volving a completely unrelated, non-viral, non-specific reaction by certain kidney cells with partially denatured or altered RBC's. This phenomenon of non-specific hemadsorption, or tissue hemadsorption (THAd), seems to be a characteristic of kidney cells and was not found to occur in cell cultures of non-kidney origin.

While this manuscript was in preparation, Neuman and Tytell reported similar findings and showed that the degree of non-specific hemadsorption could be related to the concentration of calcium ions(9). We concur with their finding that calcium is essential for adsorption. However, evidence presented here suggests that calcium ions are not the initiating factor. The usual concentration of calcium in Eagle's medium is 1.8 mM. Under these conditions we have found no evidence of non-specific adsorption of fresh RBC's. Yet RBC's which have been preconditioned or aged (in the absence of calcium) were found to yield maximum HAd patterns when tested in the same medium.

Non-specific adsorption may be avoided under the usual conditions for specific viral HAd tests simply by using fresh RBC's. However, the observed inhibition of THAd by SV_{40} infection of rhesus monkey kidney and preliminary studies with rubella in LLC-MK_2 suggest that THAd inhibition (THAdI) may offer some advantage as a practical research tool.

Summary. Hemadsorption in rhesus monkey kidney tissue culture has been shown to occur by two different mechanisms, a specific reaction of red blood cells with viral receptors and a non-specific, non-viral, reaction of partially denatured or altered red blood cells with specialized kidney cells. Non-specific tissue hemadsorption (THAd) was found not to be limited to rhesus kidney tissue culture but also occurred in kidney tissue strains and lines derived from other animal species. The finding that some viruses inhibit THAd suggests the possible use of this phenomenon as a tool in diagnostic virology.

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Effect of Thyroxine on Spontaneous Nephrosis in the Rat.* (30736)

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The occurrence of spontaneous nephrosis with proteinuria, hyperglobulinemia and hypercholesterolemia in the rat was described previously(1). Proteinuria was observed at weaning and changes in the glomerular capillary basement membrane were noted at 60 days of age. Incidence and severity of renal lesions increased with age and proteinuria became correspondingly greater. Earlier studies(2,3,4) showed that onset of nephrosis as

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well as other spontaneous diseases was delayed by dietary restriction. Since metabolism is reduced by underfeeding(5), the question arises whether increased metabolic rate accelerates onset of renal lesions. For this purpose, thyroxine was administered to adult rats for periods of 250 days to 400 days in amounts that resulted in a 50% increase in O_2 consumption.

Materials and methods. Male rats bred from a pathogen-free Sprague-Dawley colony started in 1945 were used. Details concerning laboratory conditions, diet, histologic techniques, measurement of daily food consumption, water intake and urine output, chemical and electrophoretic analysis of urine and serum proteins, and methods of determining serum cholesterol and BUN (blood urea nitrogen), have been described(1). Sodium-l-thyroxine pentahydrate was supplied in the drinking water and treatment was started when the rats were 110 or 210 days old. A stock solution was prepared by dissolving 50 mg of thyroxine in 25 ml of 95% alcohol to which 7 ml of N/10 NaOH was added, diluting to 100 ml with distilled H_2O, and then diluting 10 ml of the latter to a liter with distilled H_2O. The daily dose of thyroxine was about 85 μg/100 g. This value was derived from the daily water intake measured for a 14-day period. Body weights were recorded biweekly from time of weaning. One group of animals was autopsied at 370 days of age after receiving thyroxine for 250 days. Another group was examined at 600 days to 618 days of age, after thyroxine treatment for 410 days. Untreated rats of corresponding ages were the controls. Data on the control rats were reported previously (1). A third group was used for O_2 consumption studies. Thyroxine was given for 225 days before the test and the rats were autopsied 14 days later, at 445 days of age. Observations on O_2 consumption were repeated on 5 consecutive days. The equipment consisted of 12 metabolism chambers housed in a constant temperature, forced-air circulation, cabinet maintained at 29 $\pm$ 0.5°C. Closed-circuit units of the Benedict type(6) were used. All parts of the equipment (except the electric motor driven pumps), *i.e.*, the water sealed chambers, natron CO_2 absorption tubes, oil-sealed pumps and spirometer O_2 reservoirs were in the constant temperature cabinet. After an acclimatization period of 10-15 minutes, three 10-minute O_2 consumption readings for each rat were made and the 2 closest readings averaged, corrected to standard temperature and pressure and calculated on a body weight basis to obtain the O_2 consumption in mg O_2/kg/hr for that day. The animals were fed during the tests.

Results. Grossly, the kidneys of 370-day-old rats, untreated or treated, showed no alterations. At 600 days of age, a striking difference was noted between the 2 groups. In the untreated animals, the only discernible change was a brownish tint, whereas the kidneys of rats receiving thyroxine were yellow and greatly enlarged. In the most advanced stage of nephrosis, as evidenced by high BUN values, the surface was granular. The capsule stripped easily. On section, the parenchyma of damaged kidneys was honeycombed with cystically dilated ducts containing clear fluid.

Microscopically, the changes described previously in untreated rats(1) were more advanced at corresponding ages in animals receiving thyroxine. Severity of lesions was graded 1+ to 5+ using 370-day-old untreated rats as the basis of comparison. At this age all of the changes observed in older animals were present, but in lesser degree. Alterations consisted of glomerular capillary basement membrane thickening, hyalinization of tufts, adhesions of tufts to Bowman's capsule, thickening and fraying of the latter, dilatation of convoluted and collecting tubules both containing casts, atrophy of convoluted tubules with thickening and wrinkling of peritubular basement membranes, and occasional lymphocytic infiltration of the interstitial tissue. Medial thickening of arteries and interstitial fibrosis were infrequent findings. Lesions graded 5+ were characterized by loss of the normal architecture of the parenchyma, replacement by greatly dilated ducts filled with casts, and hyalinization of most of the glomeruli.

Kidney weights were higher in thyroxine-treated rats than in untreated animals, and increased in proportion to severity of lesions

TABLE I. Body Weights and Organ Weights of Untreated or Thyroxine-Treated Male Rats at Different Ages.

No. of rats	Avg age (days)	Body wt (g)*	Kidneys wt (g)*	Heart wt (g)*	Liver wt (g)*	Adrenals wt (g)*	Thyroid wt (g)*	Pituitary wt (g)*
				Untreated				
6	370	493 ± 8.6	4.06 ± .10	1.39 ± .44	18.7 ± .74	.0612 ± .0014	.0314 ± .0013	.0100 ± .0005
				Thyroxine-treated				
6	370	478 ± 11.4	4.73 ± .19	1.70 ± .04	23.1 ± .66	.0701 ± .0036	.0310 ± .0016	.0111 ± .0007
Significance of difference (P)					.001	.04		
				Untreated				
9	640	480 ± 14.3	4.37 ± .27	1.53 ± .02 (8)†	20.2 ± .94 (8)	.0652 ± .0031 (8)	.0298 ± .0026 (8)	.0107 ± .0003
				Thyroxine-treated (normal BUN)				
9	618	410 ± 12.4	6.00 ± .37 (8)	1.75 ± .07 (8)	20.8 ± 1.23 (8)	.0908 ± .0032 (7)	.0287 ± .0013 (8)	.0116 ± .0005 (8)
Significance of difference (P)				.01		<.001		
				Thyroxine-treated (elevated BUN)				
5	600	345 ± 7.9	7.53 ± .38	1.80 ± .10	17.9 ± .88	.1039 ± .0086 (4)	.0290 ± .0015	.0096 ± .0007

* Mean values ± S.E.

† Figures in parentheses are No. of rats used.

(Table I). Heart weights were also greater, the increase being due to left ventricular hypertrophy, a condition observed previously in aging rats with hypertension(7). Hydrothorax resulting from cardiorenal insufficiency developed in 600-day-old thyroxine-treated animals with elevated BUN values. A comparison of adrenal weights at 370 days showed 13% higher values for rats receiving thyroxine, and at 600 days the increment amounted to 39%-59%. Except for hepatic enlargement at 370 days, the weights of the thyroid, the pituitary, and the liver of thyroxine-treated animals did not differ significantly from those of untreated rats. In 600-day-old thyroxine-treated rats, the intrahepatic bile canaliculi and the extrahepatic duct were dilated. Body weights of untreated rats increased progressively with age(8) whereas the curve for treated rats remained constant (Fig. 1). Final body weight of animals receiving thyroxine was 15% to 28% lower than that of untreated animals. O_2 consumption was 51% higher in treated rats as compared with untreated ones (Table II).

Food and water consumption as well as urine output increased by 50% in thyroxine-treated rats, and in animals with advanced nephrosis accompanied by elevated BUN values a 3-fold increase in water intake and urine excretion occurred (Table III). Levels of water intake for both untreated and treated rats were higher in Table II than in Table III. This was due to the fact that the animals in Table II were fed during water intake measurements whereas the animals in Table III were not.

Degree of proteinuria corresponded roughly with severity of lesions and was greater in thyroxine-treated rats than in untreated animals (Table III). Protein excretion was

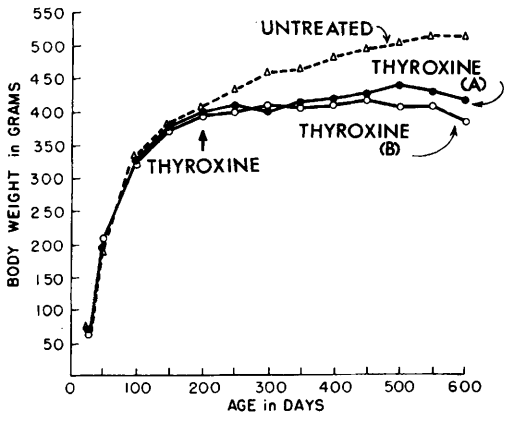

FIG. 1. Weight curves of untreated male rats, and thyroxine-treated rats with (A) normal BUN values and (B) elevated BUN values.

greatest in 600-day-old rats with renal insufficiency, as indicated by elevated BUN values. Examination of urinary proteins by electrophoresis revealed mobilities resembling serum albumin, α 2 globulin and β globulin. The albumin component predominated. There was no discernible difference between the patterns of untreated or treated rats, except for greater intensity of the various fractions in the latter. The electrophoretic patterns of the serum proteins were also alike, and consisted of a predominant albumin component and lighter β globulin, α 1 and α 2 globulins in order of diminishing intensity. The γ globulin mobility was faint and often indiscernible. Total serum proteins and serum albumin were slightly higher in 360-day-old rats receiving thyroxine as compared with untreated animals of the same age (Table IV). At 600 days, total serum proteins were reduced in thyroxine-treated animals. Hyperglobulinemia was at the same levels in untreated or treated rats except in 600-day-old treated animals with greatest proteinuria and elevated BUN values. In the latter rats hyperglobulinemia was less marked and serum al-

TABLE II. O_2 Consumption of Untreated or Thyroxine-Treated Male Rats.

No. of rats	Avg age (days)	Body wt (g)	Daily intake Food (g)*	Daily intake Water (ml)*	Thyroxine (days)†	mg/O_2/kg/hr	% increase
				Untreated			
12	425	439 ± 5.7‡	4.6 ± .09	10.7 ± .49	—	1337 ± 16.9	—
				Thyroxine-treated			
12	425	407 ± 5.3	5.3 ± .14§	16.7 ± 1.03	225	2020 ± 32.3	51

* g or ml per 100 g body wt.
† 85 μg per day.
‡ Mean values ± S.E.
§ P <.001.

TABLE III. Daily Food and Water Intake, Urine Volume, Urinary Protein Excretion, and Severity of Renal Lesions in Untreated or Thyroxine-Treated Male Rats at Different Ages.

No. of rats	Avg age (days)	Thyroxine (days)	Food (g)*	Water (ml)*†	Urine Vol (ml)*	Urine Protein (mg)*	Severity renal lesions
				Untreated			
6	360	—	4.1 ± .14‡	2.1 ± .36 (4)§	1.7 ± .22	27.6 ± 7.75	+
				Thyroxine-treated			
6	360	250	6.0 ± .22	3.6 ± .73	3.2 ± .49	49.7 ± 6.70	++ or +++
Significance of difference (P)			<.001	.10	.02	.05	
				Untreated			
9	620	—	4.2 ± .08	3.0 ± .39	2.7 ± .33	44.1 ± 5.32	+++ or ++++
				Thyroxine-treated (normal BUN)			
9	618	410	6.3 ± .16	6.1 ± .47	5.3 ± .72	81.5 ± 14.90	++++ or +++++
Significance of difference (P)			<.001	<.001	.01	.06	
				Thyroxine-treated (elevated BUN)			
5	600	407	—	12.6 ± 2.20 (4)	10.1 ± 2.25 (4)	129 ± 5.41 (4)	+++++
Significance of difference (P)				.03	.13	.03	

* g, mg, or ml per 100 g body wt.
† Fasted during 24 hr urine collection.
‡ Mean values ± S.E.
§ Figures in parentheses are No. of rats used.

TABLE IV. Serum Proteins, Serum BUN, and Serum Cholesterol in Untreated or Thyroxine-Treated Male Rats at Different Ages.

No. of rats	Avg age (days)	Serum proteins: Total (g/100 ml)*	Albumin (g/100 ml)*	Globulin (g/100 ml)*	A/G ratio	Serum BUN (mg/100 ml)*	Serum cholesterol (mg/100 ml)*
			Untreated				
6	370	6.3 ± .12	3.5 ± .08	2.9 ± .12	1.2	19.5 ± .40	105 ± 6.5
			Thyroxine-treated				
6	370	6.9 ± .22	3.9 ± .15	3.0 ± .24	1.3	21.8 ± .80	120 ± 10.4
Significance of difference (P)		.05	.04				
			Untreated				
9	640	6.3 ± .17	3.3 ± .10	3.0 ± .17	1.1	17.9 ± .51	157 ± 8.7
			Thyroxine-treated (normal BUN)				
9	618	5.5 ± .16	3.1 ± .24 (8)†	2.5 ± .14 (8)	1.3	19.9 ± 1.39	185 ± 23.0 (8)
Significance of difference		<.01		.07			
			Thyroxine-treated (elevated BUN)				
5	600	4.9 ± .28	2.6 ± .10	2.3 ± .21	1.1	72.8 ± 22.22	224 ± 37.6
Significance of difference			<.001‡	.04§			

* Mean values ± S.E.
† Figures in parentheses are No. of rats used.
‡ Difference between 3.3 ± .10 and 2.6 ± .10.
§ Difference between 3.0 ± 1.7 and 2.3 ± .21.

bumin was also reduced. Low A/G ratios existed in all groups and were related to hyperglobulinemia. Serum cholesterol was higher in 600-day-old animals than in younger ones, and the increase was greater in rats receiving thyroxine. Hematocrit values at 360 days were 45% for both untreated and treated rats, and in older animals the respective values were 41% and 37%.

Discussion. The body weight of rats receiving thyroxine for over a year remained constant but at a lower level than that of untreated animals. Evidently, the higher food intake of the treated rats was sufficient to compensate for the 50% increase in O_2 consumption. The increase of adrenal size was possibly related to an indirect thyroxine effect on ACTH release from the pituitary(9, 10,11). Prolongation of thyroxine administration resulted in larger adrenals.

Total serum proteins, serum albumin and high serum globulin levels were unaltered in 640-day-old untreated rats excreting more protein than younger animals. With thyroxine treatment, proteinuria exceeded that of untreated animals and total serum proteins were reduced. When renal insufficiency developed, a further increase in protein excretion occurred and a lowering of both serum albumin and serum globulin appeared. At this point, adaptive mechanisms were incapable of compensating for protein depletion.

The capillary basement membrane changes in the glomerular tufts of rats correspond closely with those observed by Mellors(12) in NZB/B1 mice. He described glomerular capillary basement membrane thickening and hyalinization of tufts in 7-12-month-old animals. Hypergammaglobulinemia occurred in mice examined at 7-10 months of age and specific tests for autoantibodies indicated that the renal lesions were induced by immunological, and autoimmune mechanisms. The similarity of glomerular alterations as well as the hyperglobulinemia observed in rats suggest that here, too, the spontaneous disease may be a manifestation of an autoimmune phenomenon.

The present study suggests that a metabolic factor may influence the onset of glomerular capillary basement thickening in spontaneous nephrosis. A reduction of metabolic rate, as in the case of underfeeding, delays the appearance of lesions(2,3), whereas

an increase produced by thyroxine accelerates their onset.

Summary. Long-term administration of thyroxine to adult rats at a level that produced a 50% increase in O_2 consumption, increased the severity of spontaneous nephrosis and proteinuria. The amount of excreted protein corresponded roughly with severity of renal lesions. The electrophoretic pattern of the urinary proteins of untreated or treated rats resembled that of the serum proteins. Hyperglobulinemia was present in both untreated and treated rats, but to a lesser degree in 600-day-old thyroxine-treated animals with greatest proteinuria and elevated BUN values. In the latter rats serum albumin was reduced too. Hypercholesterolemia was greater in treated rats than in untreated ones. Kidney, heart, and adrenal weights were also higher in animals receiving thyroxine, whereas body weight was lower.

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Effect of Advancing Age on Thyroid Hormone Secretion Rate of Female Rats.* (30737)

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Evidence that the thyroid undergoes atrophic and degenerative changes in old age both in animals(1,2,3,4), and man(5,6) has been reported. Thyroid function has been reported to be depressed with advancing age in mice (7), rats(8,9), chickens(10,11,12,13), turkeys(14), guinea pigs(15), rabbits(16), dogs (17), cattle(18,19), raccoon(20) and opossum(21). The present investigations were undertaken to study the effect of age on the thyroid hormone secretion rate (TSR) of female rats from weaning time until they were about 4 months of age, at 30-day intervals since no data was available on the TSR of the female rats of increasing age up to sexual maturity.

Materials and methods. The TSR estimation of 27 female rats of the Sprague-Dawley-Rolfsmeyer strain, bred in this laboratory and maintained at a temperature of 78 ± 1°F, was started when they were 25 days of age and had an average body weight of 55 g, by the technique described by Grosvenor and Turner(22). The TSR of the same rats was estimated at 30-day intervals starting at the ages of 55, 85 and 115 days respectively. During these investigations the rats had free access to water and Purina Lab Chow.

Results. The mean TSR of the 27 surviving rats as determined at the ages of 25, 55,

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