 C. Kidney of a 24 month-old C57BL/6 mouse. Note the massibe glomerular sclerosis, atrophy and hyalin degeneration. Haematoxylin-eosin, x 400

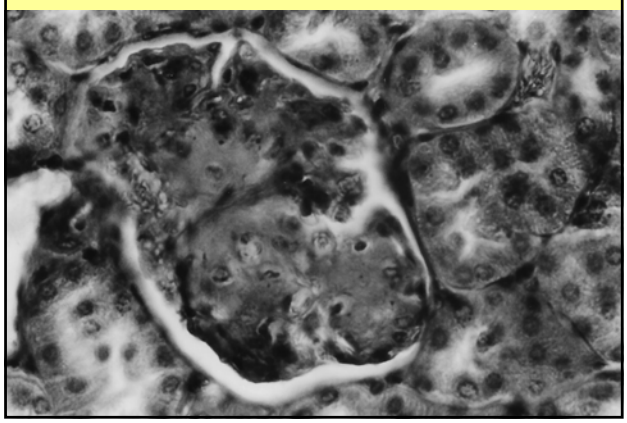


Figure 4 D. Kidney of a 24 month-old C57BL/6 mouse, after 4 month treatment with TRH in the drinking water (100 ug/ml). Note complete maintenance of glomerular structure and cellularity. Haematoxylin-eosin, x 400

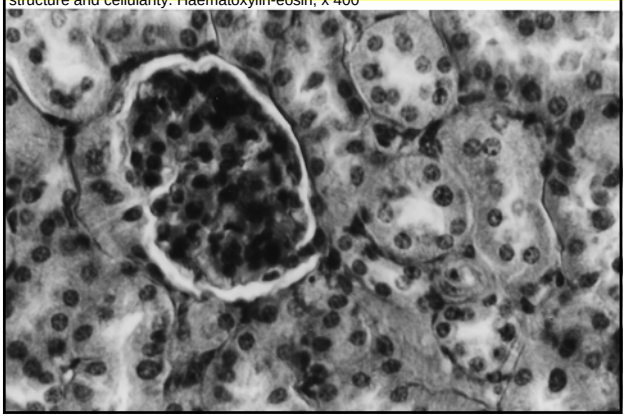
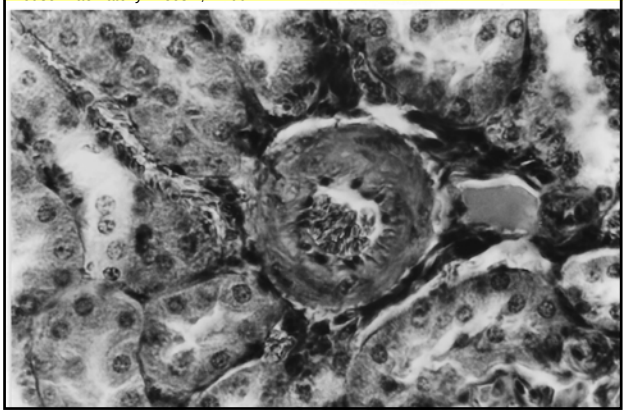


Figure 4 E . Artery degeneration (sclerosis) in the kidney of a 24 month-old C57BL/6 mouse. Haematoxylin-eosin, x 400.



AS ALREADY HINTED IN OUR PREVIOUS WORK IN 1990, TRH POSSESSES UNIQUE PROPERTIES FOR REGULATING BASIC CELL MECHANISMS WHICH ARE COMMON FOR ALL CELLS AND TISSUES IN THE BIOSPHERE. THOSE MECHANISM HAVE ANTICIPATED AND HAVE BEEN FUNDAMENTAL FOR THE ORIGIN OF LIFE AND ARE BASIC FOR MAINTANENACE OF LIFE ITSELF

WHATEVER THE MECHANISM, TRH CAN BE NOW BECOME A MAIN AGING-REVERSING "DRUG". ITS TOTAL AND DEMONSTRATED LACK OF NOXIOUS AND SIDE EFFECTS, OPENS UNLIMITED OPPORTUNITIES FOR THE PREVENTION AND THERAPY OF DEGENERATIVE DISEASES AND CANCER

TRH: welcome back on our Planet!