Aging-Reversing Properties of Thyrotropin Releasing Hormone (TRH)

Walter Pierpaoli, MD

Interbion Foundation for Basic Biomedical Research, Via San Gottardo 77, 6596 Gordola, Switzerland, pierpaoli.fnd@bluewin.ch

Abstract

Previous work from our laboratory prompted us to study the effects of short-term, acute administration of TRH or its chronic oral administration, on organs, tissues and aging-related metabolic and hormonal markers, in order to acquire more knowledge on effects, dosage and timing of administration according to its circadian cyclicity. In addition, we wanted to verify its anti-aging effects on two most fundamental functions, namely gonadal- reproductive and kidney-urinary. The results demonstrate that both a short-term, acute or a chronic, long-term oral administration of TRH to old, aging mice, results into positive changes and rapid correction to more juvenile levels of most typical aging-related hormonal and metabolic alterations. Remarkably, 4-month oral treatment with TRH maintains testes function in aging mice. As hinted by the significant increase of testes weight, TRH taken from the drinking water produces a maintenance and/or reconstitution of testes structure and function

as shown by active proliferation and formation of mature spermatogonia and intensive spermatogenesis in the follicles. 4-month oral treatment with TRH protects the kidneys from amyloid and hyalin infiltration of both tubuli and glomeruli, which is typical of aging mice. In fact, massive deposits of amyloid and hyalin material are clearly infiltrating the shrunken glomeruli of untreated mice with loss of filtration capacity, while hardly present in TRH-treated mice. Massive hyalin degeneration can also be observed in the tubular vessels of the untreated control mice.

These experiments with parenteral and oral administration of TRH show a most remarkable aging-delaying and apparently even aging-reversing effects of the neuropeptide TRH. Again, similarly to melatonin, we are confronted with an anti-aging agent with a broad spectrum of activities which must be necessarily linked to a most fundamental role in the regulation of metabolic and hormonal functions.

Introduction

Work in our laboratory has shown the remarkable and varied effects of TRH
in the rapid reversal of aging-related changes and alterations in old rodents (1,2). It was also demonstrated that these restoring effects of TRH were not exerted via the thyroid gland, with the obvious increase of synthesis or secretion of thyroid hormones, but depended on a newly found and rather unexpected direct activity of TRH (1,2). We suggested that the tripeptide TRH, thanks to its ubiquitous localization in nature and its high concentration in key tissues such as the pineal gland, the pancreatic insulin-producing beta cells and the anterior hypothalamic area (3,4), represents a vehicle for most fundamental energy-regulating and hormone-synchronizing activities (5).
In fact, the restoring effects of TRH could be seen in all models used, such as aging and immunodeficient animals (1), virus-infected and stressed mice (1), hypothalamus lesioned mice (2) and in particular in old animals with typical adiposity and progressive decay of neuroendocrine, immune and hormonal functions (6). TRH associated to melatonin normalizes lipid

metabolism and prolongs longevity in aging mice (7). All this previous work prompted us to study the effects of short-term, acute administration of TRH or its chronic oral administration, on organs, tissues and aging-related metabolic and hormonal markers, in order to acquire more knowledge on effects, dosage and timing of administration according to a possible circadian cyclicity of its production and/or release (8,9). In addition, we wanted to verify its anti-aging effects on two most fundamental functions, namely gonadal- reproductive and kidney-urinary. The results obtained demonstrate that TRH possesses in fact most remarkable restorative, aging-reversing properties in the models chosen.

Materials and methods

Animals. Inbred, male or female C57BL/6 and BALB/c mice raised in our animal rooms have been used. They were given water and pellets *ad libitum* and kept in air-conditioned rooms at a temperature of 20 to 22 °C. Lighting was: 7AM light on and 7PM light off.

TRH. Synthetic and analytically pure tripeptide (TRH-tartrate) was a generous gift from Senn Chemicals, Dielsdorf, Switzerland. Its was dissolved in sterile bidistilled water and injected immediately after the preparation of the solutions. It was injected intraperitoneally (ip) for short or longer periods according to the experiment.

Blood assays. In the morning between 8 and 10 A, blood samples were taken from the retro-orbital plexus under rapid aether anaesthesia. Clotted blood was centrifuged and sera samples were taken and frozen until the different analyses were performed. Peripheral blood counts were done on fresh samples immediately after bleeding.

Oral treatment with TRH. When TRH was given chronically in the drinking water, the dosage used was 100 micrograms per ml. Water with or without TRH was changed every two days.

Light microscopy. Organs we taken after sacrifice, weighed and fixed in Bouin fluid. They were embedded in paraffin and 3 to 5 micron slices were stained with haematoxylin-eosin

Results

The results illustrated in Tables 1-6 demonstrate that both a short-term, acute or a chronic, long-term oral administration of TRH to old, aging mice, results into positive changes and rapid correction to more juvenile levels of most typical aging-related hormonal and metabolic alterations.

Acute parenteral treatment with TRH. As seen in Table 1, a 10-day treatment with TRH produces a rapid decline of triglycerides in aging and old BALB/c and C57BL/6 mice, with less evident and significant effect on levels of cholesterol. Acute evening treatment with 10ug TRH for two weeks also significantly modifies levels of cholesterol and triglycerides of aging, 15-month old mice towards more juvenile values (Tables 2A and B). As shown in Tables 3A and B, where older, 18 month-old mice were used, morning but also to a minor extent evening administration of TRH for two weeks remarkably modifies to more juvenile values blood levels of cholesterol, triglycerides, phospholipids, sodium, chloride, calcium and creatine.

Chronic oral treatment with TRH.

A) Effect on blood parameters. As shown in Table 4, a two-month oral treatment with TRH at the concentration of 100ug/ml in the drinking water of 20-month old C57BL/6 mice significantly increases peripheral blood levels of lymphocytes and lowers blood levels of cholesterol and triglycerides. It also positively corrects levels of thyroxin and plasma zinc.

When the treatment was prolonged for further two months, it can be seen in Table 5 that TRH significantly and positively corrected blood levels of lymphocytes, triglycerides and thyroid hormones. Levels of cholesterol and testosterone show a positive, respectively lower and higher but not significant variation (Table 5).

B) Effect on organs. The animals were bled and sacrificed at the end of the four-month period of TRH oral administration. Their organs were immediately weighed.

As seen in Table 6, a four-month oral administration of TRH in the drinking water results into significant changes of organ weight towards more juvenile values.

TRH oral treatment produces an increase of thymus, testes, heart and kidney weight and a reduction of adrenal weight.

C) Effect on longevity. Day and night treatment with TRH in the drinking water (100 ug/ml) of C57BL/6 mice starting at 16 months of age, die not produce and significant prolongation of their longevity (Figs 1 and 2).

Effect of chronic oral administration of TRH on kidney and testis structure and function. Light microscopy analysis.

A) Testes. As seen in Figure 3A-D, 4-month oral treatment with TRH remarkably maintains testes function in aging mice. As hinted by the significant increase of testes weight (see above), TRH taken from the drinking water produces a maintenance and/or reconstitution of testes structure and function as shown by active proliferation and formation of mature spermatogonia and intensive spermatogenesis in the follicles (B and D, when compared to untreated controls (A and C). Apparently TRH greatly enhances the final maturation process of spermatogonia to spermatozoa.

B) Kidneys . As illustrated in Figure 4A-E, a 4-month treatment with TRH protects the kidneys from amyloid and hyalin infiltration of both tubuli and glomeruli, which is typical of aging mice. In fact, massive deposits of amyloid and hyalin material are clearly infiltrating the shrunken glomeruli of untreated mice with loss of filtration capacity (A and C), while hardly present in TRH-treated mice (B and D) . Massive hyalin degeneration can also be observed in the walls of blood vessels and in tubules of the untreated control mice (E).

Discussion

The extensive and repeated experiments with subcutaneous and oral administration of TRH reported above indicate a most remarkable aging-delaying and apparently *even aging-reversing* effects of the neuropeptide TRH. Again, similarly to melatonin (10), we are confronted with an

anti-aging agent with a broad spectrum of activities which must be necessarily linked to a most fundamental role in the regulation of metabolic and hormonal functions(10,11). In fact, it is impossible to believe that the variety of protective activities observed on stress, immunity, viruses, thyroid, lipids and gonads depend on specific and limited tissue or cell-targeted effects. On the contrary, as suggested earlier (5), we think that TRH, which is ubiquitous in nature (3), provides to basic mechanisms centrally located in the CNS such as the anterior hypothalamic area and the pineal gland, and in high-demanding endocrine tissues such as the pancreatic beta cells, for regulation of rapid adaptive responses such as regulation of sugar levels in the blood (12-14). In fact and contrarily to "slow melatonin", TRH administration has been shown in many models to rapidly and positively modify immunological and hormonal derangements (1,2,6,7).

The most remarkable and rapid effects of TRH seem to concern the adjustment of aging-altered lipids within a few days. This is shown in Tables 1-3. As seen in Table 2 and 3, the lipid-lowering effects of TRH are more pronounced in older mice (compare Tables 2 with Tables 3). Also a significant improvement of kidney function, bone metabolism, glucose levels, sodium and chloride can be seen in 18-month-old TRH-treated mice, in particular in the mice treated in the morning.

When TRH was administered chronically in the drinking water at the dose of 100micrograms/ml water to 20-month old C57BL/6 male mice, rapid and significant improvement of all typical aging-related alterations measured were observed after two (Table 4) and after four (Table 5) months of TRH oral supply. The positive changes concerned immunological values such as peripheral blood lymphocyte number, key-metabolic derangements such as lipid levels, thyroid hormones and finally zinc, a mineral whose decline precisely follows aging
(Tables 4 and 5).

The examination of the organs of the mice measured after four months of TRH administration showed a positive correction which corresponded to the metabolic and hormonal changes observed in peripheral blood. In fact, the adrenals were not enlarged as usually seen in aging-related hyper-corticosurrenalism while the thymus and the testes maintained their weight in relation to cellularity and function (Table 6). A striking evidence for maintenance of function and regression of aging was seen in the light microscopy analysis of the testes and kidneys. A four-month oral administration of TRH in C57BL/6 mice starting and 20 months of age, produced a clear-cut re-activation of spermatogenesis, as already hinted by testes weight and testosterone levels
(Figures 3A-D). TRH also prevented typical hyalin and amyloid degeneration of kidney glomerular and tubular structures which affects all senescent mice(Figures 4A-E).

The observations reported above, although critical for the attribution of aging-delaying or -reversing properties to the neuropeptide TRH and for its possible clinical applications, do not

certainly allow to speculate on its mechanism of action (12, 15, 16). We can only suggest that TRH may be partly responsible, at least in mammals and via its stimulating effects on hypophyseal TSH, to maintain thyroid function and prevent its aging-related decay. In addition, the chronic (day and night) administration of TRH may not be suitable to evaluate the effects of a biologically active peptide such as TRH. We must in fact elaborate a method of administration in patients with different pathologies, which is closer to its circadian levels. In fact, the beneficial effects of TRH may become more evident when its administration will follow a day-night variability (4,9). Independently from its proposed anti-aging effects, TRH is anyhow suitable for a rapid correction of most common metabolic and hormonal derangements of aging, alone or possibly in combination with melatonin (7). We are confident that accurate studies will allow to obtain a supplementation suitable to "reset the hormonal clock" and to delay or even to reverse aging (17)

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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 2A. *Acute treatment with TRH lowers cholesterol and triclycerides i*

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 *

TABLE-B2. Acute treatment with TRH lowers cholesterol and triglycer

Parameters measured	*Young Untreated mice (N=10)*	*Old mice + saline (N=7)*	*Old mice + TRH eveni (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 (µmol/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 c
t-Test). 15 month old C57BL female mice were injected in the evening (6p.m) for 15 cons
with TRH-tartrate (10µg/0.2 ml/mouse) or saline (0.2ml/mouse).

TABLE 3A. *Acute treatment with TRH improves age-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)	54.9±0.95**	59.6±1.2	58.7±2.2	59.0±1.9
Albumin (g/L)	33.7±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*

TABLE-3. *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
Creatinenzymatic (µmol/L)	52.1±3.8	79.2±9.8	30.5±2.0*	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)	21.1±0.58**	26.0±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, (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 juve peripheral blood lymphocytes, thyroxin and plasma zinc (Zn) levels and levels in old C57 BL male mice*

Parameters measured	*Old control Mice (N=6)*	*Old mice +TRH (N=6)*	*Young Untreated mice (N=5)*
Blood leucocytes (No/mm³ x 10³)	12.3 3.3	15.0 3.5	13.0 3.0
% lymphocytes	63.3 9.1	75.8 5.1	84.0 3.6
Blood lymphocytes (No/mm³ x 10³)	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 (µgdL)	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 ju levels peripheral blood lymphocytes and thyroid hormones, and low 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 in the drinking water at the concentration of 100 µg/ml.

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

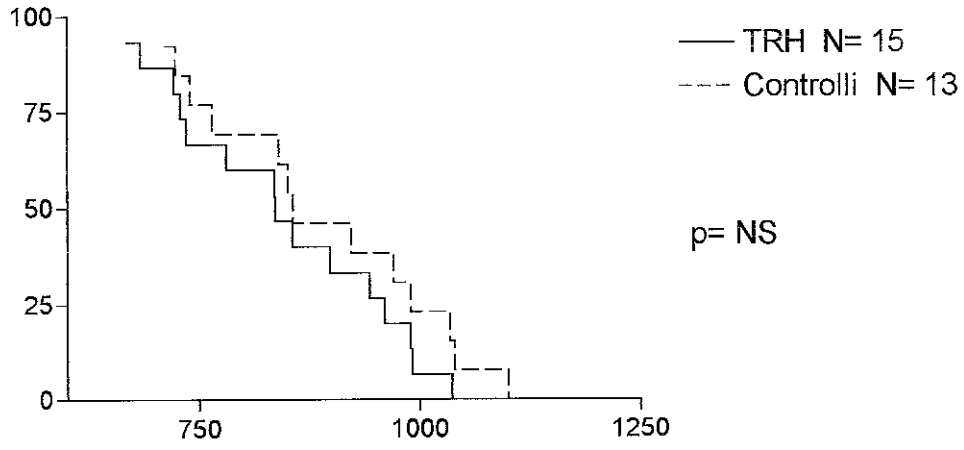

Figure 2

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

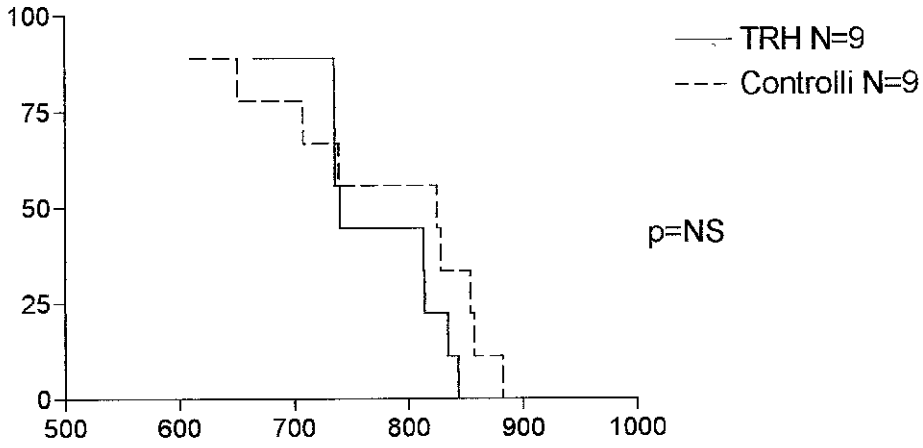

Figure 3 A. Testicle of a 24 month-old C57BL/6. Note atrophy of follicles and abrogation of spermatogenesis. Haematoxylin-eosin, x 200

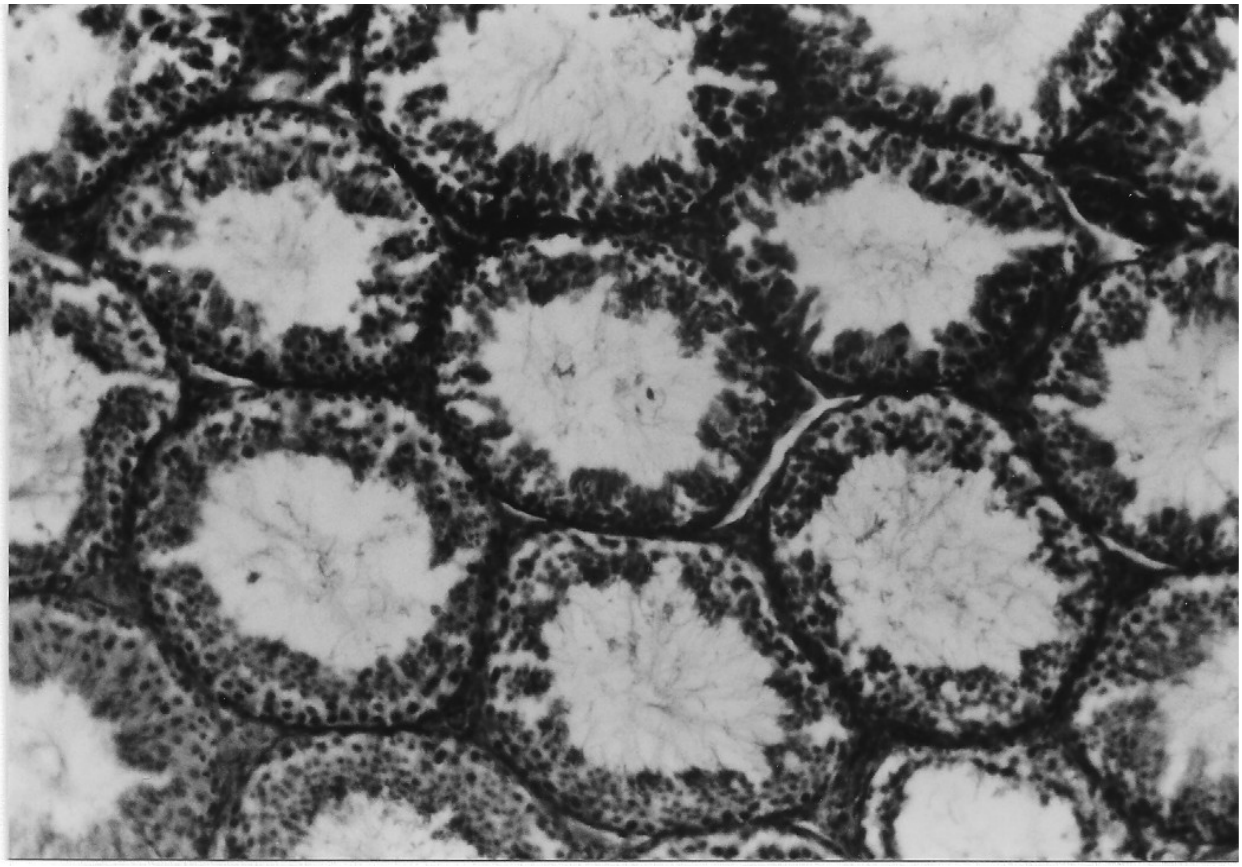

Figure 3 B Testicle of a 24 month-old C57BL/6 mouse, receiving TRH in the drinking water for 4 months at the dosage of 100ug/ml. Note total recovery of spermatogenesis. Haematoxylin-eosin, x 200

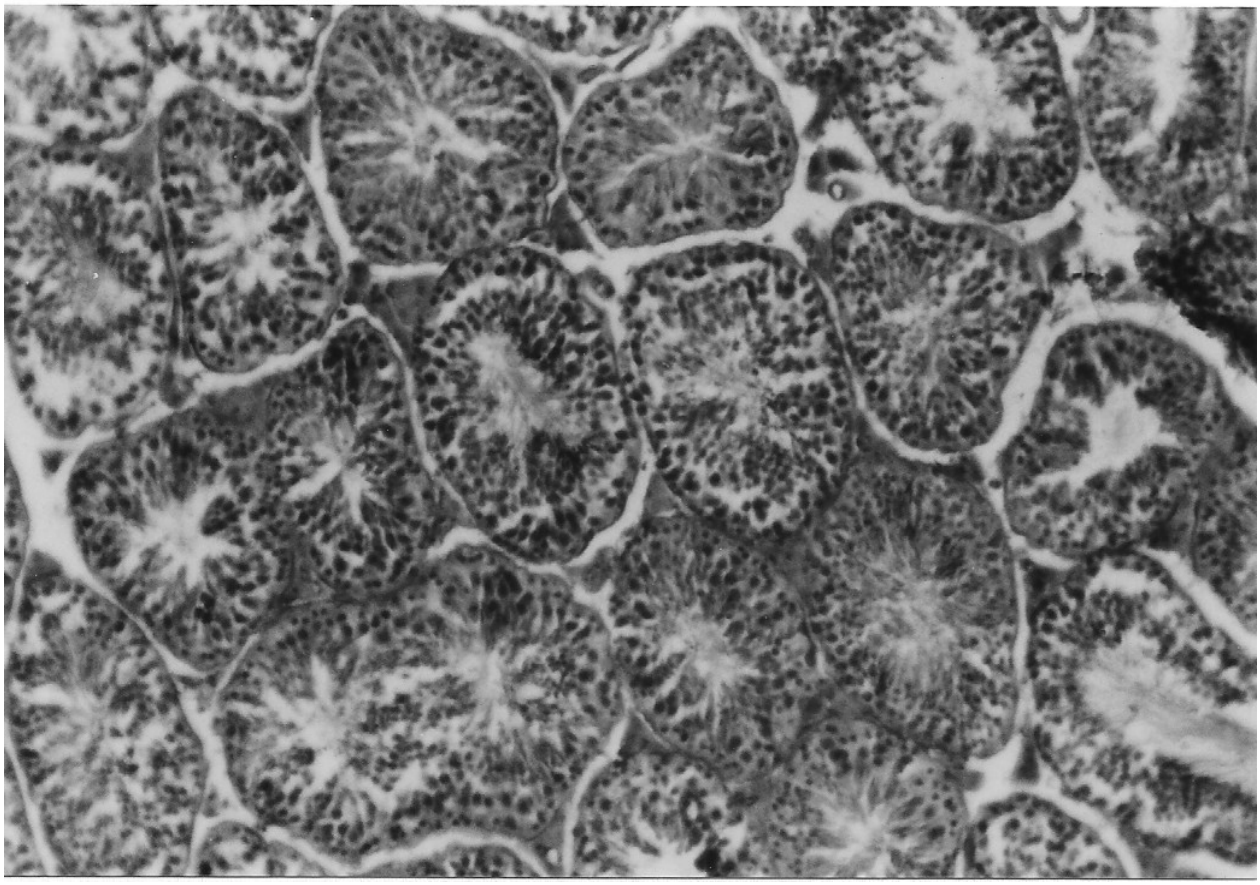

Figure 3 C Testicle of a 24 month-old C57BL/6 mouse. Note atrophy and absence of active spermatogenesis. Haematoxylin-eosin, x 400

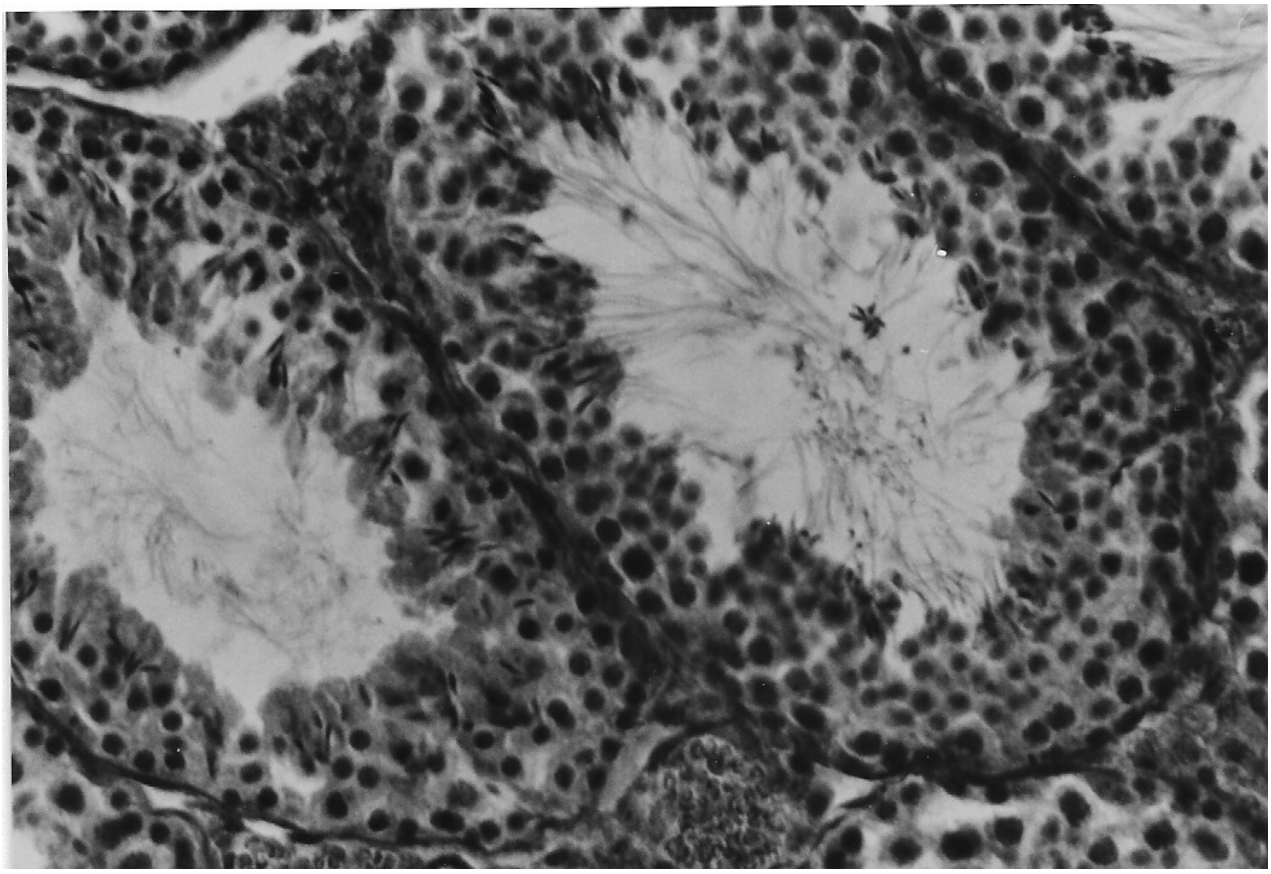

Figure 3 D Testicle of a 24 monht-old C57BL/6 mouse, after four month treatment with TRH in the drinking water (100 ug/ml). Note complete reconstitution of spermatogenesis and maintenance of function. Haematoxylin-eosin, x 400

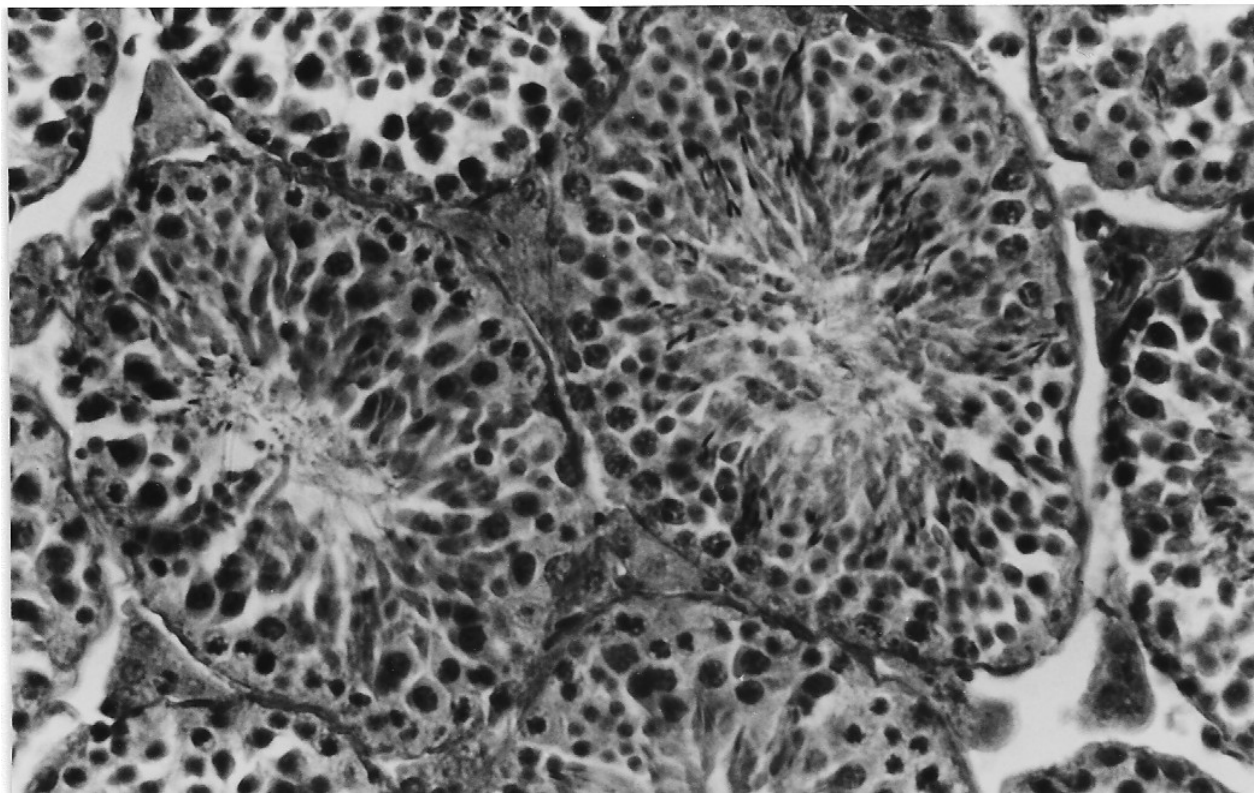

Figure 4 A. Kidney of a 24 month-old C57BL/6 mouse. Note massive hyalin degeneration and glomerular atrophy. Haematoxylin-eosin, x 200

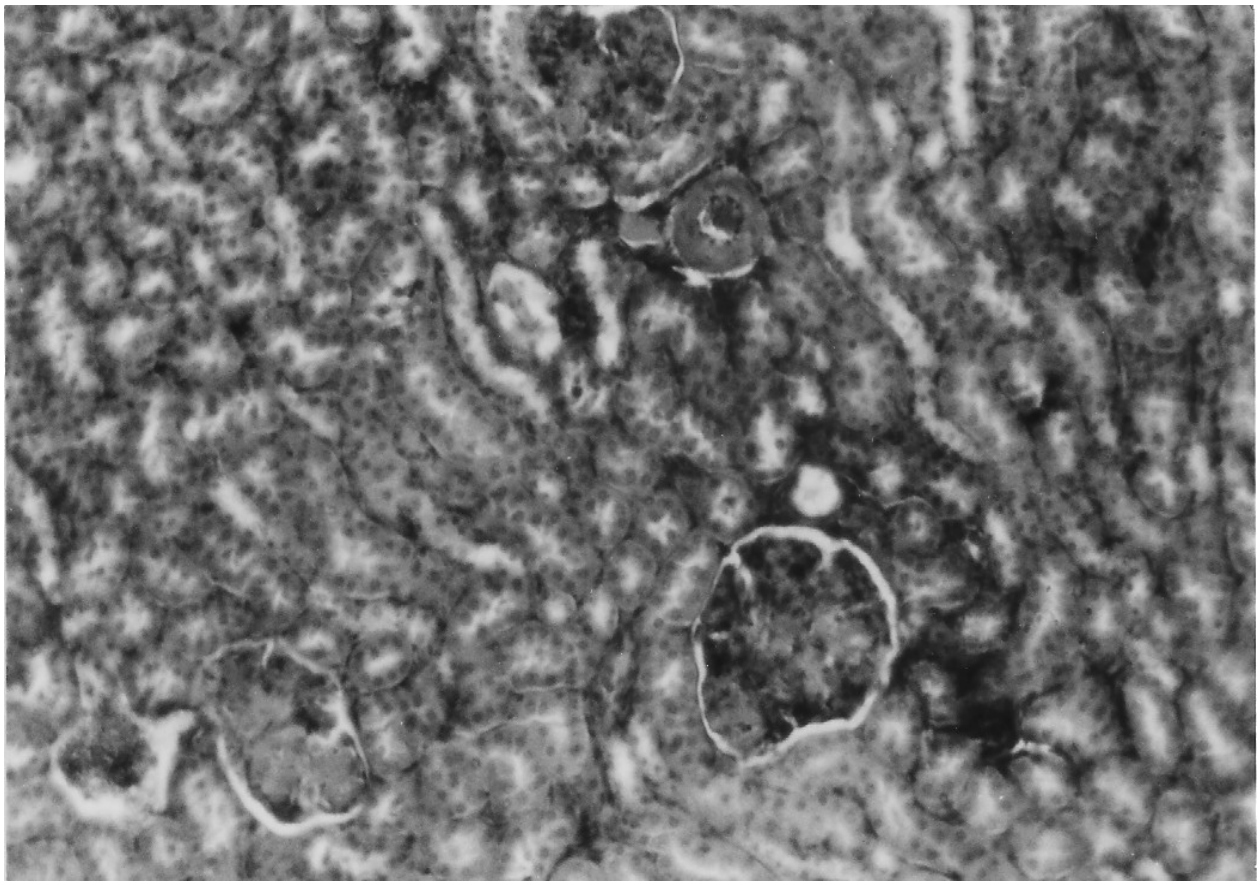

Figure 4 B. Kidney of a 24 month-old C57BL/6 mouse after 4 month treatment with TRH in the drinking water (100 ug/ml). Note the perfect maintenance and/or reconstitution of kidney structure and glomerular cellularity. Haematoxylin-eosin, X 200

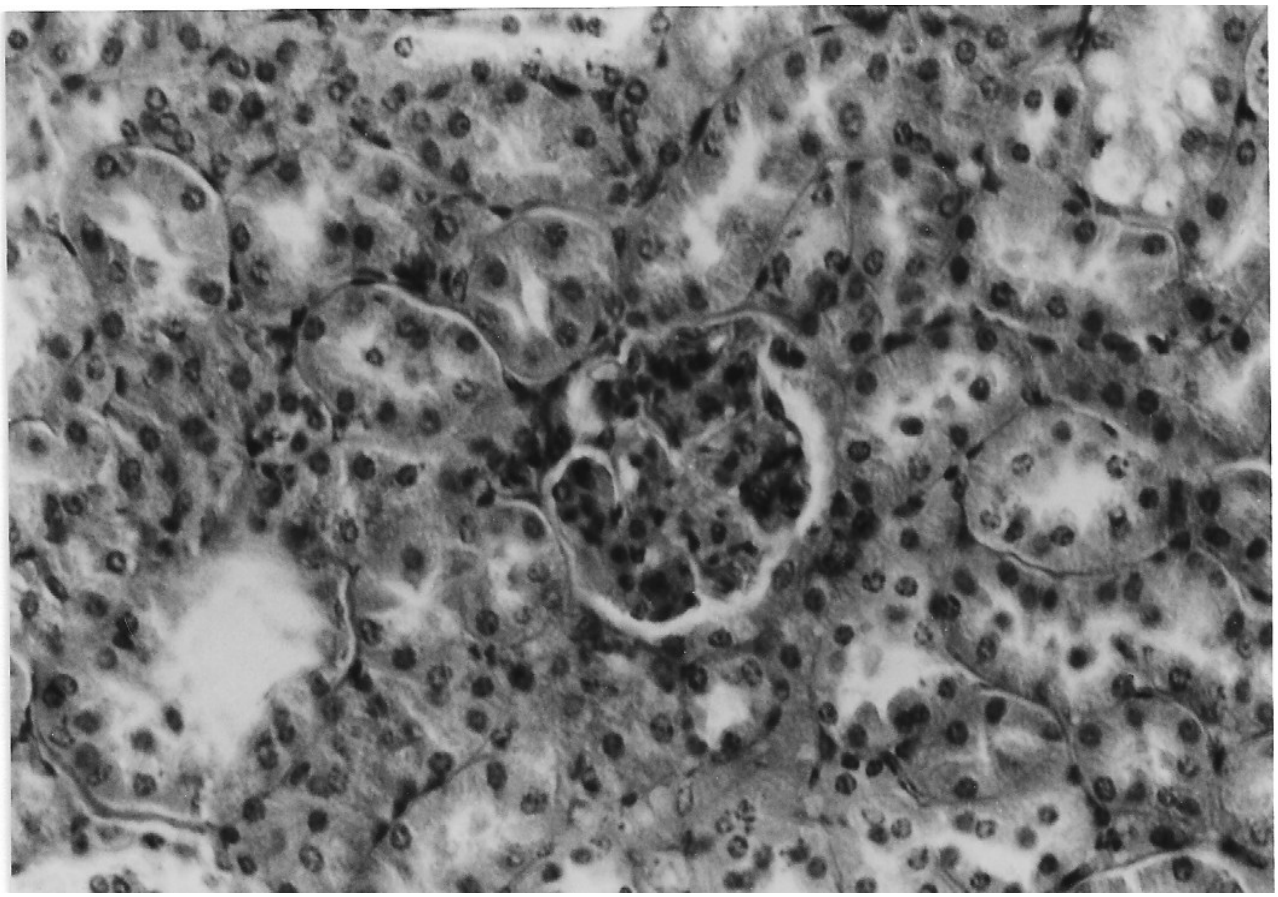

Figure 4 C. Kidney of a 24 month-old C57BL/6 mouse. Note the massibe glomerular sclerosis, atrophy and hyalin degeneration. Haematoxylin-eosin, x 400

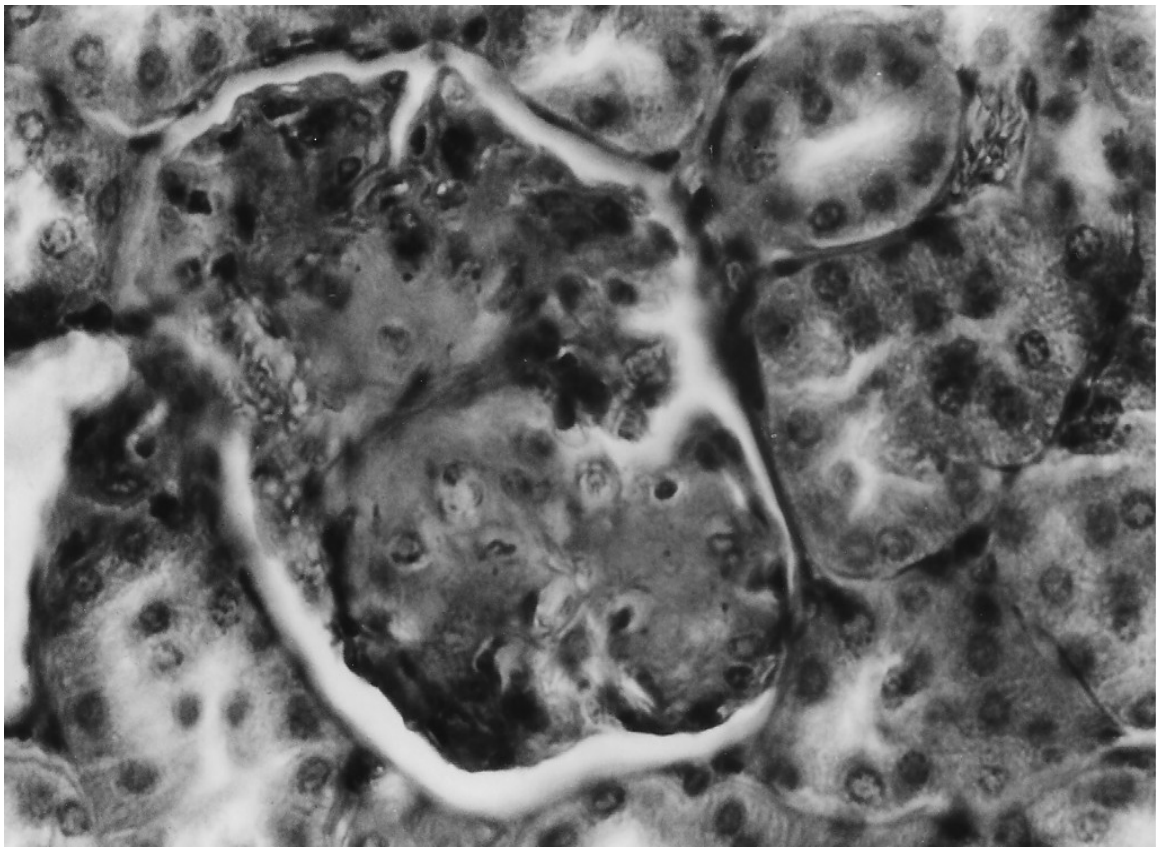

Figure 4 D. Kidney of a 24 month-old C57BL/6 mouse, after 4 month treatment with TRH in the drinking water (100 ug/ml). Note complete maintenance of glomerular structure and cellularity. Haematoxylin-eosin, x 400

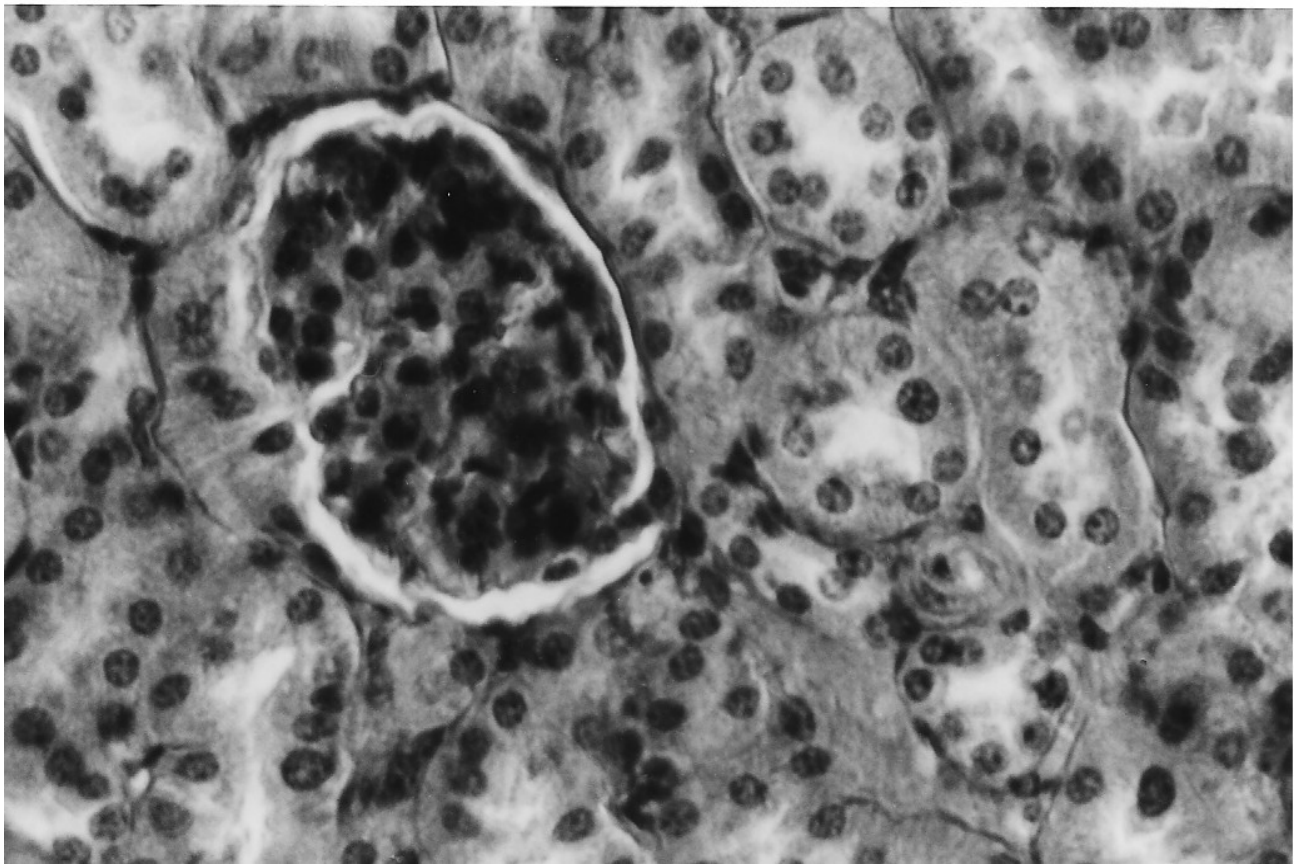

Figure 4 E . Artery degeneration (sclerosis) in the kidney of a 24 month-old C57BL/6 mouse. Haematoxylin-eosin, x 400.