Mechanisms of Ageing and Development, 43 (1988) 137—152
Elsevier Scientific Publishers Ireland Ltd.

ALBUMIN ELIMINATION IN FEMALE WAG/Rij RATS WITH AGE: A LONGITUDINAL STUDY

G.J.M. JEAN HORBACH[a], STEPHEN K. DURHAM[a], SING HIEM YAP[b] and CORNELIS F.A. VAN BEZOOIJEN[a]

[a]TNO Institute for Experimental Gerontology, P.O. Box 5815, 2280 HV Rijswijk and [b]Division of Gastrointestinal and Liver Diseases, Department of Medicine, University of Nijmegen, Nijmegen (The Netherlands)

(Received July 7th, 1987)
(Revision received November 6th, 1987)

SUMMARY

A longitudinal study was performed to examine total albumin elimination and urinary albumin excretion in the female WAG/Rij rat. Complete necropsies were performed following the spontaneous death of the animals. The survival characteristics of this group was similar to that of survival cohorts. An increase in total albumin elimination, urinary protein excretion and urinary albumin excretion was observed with age. A proportional increase in the contribution of albumin to the urinary protein excretion was also observed. However, the observed increase in urinary albumin excretion could not totally account for the increase in total albumin elimination.

The predominant kidney lesion was chronic progressive nephrosis. The histological severity of the renal lesions were closely correlated with the increase in urinary albumin and total protein loss. It is concluded that the increase in total albumin elimination in rats in this study was due to age-related changes and not to cohort effects.

Key words: Albumin elimination; Urinary excretion; Longitudinal study; Aging; Rat

INTRODUCTION

Numerous studies have examined albumin metabolism and aging [1—7]. The results of these studies indicate that the levels of albumin synthesis are higher in old rats as compared to young rats. This increase was due to an increase in the content of albumin messenger ribonucleic acids [4], which resulted from an increased stability of this mRNA [8,9].

0047-6374/88/$03.50
Printed and Published in Ireland

A previous cross-sectional study documented that the absolute albumin elimination rate in female WAG/Rij rats increased with age [10]. The increase observed between 12 and 24 months of age was due to physiological changes in the animals, whereas the increase observed between 24 and 36 months of age resided in changes in the albumin molecule. An age-related increase in the urinary albumin excretion was also observed in this study, but this increase could not totally account for the increase in total albumin elimination in this rat strain.

The predominant disadvantage of cross-sectional studies is that they do not exclude cohort effects upon the parameter being studied. It is necessary to examine temporal changes in a specified function in an *individual* animal in order to ascertain the influence of aging per se on that function. A longitudinal study on albumin elimination was performed to determine if aging exerts a uniform effect on all individuals. By comparing these results with those from the cross-sectional study, information will be gathered on those changes in this elimination process that are caused by aging alone.

MATERIALS AND METHODS

Animals and materials

Thirty, 3-month-old female WAG/Rij rats having a mean weight (± S.D.) of 136 ± 17 g, were maintained under "clean conventional" conditions [11].

DEAE Affi-Gel Blue was obtained from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Goat anti-(rat total serum proteins) antiserum used for immuno-electrophoresis (GARa/ielfo) and rabbit anti-(rat serum albumin) antiserum (RARa/Alb) were obtained from Nordic Immunological Laboratories (Tilburg, The Netherlands). ^{125}I was obtained from the Radiochemical Centre (Amersham, Bucks, U.K.).

Assays

(1) Albumin isolation, purification and labeling with ^{125}I. The isolation and purification of albumin was achieved by chromatography of fresh rat serum on DEAE Affi-Gel Blue as described by Horbach *et al.* [10].

The isolated rat serum albumin was iodinated by the method of McFarlane [12] as modified by Helmkamp *et al.* [13] to a final ratio of 1 mol of ^{125}I/mol of albumin.

(2) Plasma radioactivity curves. Iodinated albumin (5 μCi) was injected intravenously into the external jugular vein of the rats at 3, 12, 24 and 36 months of age. Trichloroacetic acid-precipitable radioactivity was determined in 20-μl aliquots of plasma at several time points over 5 days. From the plasma radioactivity curves, the following characteristics were calculated: (a) Elimination half-life: $t_{1/2,\,el}$; (b) Apparent volume of distribution: V_d,· (c) Clearance: Cl. These characteristics were calculated as described previously [10].

(3) Plasma albumin concentration, plasma protein concentration, the whole-body

albumin pool and the absolute rate of albumin elimination. The albumin concentration in plasma was determined by radial immunodiffusion as described by Mancini *et al.* [14] and modified by Radl *et al.* [15]. With the use of the plasma albumin concentration, the data for apparent volume of distribution and clearance can be extrapolated in terms of the whole-body albumin pool and the absolute rate of albumin elimination as described previously [10].

The protein concentration in plasma was determined by the method of Lowry *et al.* [16].

(4) Urinary albumin and total protein excretion. Animals were kept in metabolic cages for 24 h and supplied with water and food *ad libitum.* The urine was collected and the amount of albumin excreted was determined by radial immunodiffusion as described by Mancini *et al.* [14] and modified by Radl *et al.* [15]. The amount of total protein excreted was determined by the method of Lowry *et al.* [16], as modified by Bensadoun and Weinstein [17].

(5) Quantitative determination of the total liver RNA content and the albumin mRNA content. Total post-nuclear RNA was isolated according to the method of Taylor and Schimke from the livers of the rats that reached the age of 36 months [18]. The RNA content was determined by the method of Fleck and Munro [19]. Albumin mRNA quantitation was performed by analytical RNA-cDNA hybridization as described by Housman *et al.* [20].

(6) Histology. Complete necropsies were performed on all animals in this study. Most animals died spontaneously. Some rats were killed in extremis, as were animals which survived to 36 months of age. Tissue specimens were fixed by immersion in 10% buffered formalin, embedded in paraffin by standard methods, sectioned at 3 μm, and stained with hematoxyline-phloxine-saffron (HPS). Several kidney specimens were sectioned at 2 μm, and stained with PASM-Azan to demonstrate basement membranes. The following tissue specimens were routinely examined by light microscopy: skin, salivary glands, mammary gland, lung, heart, esophagus and all segments of the gastrointestinal tract, liver, pancreas, kidney, urinary bladder, ovary, uterus, vagina, preputial gland, spleen, thymus, longitudinal section of the femur and sternum and their associated bone marrow, trachea, thyroid, adrenals, pituitary, brain and meninges, and cervical, inguinal, axillary, mediastinal, and mesenteric lymph nodes. Four cross-sections through the head were also examined.

A longitudinal section of each kidney was examined without knowledge of group and graded histologically. The grading scale for the renal lesions was based on the severity of the individual basic lesions and on the number of nephrons affected as described by Gray *et al.* [21]: Grade 0, normal or minimal lesions; Grade 1, mild lesions; Grade 2, moderate lesions; and Grade 3, marked lesions. An animal was placed in the higher of the two numerical categories if there was any dissimilarity in the histological score between individual kidneys.

(7) Statistical analysis. Measured characteristics in the longitudinal study were analyzed using the Student's *t*-test for correlated groups.

The correlation between the amount of protein or albumin in the urine and the histological score of the severity of renal lesions was calculated using Spearman's rank correlation coefficient [22]. Animals that died more than 16 weeks following the last determination of urinary protein were not included in the statistical analysis.

RESULTS

Survival characteristics

The survival curve of the animals in this study is similar to the described survival characteristics for this strain [23]. A survival curve for a control group of 50 female WAG/Rij rats from three different cohorts is presented for comparison (Fig. 1). The death rate of animals was low for the initial 24 months as only three rats died during the first 2 years of the study. The death rate progressively increased after 24 months of age and by 30 months, only 12 animals remained. Six animals survived until 36 months of age.

The influence of age on the measured characteristics

Body weight. There was an increase in body weight for all rats between 3 and 12 months and between 12 and 24 months of age. The body weight did not change

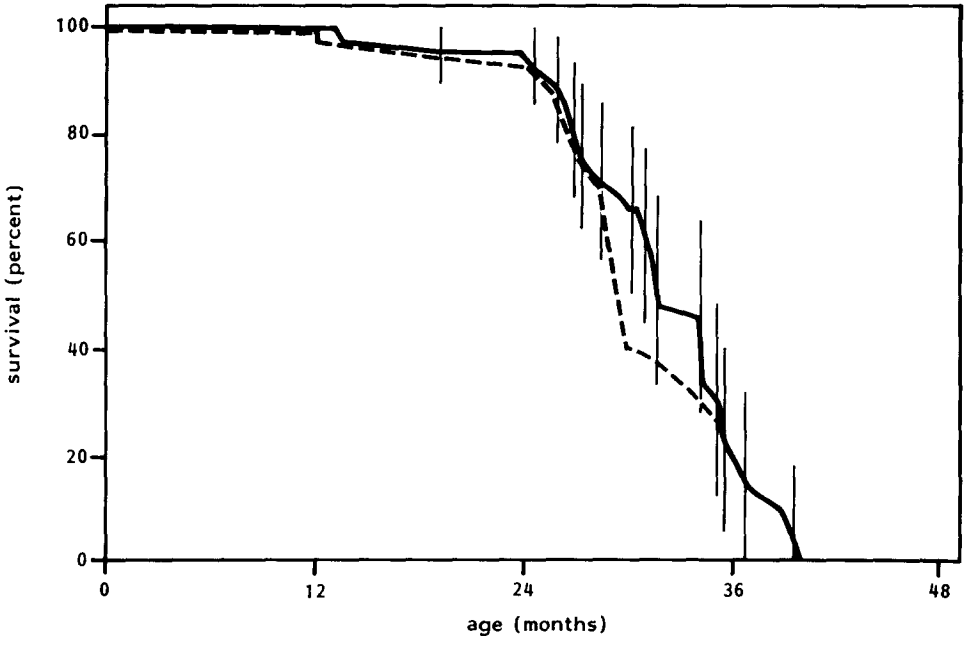

Fig. 1. Survival curves of female WAG/Rij rats. ———, 30 rats used in this study; ————, 50 rats from three separate cohorts, born in the same period as the rats used in this study. The vertical bars represent the 95% confidence limits.

TABLE I

THE INFLUENCE OF AGE ON THE BODY WEIGHT AND PLASMA VOLUME OF FEMALE WAG/Rij RATS

Age (months)	*N*	*Body wt (g)*	*Plasma volume (ml)*	*Plasma volume/ 100 g body wt (ml)*
3	30	136 ± 17	6.1 ± 0.5	4.4 ± 0.2
12	29	192 ± 12[a]	6.6 ± 0.5[a]	3.5 ± 0.2[a]
24	27	219 ± 35[a,b]	6.8 ± 1.4[a]	3.1 ± 0.2[a]
30	12	209 ± 25[a,b]	N.D.	N.D.
36	6	199 ± 19[a,c]	7.3 ± 0.8[a,b,c]	3.7 ± 0.5[a,b,c]

Values are expressed as means ± S.D.
N, the number of animals.
[a]Value differs significantly ($P < 0.05$) from 3-month value.
[b]Value differs significantly ($P < 0.05$) from 12-month value.
[c]Value differs significantly ($P < 0.05$) from 24-month Value.
N.D., not determined.

significantly in animals between 24 and 30 months of age. The six survivors at 36 months of age had a lower body weight compared to values obtained at 24 and 30 months of age.

Plasma volume. The plasma volume increased between 3 and 12 months, and between 24 and 36 months of age, but remained constant between 12 and 24 months of age (Table I). The increase in plasma volume was not related to a changed body weight.

TABLE II

THE INFLUENCE OF AGE ON THE ELIMINATION HALF-LIFE OF ALBUMIN IN FEMALE WAG/Rij RATS

Age (months)	*N*	*Elimination half-life (h)*	
		Albumin from 3-month-old rats	*Age-matched albumin*
3	30	45.3 ± 6.5	45.3 ± 6.5
12	29	44.9 ± 4.5	40.4 ± 3.6
24	27	38.5 ± 3.1[a,b]	37.3 ± 3.9[a]
36	6	37.1 ± 3.3[a,b]	41.5 ± 4.6

Values are expressed as means ± S.D.; *N*, the number of animals.
[a]Value differs significantly ($P < 0.05$) from 3-month value.
[b]Value differs significantly ($P < 0.05$) from 12-month value.

TABLE III

THE INFLUENCE OF AGE ON THE CLEARANCE AND APPARENT VOLUME OF DISTRIBUTION OF ALBUMIN IN FEMALE WAG/Rij RATS

Age	*N*	*Clearance (ml/h)*		*Apparent volume of distribution (ml)*	
		Albumin from 3-month-old rats	*Age-matched albumin*	*Albumin from 3-month-old rats*	*Age-matched albumin*
3	30	0.226 ± 0.021	0.226 ± 0.021	14.7 ± 1.5	14.7 ± 1.5
12	29	0.226 ± 0.026	0.246 ± 0.020	14.6 ± 1.7	14.3 ± 1.4
24	27	0.343 ± 0.098[a,b]	0.366 ± 0.096[a,b]	18.4 ± 4.3[a,b]	18.7 ± 4.6[a,b]
36	6	0.338 ± 0.091[a,b]	0.402 ± 0.075[a,b,c,d]	17.9 ± 1.7[a,b]	24.1 ± 3.9[a,b,c,d]

Values are expressed as means ± S.D.

N, number of animals.

[a]Value differs significantly ($P < 0.05$) from 3-month value.

[b]Value differs significantly ($P < 0.05$) from 12-month value.

[c]Value differs significantly ($P < 0.05$) from 24-month value.

[d]Value differs significantly ($P < 0.05$) from value obtained when using albumin from 3-month-old animals.

Elimination half-life of albumin. The elimination half-life was lower at 24 months of age as compared to 3 months of age following injection of age-matched albumin (Table II). A significant decrease in $t_{\frac{1}{2},el}$ was observed between 12 and 24 months of age when albumin from 3-month-old rats was injected.

Albumin clearance. After injection of 3-month-old albumin, an increase in the clearance values between 12 and 24 months of age was observed (Table III). No significant change in the clearance was observed after 24 months of age.

The clearance values obtained after injection of albumin isolated from rats of the same age as the recipients are higher at 24 and 36 months of age than those at 3 and 12 months of age (Table III). In contrast with the results obtained using albumin isolated from 3-month-old rats, higher values were obtained at 36 months of age compared with those obtained at 24 months of age when animals were injected with albumin from an age-matched donor. The increase in clearance of age-matched albumin between 12 and 36 months of age was found in all rats. At 36 months of age, a significant difference is observed between the clearance of age-matched albumin and of albumin isolated from 3-month-old rats.

Apparent volume of distribution of albumin. Age-related changes in the apparent volume of distribution are also dependent on the age of the albumin donor rats. The apparent volume of distribution of age-matched albumin is significantly higher in 36-month-old rats than that of albumin isolated from 3-month-old rats (Table III). Independent of the origin of administered albumin, all animals between 12 and 24 months of age had an increase in this characteristic. The exception is rat I-1, whose volume of distribution remained constant. A further increase was observed with the age-matched albumin in animals between 24 and 36 months of age (Table III).

Plasma albumin and protein concentrations. Plasma albumin and protein concentrations did not differ between any age group examined in this study (Table IV), as albumin accounted for about 45% of the total plasma proteins in all age groups.

TABLE IV. THE INFLUENCE OF AGE ON PLASMA ALBUMIN AND TOTAL PROTEIN CONCENTRATION IN FEMALE WAG/Rij RATS

Age (months)	*N*	*Plasma albumin (mg/ml)*	*Plasma protein (mg/ml)*	*Percentage albumin of total protein (%)*
3	30	33.6 ± 3.3	66.1 ± 5.1	50.9 ± 4.3
12	29	31.8 ± 1.6	76.6 ± 10.2	42.2 ± 5.8
24	27	31.3 ± 6.0	76.3 ± 5.9	42.5 ± 3.1
30	12	32.1 ± 4.6	66.3 ± 8.4	47.0 ± 6.7
36	6	33.2 ± 2.5	71.3 ± 5.9	47.0 ± 6.7

Values are expressed as means ± S.D.
N, the number of animals.

TABLE V

THE INFLUENCE OF AGE ON THE ABSOLUTE ALBUMIN ELIMINATION RATE AND THE WHOLE-BODY ALBUMIN POOL IN FEMALE WAG/Rij RATS

Age (months)	*N*	*Absolute elimination rate (mg/24 h)*	*Absolute elimination rate/100 g body wt (mg/24 h)*	*Whole-body albumin pool (mg)*	*Whole-body albumin pool/100 g body wt (mg)*
3	30	184 ± 26	135 ± 21	493 ± 61	351 ± 40
12	29	187 ± 18	99 ± 19[a]	454 ± 53	237 ± 31[a]
24	27	278 ± 79[a,b]	133 ± 35[b]	620 ± 170[a,b]	302 ± 53[b]
36	6	323 ± 49[a,b,c]	162 ± 25[a,b,c]	810 ± 140[a,b,c]	407 ± 71[a,b,c]

Values are expressed as means ± S.D.

N, the number of animals.

[a]Value differs significantly ($P < 0.05$) from 3-month value.

[b]Value differs significantly ($P < 0.05$) from 12 month value.

[c]Value differs significantly ($P < 0.05$) from 24-month value.

TABLE VI

THE INFLUENCE OF AGE ON URINARY EXCRETION OF TOTAL PROTEIN AND ALBUMIN IN FEMALE WAG/Rij RATS

Age (months)	*N*	*Protein excretion (mg/24 h)*	*Albumin excretion (mg/24 h)*	*Percentage albumin of excreted protein (%)*
3	30	2.9 ± 1.2	0.12 ± 0.04	4.5 ± 2.0
12	29	2.4 ± 0.9	0.21 ± 0.13	9.5 ± 4.9
24	27	6.0 ± 3.9[a,b]	1.3 ± 1.6[a,b]	15.7 ± 9.9[a,b]
30	12	30 ± 16[a,b,c]	10.9 ± 7.8[a,b,c]	31 ± 12[a,b,c]
36	6	48 ± 14[a,b,c]	19.5 ± 5.1[a,b,c]	40.9 ± 4.1[a,b,c]

Values are expressed as mean ± S.D.
N, the number of animals.
[a]Value differs significantly ($P < 0.05$) from 3-month value.
[b]Value differs significantly ($P < 0.05$) from 12-month value.
[c]Value differs significantly ($P < 0.05$) from 24-month value.

Absolute albumin elimination rate and whole-body albumin pool. Calculations on the absolute albumin elimination rate and whole-body albumin pool were only performed on data obtained with age-matched albumin. Both characteristics uniformly increase at between 12 and 36 months of age, with the exception of rat I-1 whose elimination rate showed a slight increase only after 24 months of age and whose whole-body albumin pool remained constant up to 36 months of age. The age-related increase in absolute albumin elimination and whole-body albumin pool were not related to a changed body weight (Table V).

Albumin and protein excretion via the urine. The urinary excretion of albumin and total protein increases with age up to 30 months of age (Table VI). Only 3 out of

TABLE VII

TOTAL LIVER RNA CONTENT AND ALBUMIN mRNA LEVELS IN THE SIX 36-MONTH-OLD SURVIVOR RATS

Rat No.	*Liver wt (g)*	*RNA content (mg/g of liver)*	*Albumin mRNA content (μg)*
I-1	6.11	6.41	156.4
I-3	7.01	7.24	173.4
II-0	7.30	5.43	130.5
VII-0	6.33	6.55	136.0
VII-2	7.32	6.80	182.9
VIII-0	5.44	6.45	137.2
Mean ± S.D.	6.58 ± 0.75	6.48 ± 0.60	153 ± 22

TABLE VIII

PATHOLOGICAL LESIONS OBSERVED IN FEMALE WAG/Rij RATS USED IN THIS STUDY

Organs	*Lesions*	*No. of animals*	*Percentage of animals*	*Mean age (range) in months*
Liver	Foci of cellular alteration	17	56.7	31 (24—36)
	Biliary cysts	3	10.0	34 (31—36)
	Hepatocellular necrosis	3	10.0	33 (30—34)
	Angiectasis	2	6.7	31 (30—32)
	Macrophage aggregation in sinusoids	2	6.7	32 (30—34)
	Myeloid leukemia	2	6.7	29.5 (27—32)
	Spongiosis hepatis	2	6.7	30 (30)
	Metastatic osteosarcoma	1	3.3	31
	Hepatocellular atrophy	1	3.3	25
	Granulamatous inflammation	1	3.3	35
	Nodular hyperplasia	1	3.3	35
Thyroid	Medullary thyroid carcinoma	19	63.3	31(24—36)
	Parathyroid hyperplasia	3	10.0	31 (27—36)
Pituitary gland	Pituitary adenoma	21	70.0	32 (24—36)
	Pituitary carcinoma	1	3.3	24
	Pars intermedia cysts	1	3.3	36
Ovary	Multiple cysts	7	23.3	32 (24—36)
Kidney	Chronic progressive Nephrosis	21	70.0	31 (24—36)
	Tubular abcess	1	3.3	32
	Nephroblastoma	1	3.3	25
Heart	Moderate myocardial fibrosis	3	10.0	36 (36)
	Congestive cardiomyopathy	1	3.3	27
	Focal thrombus	1	3.3	27
Pancreas	Islet cell carcinoma	2	6.7	32.5 (29—36)
	Pancreatic duct ectasia	2	6.7	31 (30—32)
Thymus	Mixed thymoma	2	6.7	32.5 (29—36)
	Thymic hyperplasia	1	3.3	30
Uterus	Cystic glands	2	6.7	36 (36)
	Endometrial polyp	2	6.7	36 (36)
	Endometritis	1	3.3	34
	Unilateral hydrometra	1	3.3	34
	Fibroma	1	3.3	24
	Stromal cell sarcoma	1	3.3	25
Spleen	Myeloid leukemia	2	6.7	29.5 (27—32)
	Hemorrhage	1	3.3	36

TABLE VIII (*continued*)

Organs	*Lesions*	*No. of animals*	*Percentage of animals*	*Mean age (range) in months*
Mammae	Fibroadenoma	4	13.3	34 (30—36)
	Tubulopapillary carcinoma	3	10.0	34 (31—36)
	Fibroma	1	3.3	26
	Adenocarcinoma	1	3.3	24
	Lobular hyperplasia with focal atypia	1	3.3	36
Lymph nodes	Capillary hemangioma	1	3.3	34
	Myeloid leukemia	1	3.3	27
Adrenal gland	Cortical adenoma	1	3.3	36
Stomach	Severe ulcerative gastritis	2	6.7	25.5 (24—27)
Oral cavity	Squamous cell carcinoma	1	3.3	33
Nasal cavity	Focal suppurative rhinitis	1	3.3	32

the 6 survivors had an increase in both albumin and total protein excretion between 30 and 36 months of age. Changes in urinary albumin excretion always reflected an equivalent change in total protein excretion. There was an enormous heterogeneity in the age at which the increase in urinary albumin and protein loss starts. Most rats show this increase between 12 and 24 months of age, others after 24 months of age. No increase was observed in one animal (rat VII-3) that died at 31 months of age.

In all cases, albumin becomes an increasingly important constituent of the excreted protein after the start of the increase in protein excretion *via* the urine (Table VI). Only 4.5% of the excreted protein is albumin-like in 3-month-old animals, whereas at 36 months of age, this value has increased to 41%, which is similar to the proportion of plasma proteins that are " albumin-like".

Total liver RNA content and albumin mRNA content

The content of total RNA and albumin mRNA in the liver was determined in the six survivor rats at 36 months of age (Table VII). No distinct linear relationship exists between the amount of albumin mRNA in the livers of the six survivor rats and the levels of absolute albumin elimination ($r = 0.582$; $P = 0.23$).

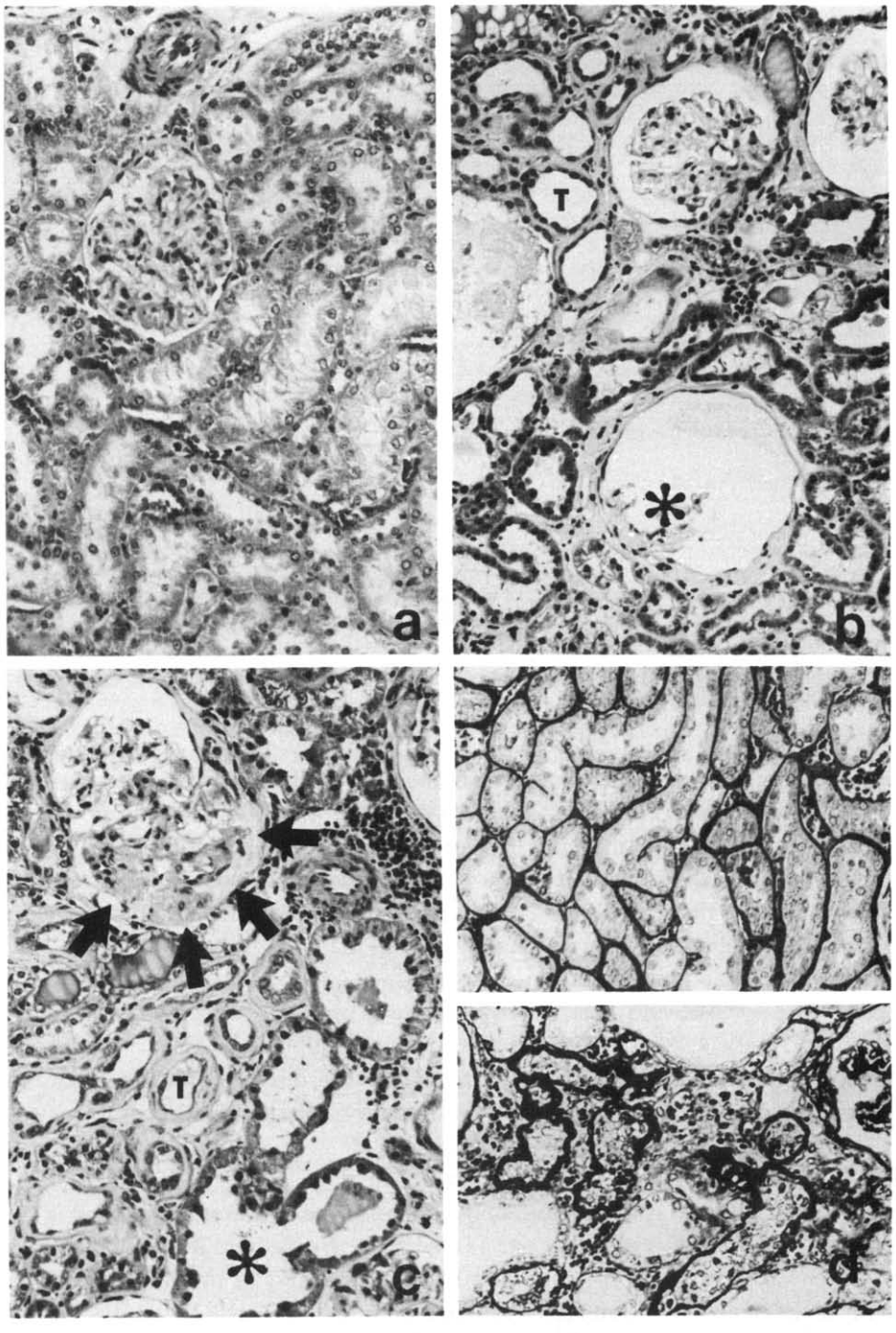
a
T
*
b
T
*
c
d

TABLE IX

THE MEAN AMOUNT OF URINARY ALBUMIN AND PROTEIN EXCRETION IN RELATION TO THE HISTOLOGICAL SCORE OF RENAL LESIONS

Histological score	*N*	*Mean urinary protein (range) in mg/24 h*	*Mean urinary albumin (range) in mg/24 h*
0	6	5.5 (2.7—8.2)	0.64 (0.33—1.1)
1	5	22 (8.7—30)	6.9 (1.5—11)
2	7	44 (20—74)	18 (7.8—28)

N, the number of animals.

Histological data

A wide variety of pathological lesions were observed in this study. The most frequently observed lesions included: pituitary tumors, medullary thyroid carcinomas, biliary cysts and foci of cellular alteration in the liver, mammary tumors, and ovarian cysts (Table VIII). These lesions are common in aged rats of this strain [23]. Pathological changes that could result in a marked loss of protein, such as protein-losing enteropathy and severe hemorrhage, were not observed in this study. Generalized neoplasia, which could influence protein turnover, was observed in three cases: (IV-1, myeloid leukemia; V-3, myeloid leukemia, and; VI-2, metastatic osteosarcoma. However, cases V-3 and VI-2 were not included in the statistical analysis due to the long time interval between the last urinary protein determination and death. Histologic evidence of increased protein synthesis in the liver, recognized as focal areas of hepatocellular basophilia, was only observed in one animal (VIII-0). However, the light microscope is a relatively insensitive device for this purpose.

The most frequently observed renal lesion was chronic progressive nephrosis (CPN), a common disease of laboratory rats [23]. The characteristic lesions of this disease include: glomerular changes of thickened Bowman's capsule and mesangium, cast formation, and atrophic proximal tubule with thickened basement

Fig. 2. (a) Grade 0 (normal): There are no morphologic alterations (hematoxyline-phloxine-saffron, HPS, × 210). (b) Grade 1 (mild): There is focal, segmental thickening of Bowman's capsule (arrows) and atrophy of the glomerular tuft (asterisk). Tubules (T) are lined by mildly attenuated epithelial cells with moderately thickened basement membranes (HPS, × 210). (c) Grade 2 (moderate): There is marked segmental sclerosis of the glomerulus with thickened basement membranes and adhesions to Bowman's capsule (arrows). Several tubules (T) are atrophied, lined by markedly atenuated to hyperplastic epithelial cells with pronounced thickening of the basement membrane. Other tubules (asterisk) are irregularly dilated, contain proteinaceous material, and are lined by hypertrophied epithelial cells. The interstitium contains a focal aggregate of lymphocytes. (HPS, × 210). (d) Special stains demonstrate comparative thickening of basement membrane between normal (upper photograph) and affected animal (lower photograph) (PASM-Azan, × 210).

membrane [22]. All grades of histological severity, except for Grade 3, were observed in the kidneys of animals in this study (Figs. 2a—d).

There was a significant correlation between the histological score of renal lesions and the total amount of urinary protein ($r = 0.8613$; $P < 0.001$) and urinary albumin ($r = 0.9029$; $P < 0.001$) (Table IX).

DISCUSSION

The results of this study indicate that changes in both albumin clearance and apparent volume of distribution occur with aging as all animals had higher levels for these two parameters at 24 months of age as compared to 3 or 12 months of age. No significant change was observed between 3 and 12 months of age or between 24 and 36 months of age. These results are similar to those reported in a cross-sectional study in this strain [10]. The elimination half-life was significantly decreased between 12 and 24 months of age. This change was not observed in the cross-sectional study, probably because of the small number of animals used in that study.

The age of the animal from which the albumin was obtained had no effect on albumin clearance and the apparent volume of distribution when the donor was between the ages of 3 and 24 months. However, there was an influence on the clearance and apparent volume of distribution when the age of the albumin donor exceeded 24 months. These findings indicate that changes in these two characteristics, up to 24 months of age, are only due to age-related changes in the physiology of the recipients. The increase in the clearance and apparent volume of distribution between 24 and 36 months of age was only observed after injection of age-matched albumin and could therefore be attributed to a change in the albumin molecule.

No significant change in plasma albumin or plasma protein concentration was detected during the life-span of the animals in this study. Some variation with age was observed, but this variation is smaller than the inter-individual variation. Albumin comprises about 45% of the total plasma protein pool in all age-groups. Since the plasma albumin concentration remains constant with age, the age-related changes in clearance and apparent volume of distribution observed after injection of age-matched albumin are reflected in the absolute albumin elimination rate and whole-body albumin pool, respectively. Therefore, the oberved age-related increase in clearance and apparent volume of distribution after injection of age-matched albumin also holds for these characteristics.

Numerous studies in several rat strains have documented that urinary excretion of albumin and other proteins increases with age [6,24,25]. Similar changes were observed in this study in the female WAG/Rij rat as the excretion of albumin and other proteins increased up to 30 months of age. Similar results were also obtained in a previous cross-sectional study in this strain [10]. Some variation occurred in these parameters after 30 months of age; excretion rates increased in some rats while decreasing in others.

One phenomenon which occurred in all animals in this study was a proportional increase in the contribution of albumin to the urinary protein excretion. In 3-month-old rats, only 4.5% of total urinary protein was albumin, but this component had increased to 41% by 36 months of age. Since this percentage approaches the values found in plasma, it most likely represents non-selective protein leakage into the urine in old rats. The age of onset of this increased contribution of albumin varied as some rats had high percentages at 12 months of age.

Aging rats have a wide variety of pathological lesions, some of which are strain specific [23]. One objective of this study was to correlate albumin elimination and urinary protein loss with the occurrence of these lesions. The only significant correlation that was observed in this study was between urinary protein and albumin loss and the histological severity of renal lesions. One complicating factor in this study is the time interval between the last determination on urinary protein loss and the time of death. Histological scoring was not included if this time span was more than 16 weeks, since major pathological changes could occur within this time frame. The age-related increase in the relative contribution of albumin to total urinary protein excretion can probably be explained by the increased glomerular permeability to larger proteins [25].

Other investigators examined albumin degradation and aging in female rats and reported a four-fold increase in the degradation half-life with age [26]. The difference between those results and the present study resides in the fact that degradation is only a part of the processes involved in plasma protein elimination.

A previous study documented an increased albumin elimination with age that was accompanied by increased albumin mRNA levels [4]. Levels of albumin mRNA were determined in the six 36-month-old survivor rats in this longitudinal study in order to compare these values with the albumin elimination rate. Although both factors are high in comparison to values found in young rats, no linear relationship was observed between the magnitude of the albumin elimination rate and the albumin mRNA levels. It remains to be established whether this lack of correlation relates to the number of animals being too small, or to any relationship being rather complex.

REFERENCES

1 J.C. Chen, P. Ove and A.I. Lansing, *In vitro* synthesis of microsomal protein and albumin in young and old rats. *Biochim. Biophys. Acta, 312* (1973) 598—607.

2 C.F.A. van Bezooijen, T. Grell and D.L. Knook, Albumin synthesis by liver parenchymal cells isolated from young, adult and old rats. *Biochem. Biophys. Res. Commun., 71* (1976) 513—519.

3 C.F.A. van Bezooijen and D.L. Knook, Aging changes in bromsulfophtalein uptake, albumin and total protein synthesis in isolated hepatocytes. In D. Platt (ed.), *Liver and Aging,* Schattauer Verlag, Stuttgart and New York, 1977, pp. 227—241.

4 G.J.M.J. Horbach, H.M.G. Princen, M. van der Kroef, C.F.A. van Bezooijen and S.H. Yap, Changes in the sequence content of albumin mRNA and in its translational activity in the rat liver with age. *Biochim. Biophys. Acta, 783* (1984) 60—66.

5 R.E. Beauchene, L.M. Roeder and C.H. Barrows, The interrelationships of age, tissue protein synthesis and proteinuria. *J. Gerontol., 25* (1970) 359—363.

6 K. Salatka, D. Kresge, L. Harris Jr., D. Edelstein and P. Ove, Rat serum protein changes with ages. *Exp. Gerontol., 6* (1971) 25—36.

7 P. Ove, M. Obenrader and A.I. Lansing, Synthesis and degradation of liver proteins in young and old rats. *Biochim. Biophys. Acta, 277* (1972) 211—221.

8 G.J.M.J. Horbach, H. van der Boom, C.F.A. van Bezooijen and S.H. Yap, Molecular aspects of age-related changes in albumin synthesis in rats. In C.F.A. van Bezooijen, F. Miglio and D.L. Knook (eds.), *Liver, Drugs and Aging,* Vol. 7, EURAGE, Rijswijk, 1986, pp. 121—126.

9 G.J.M.J. Horbach, *Albumin Metabolism and Aging,* Thesis, University of Maastricht (1986).

10 G.J.M.J. Horbach, S.H. Yap and C.F.A. van Bezooijen, Age-related changes in albumin elimination in female WAG/Rij rats. *Biochem. J., 216* (1983) 309—315.

11 C.F. Hollander, Current experience using the laboratory rat in aging studies. *Lab. Anim. Sci., 26* (1976) 320—328.

12 A.S. McFarlane, Labelling of plasma proteins with radioactive iodine. *Biochem. J., 62* (1956) 135—143.

13 R.W. Helmkamp, H.A. Contreras and W.F. Bale, ^{131}I-labelling of proteins by the iodine monochloride method. *Int. J. Appl. Radiat. Isot., 18* (1967) 737—746.

14 G. Mancini, A.O. Carbonara and J.F. Heremans, Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry, 2* (1965) 235—254.

15 J. Radl, F. Skvaril, J. Masopust and K. Kithier, Quantitative estimation of human immunoglobulins. II. The development of serum immunoglobulin levels in man. *J. Hyg., Epidemiol., Microbiol., Immunol., 14* (1970) 488—502.

16 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, Protein measurement with the folin phenol reagent. *J. Biol. Chem., 193* (1951) 265—275.

17 A. Bensadoun and D. Weinstein, Assay of proteins in the presence of interfering materials. *Anal. Biochem., 70* (1976) 241—250.

18 J.M. Taylor and R.T. Schimke, Synthesis of rat liver albumin in a rabbit reticulocyte cell-free protein-synthesizing system. *J. Biol. Chem., 248* (1973) 7661—7668.

19 A. Fleck and H.N. Munro, The precision of ultraviolet absorption measurements in the Schmidt-Thannhauser procedure for nucleic acid estimation. *Biochim. Biophys. Acta, 55* (1962) 571.

20 D. Housman, A Skoultchi, B.G. Forget and E.J. Benz, Use of a cDNA as a hybridization probe for globin mRNA. *Ann. N.Y. Acad. Sci., 241* (1974) 280—289.

21 J.E. Gray, M.J. van Zwieten and C.F. Hollander, Early light microscopic changes of chronic progressive nephrosis in several strains of aging laboratory rats. *J. Gerontol., 37* (1982) 142.

22 G.W. Snedecor and W.G. Cochran, *Statistical Methods,* 7th Edn. Iowa State University Press, Ames, USA, 1980.

23 J.D. Burek, *Pathology of Aging Rats.* CRC Press, West Palm Beach, 1978.

24 M. Obenrader, J. Chen, P. Ove and A.I. Lansing, Etiology of increased albumin synthesis in old rats. *Exp. Gerontol., 9* (1974) 173—180.

25 J.M. Alt, H. Hackbarth, F. Deerberg and H. Stolte, The study of urinary protein excretion in male rats as compared with human proteinuria. *Lab. Anim., 14* (1980) 95—101.

26 R.G. Goya, Y.E. Sosa, J. Chang and J. Meites, Degradation of immunoreactive albumin in young and old conscious female rats. *Mech. Ageing Dev., 37* (1986) 69—78.