Effects of intravenous administration of distilled water and bovine serum albumin on the kidney of rat

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Effects of Intravenous Administration of Distilled Water and Bovine Serum Albumin on the Kidney of Rat

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Abstract

Distilled water (DW) or bovine serum albumin (BSA) was injected intravenously into rats over a period of 19 days. DW caused intravessel hemolysis, the acceleration of hematopoiesis and hemosiderine presipitation in urinary epithelial cells. On the other hand, BSA caused high concentration of protein in blood and urine, the eosinophilic granules in urinary epithelial cells, chronic nephrosis and other alterations.

Though both DW and BSA treatment induced to make deposits in urinary epithelial cells, granules induced by the DW were different from eosinophilic bodies, and those induced by the BSA were small in size, but similar in property with eosinophilic bodies.

Therefore, the eosinophilic granules induced by BSA are assumed to be a presursor of eosinophilic bodies, and eosinophilic bodies may be formed not by intravessel hemolysis, but by hyperproteinuria.

Introduction

In toxicological studies, many drugs have been known to induce renal alterations[4]. Rat has been used frequently for these studies. However, some spontaneous alterations are found in the rat's kidney, especially some kinds of deposits in the uriniferous tubules which sometimes prevent accurate evaluation for toxicity. Among these deposits, eosinophilic bodies are often observed[1], but the pathogenesis is not well known. The formation of eosinophilic bodies in rats is assumed to be related to Fe-positive particles (personal communication) or to urinary protein. Urinary protein (mostly albumin) in rats is larger in amount than that in human[5].

For detail examination, distilled water (DW) or bovine serum albumin (BSA) was injected intravenously to rats.

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Materials and Methods

1. Animals

Twenty-five Sprague-Dawley rats of each sex were used in this study. Male and female rats were 10 and 9 weeks old, respectively. Animals were housed in an air-conditioned barrier-system, where temperature was kept at 23±3°C and relative humidity 55±10% and ventilation was conducted 12–18 times per hour. Two or 3 animals were kept in each cage. They were supplied with commercial diet (CRF-1) and tap water *ad libitum*.

2. Experimental design

Experimental design was shown in Table 1.

i) Experiment I: Introduction of intravessel hemolysis

Through *Vena caudae*, 4m*l* or 20m*l* of DW/kg body weight was administered daily.

ii) Experiment II: Protein overloading

1.25 g or 5 g of BSA/kg body weight was also administered daily as 6.25 or 25 w/v% saline solution, respectively. BSA was purchased from WAKO PURE CHEMICAL INDUSTRIES, LTD., Tokyo.

20 m*l*/kg of physiological saline was also administered daily as control. Five groups of each sex were examined in all. Each group consisted of 5 animals to which the half dosages, respectively, were administered twice a day (at 9 : 30 a.m. and 3 : 00 p.m.) for 19 days. The animals were administered only at 9 : 30 a.m. on the final day.

3. Urinary analysis

The animals were put to individual metabolic cages at 5 : 00 p.m. on the final day, and the urine was collected over a period of 16 hrs. The samples were assayed as to total volume, specific gravity (ATAGO Uricon), and amounts of Na^+, K^+ (CORNING 480–Flamephotometer), Cl^- (HIRANUMA CL–6 Chloridecounter) and protein (Biuret method: TOSHIBA TBA–380 Biochemical analyzer).

4. Hematological analysis

The blood was collected from the common *Vena femoralis* of rats anesthetized with ether. Values of hemoglobin, hematocrit, red blood cell (RBC) and white blood cell (WBC) counts (SYSMEX Microcellcounter) and reticulocyte ratio (Brecher's method) were measured.

5. Biochemical analysis of serum

The blood collected from the common *Aorta abdominalis* was kept 20°C for 1 hr and centrifuged at 3,000 rpm. GOT and GPT activities (UV method), and amounts of glucose (Mutarotase-GOD method), total bilirubin (T-bilirubin, Stabilized diazo reagent method), total protein (T-Protein, Biuret method), urea nitrogen (BUN, Enzyme method), creatinine (Jaffe's method), uric acid (Uricase method),

Table 1. Experimental design.

	Substance	Dose[a] (m*l* or g/kg/day)	Number of animals	
			male	female
Control	Physiological saline	20 m*l*	5	5
Experiment I	DW[b]	4 m*l*	5	5
	DW	20 m*l*	5	5
Experiment II	BSA[c]	1.25 g	5	5
	BSA	5 g	5	4

a) All animals were injected intravenously twice a day, so the amount of substance for one administration is half of the presented dose.
b) DW: Distilled water.
c) BSA: Bovine serum albumin.

Na^+, K^+ (CORNING 480–Flamephoto-meter), and Cl^- (HIRANUMA CL–6 Chloridecounter) in the serum were measured. All tests were carried out using TBA–380 Biochemical analyzer except Na^+, K^+ and Cl^-.

6. Electrophoresis

Electrophoresis of the serum and urinary protein was carried out in Experiment II and control group using cellulose acetate membranes (SCHEICHER & SCHULL Electrophoresis foils) at constant voltage of 130 V for 70 minutes. Membrane was stained with Ponceau 3R solution (WAKO) and scanned at the wave length of 520 nm (KAYAGAKI Programable Densitometer ADC–20EX).

7. Autopsy and organ weight

Animals were bled from the *Aorta abdominalis* under ether-anesthetization and sacrificed 24 hrs after final administration. After autopsy, liver, kidney and spleen were weighed and fixed with 10% formaline.

8. Histopathological analysis

The organs mentioned above were embedded in paraffin after fixation. Three staining methods were employed; hematoxylin-eosin (HE), periodic acid Schiff (PAS) and Berline blue. These samples were examined under a microscope.

9. Statistical analysis

Results of urinary tests, hematological tests and biochemical tests of serum, and absolute and relative organ weights were statistically analyzed. Distribution was checked by Bartlett's test, and the analysis for all groups was done by one dimension analysis or Kruskal-Wallis's test. Then significant difference was compared with control group by Dunnett's test or Mann-Whitney's U-test.

Result

1. Experiment I; DW treatment

1) Urinary analysis

The excretion of red urine was observed for

Table 2. Results of urinary analysis of rats administered distilled water intravenously.

Dosage group		Male			Female		
Dose (m*l*/kg/day)		Control —	Low 4	High 20	Control —	Low 4	High 20
Number of animals		5	5	5	5	5	5
Volume	(m*l*)	15.42 ±7.53	11.12 ±2.33	10.60 ±3.61	12.84 ±3.92	4.74* ±2.46	8.14 ±3.29
Specific gravity		1.024 ±0.006	1.030 ±0.007	1.031 ±0.005	1.022 ±0.004	1.042** ±0.011	1.032 ±0.008
Protein	(g/d*l*)	0.18 ±0.04	0.24 ±0.05	0.20 ±0.00	0.10 ±0.00	0.10 ±0.00	0.10 ±0.00
Urobilinogen	(EU/d*l*)	0.10 ±0.00	0.10 ±0.00	0.00 ±0.00	0.10 ±0.00	0.10 ±0.00	0.10 ±0.10
Na^+	(mEq/*l*)	11.40 ±6.69	22.10 ±11.60	16.30 ±1.60	12.60 ±4.77	29.90 ±17.15	20.50 ±7.68
K^+	(mEq/*l*)	37.60 ±8.36	57.70 ±27.89	67.00 ±18.87	29.60 ±7.26	80.50* ±34.73	59.10 ±31.45
Cl^-	(mEq/*l*)	23.8 ±12.3	45.6 ±36.1	44.6 ±12.9	29.4 ±11.4	66.4 ±51.9	47.2 ±24.2

Values are expressed as Mean ± SD.
*(**) Significantly different from control group by Dunnett procedure $p < 0.05$ (0.01).

Table 3. Results of hematological analysis of rats administered distilled water intravenously.

	Male			Female		
Dosage group	Control	Low	High	Control	Low	High
Dose (m*l*/kg/day)	—	4	20	—	4	20
Number of animals	5	5	5	5	5	5
RBC (10^4/mm^3)	803.0 ±29.1	755.4* ±24.9	682.6** ±20.9	697.0 ±29.0	686.2 ±25.2	622.2** ±45.3
Hematocrit (%)	45.48 ±1.64	43.44 ±1.28	40.92** ±1.25	40.80 ±1.90	41.14 ±2.10	38.94 ±0.07
Hemoglobin (g/d*l*)	14.88 ±0.31	13.94** ±0.36	12.86** ±0.36	13.72 ±0.61	13.62 ±0.48	12.74* ±0.41
Reticulocyte (/10^3 RBC)	15.6 ±4.3	27.0 ±6.4	62.0** ±13.6	30.6 ±7.2	39.6 ±8.7	71.4** ±10.9
WBC (10^2/mm^3)	78.2 ±23.1	85.8 ±22.5	67.6 ±15.2	96.2 ±30.8	81.0 ±16.0	80.0 ±13.1

Values are expressed as Mean ± SD.
* (**) Significantly different from control group by Dunnett procedure $p<0.05$ (0.01).

Table 4. Results of biochemical analysis of serum of rats administered distilled water intravenously.

	Male			Female		
Dosage group	Control	Low	High	Control	Low	High
Dose (m*l*/kg/day)	—	4	20	—	4	20
Number of animals	5	5	5	5	5	5
GOT (I.U.)	92.2 ±12.6	83.4 ±13.0	88.6 ±13.2	93.8 ±19.4	90.8 ±13.1	94.8 ±7.1
GPT (I.U.)	17.6 ±0.9	17.4 ±3.0	17.6 ±0.9	13.8 ±1.9	15.5 ±2.5	16.6 ±2.9
Glucose (mg/d*l*)	124.8 ±16.5	130.0 ±11.7	128.0 ±15.6	105.2 ±20.3	96.0 ±11.9	115.0 ±24.0
T-Bilirubin (mg/d*l*)	0.14 ±0.09	0.10 ±0.00	0.10 ±0.00	0.12 ±0.04	0.13 ±0.05	0.10 ±0.00
T-Protein (g/d*l*)	5.82 ±0.26	5.78 ±0.18	5.78 ±0.22	6.06 ±0.35	6.10 ±0.54	6.14 ±0.49
BUN (mg/d*l*)	15.8 ±2.0	16.4 ±2.3	16.2 ±1.1	21.2 ±1.1	22.3 ±3.0	20.6 ±2.3
Creatinine (mg/d*l*)	0.50 ±0.34	0.46 ±0.40	0.46 ±0.43	0.62 ±0.04	0.60 ±0.08	0.56 ±0.05
Uric Acid (mg/d*l*)	1.52 ±0.34	1.54 ±0.40	1.76 ±0.43	1.30 ±0.12	1.53 ±0.43	1.40 ±0.23
Na^+ (mEq/*l*)	152.10 ±1.39	150.90 ±1.02	150.70 ±1.68	145.10 ±0.42	147.00 ±1.87	145.30 ±1.92
K^+ (mEq/*l*)	4.824 ±0.505	5.108 ±0.376	5.498 ±0.628	4.670 ±0.154	4.908 ±1.087	4.526 ±0.359
Cl^- (mEq/*l*)	109.6 ±3.2	109.4 ±2.7	108.8 ±2.8	107.4 ±6.3	109.5 ±1.3	107.2 ±1.3

Values are expressed as Mean ± SD.
* (**) Significantly different from control group by Dunnett procedure $p<0.05$ (0.01).

all animals just after the treatment. The red color became deeper in high dosage. No notable changes were seen quantitatively (Table 2).

2) Hematological analysis

As shown in Table 3, the decrease in RBC counts, hematocrit and hemoglobin values, and the increase in reticulocyte ratio were observed in the group of high dosage irrespective of sex and in the male group of low dosage, indicating that the anemia and the acceleration of hematopoiesis occurred in these groups.

3) Biochemical analysis

No alterations were observed in all groups (Table 4).

4) Organ weight

There were no significant difference among all groups (Table 5).

5) Autopsy findings

Dilatation of renal pelvis was found in 2 males and 2 females in the group of high dosage.

6) Histopathological findings

There were no distinctive changes in the liver and spleen of all rats. As shown in Table 6, Fe-positive hemosiderine pigments (Photo 1) were found in the urinary epithelial cells in the kidneys of all rats. These pigments were small, crystalline and brown in PAS-staining (Photo 2).

2. Experiment II; BSA treatment

1) Urinary analysis

As shown in Table 7, the large amount of protein was found in the group of high dosage irrespective of sex. The increase in potassium excretion was also observed in all BSA administered groups. Though some deviation were seen in specific gravity and chloride excretion, they were not considered to be

Table 5. Absolute and relative organ weights of rats administered distilled water intravenously.

Dosage group / Dose (ml/kg/day)		Male			Female		
		Control —	Low 4	High 20	Control —	Low 4	High 20
Number of animals			5	5	5	5	5
Liver	(g)	9.018 ±1.041	9.364 ±1.146	9.118 ±1.172	5.943 ±0.353	5.918 ±0.480	6.180 ±0.830
	(%)	2.309 ±0.139	2.432 ±0.219	2.477 ±0.230	2.482 ±0.098	2.514 ±0.024	2.595 ±0.150
Kidney	R (g)	1.308 ±0.171	1.336 ±0.151	1.408 ±0.142	0.770 ±0.098	0.786 ±0.069	0.848 ±0.069
	(%)	0.347 ±0.035	0.347 ±0.032	0.380 ±0.029	0.321 ±0.038	0.334 ±0.018	0.358 ±0.028
	L (g)	1.250 ±0.118	1.290 ±0.139	1.366 ±0.147	0.776 ±0.043	0.790 ±0.087	0.830 ±0.080
	(%)	0.332 ±0.023	0.335 ±0.032	0.369 ±0.033	0.324 ±0.015	0.335 ±0.019	0.350 ±0.036
Spleen	(g)	0.753 ±0.125	0.640 ±0.138	0.850 ±0.157	0.540 ±0.034	0.548 ±0.073	0.566 ±0.072
	(%)	0.206 ±0.031	0.167 ±0.026	0.229 ±0.036	0.226 ±0.022	0.234 ±0.032	0.238 ±0.024

Values are expressed as Mean ± SD.
* (**) Significantly different from control group by Dunnett procedure $p < 0.05$ (0.01).

Table 6. Histopathological findins in the kidney of rats administered distilled water or bovine serum albumin intravenously.

		Male					Female				
		Saline	DW[a] (ml/kg/day)		BSA[b] (g/kg/day)		Saline	DW (ml/kg/day)		BSA (g/kg/day)	
	Grade		4	20	1.25	5		4	20	1.25	5
Number of animals		5	5	5	5	5	5	5	5	5	4
Eosinophilic granules in urinary epithelial cells	−	5	5	4	1	2	5	5	5	5	0
	±	0	0	0	0	3	0	0	0	0	0
	+	0	0	1	4	0	0	0	0	0	4
Hemosiderosis in urinary epithelial cells	−	5	0	0	5	5	5	0	0	5	4
	±	0	0	0	0	0	0	3	0	0	0
	+	0	5	1	0	0	0	2	2	0	0
	++	0	0	4	0	0	0	0	3	0	0
Vacuoles in urinary epithelial cells	−	5	5	5	5	0	5	5	5	2	0
	±	0	0	0	0	1	0	0	0	3	0
	+	0	0	0	0	2	0	0	0	0	0
	++	0	0	0	0	1	0	0	0	0	2
	+++	0	0	0	0	1	0	0	0	0	2
Urinary cast	−	5	5	5	5	0	5	5	5	5	0
	+	0	0	0	0	2	0	0	0	0	0
	++	0	0	0	0	1	0	0	0	0	0
	+++	0	0	0	0	2	0	0	0	0	4
Dilatation of uriniferous tubules	−	5	5	5	5	0	5	5	5	5	0
	±	0	0	0	0	4	0	0	0	0	0
	+	0	0	0	0	1	0	0	0	0	1
	++	0	0	0	0	0	0	0	0	0	2
	+++	0	0	0	0	0	0	0	0	0	1
Lymphocytic infiltration	−	4	5	5	5	0	5	5	5	5	0
	±	1	0	0	0	2	0	0	0	0	0
	+	0	0	0	0	3	0	0	0	0	0
	++	0	0	0	0	0	0	0	0	0	1
	+++	0	0	0	0	0	0	0	0	0	3

Values are number of animals with particular findings.

a) DW: Distilled water.

b) BSA: Bovine serum albumin.

Tbled 7. Results of urinary analysis of rats administered bovine serum albumin intravenously.

Dosage group	Male			Female		
Dose (m*l*/kg/day)	Control —	Low 1.25	High 5	Control —	Low 1.25	High 5
Number of animals	5	5	5	5	5	4
Volume (m*l*)	15.42 ±7.53	11.46 ±7.75	7.42 ±2.26	12.84 ±3.92	8.02 ±4.19	13.70 ±6.93
Specific gravity	1.024 ±0.006	1.034 ±0.011	1.045** ±0.007	1.022 ±0.004	1.035 ±0.011	1.025 ±0.009
Protein (g/d*l*)	0.18 ±0.04	0.18 ±0.04	1.58* ±0.39	0.10 ±0.00	0.10 ±0.00	0.75* ±0.44
Urobilinogen (EU/d*l*)	0.10 ±0.00	0.10 ±0.00	0.10 ±0.00	0.10 ±0.00	0.10 ±0.00	0.10 ±0.00
Na^+ (mEq/*l*)	11.40 ±6.69	16.30 ±9.22	15.10 ±10.14	12.60 ±4.77	28.20 ±21.25	6.75 ±3.66
K^+ (mEq/*l*)	37.60 ±8.36	62.10 ±29.72	79.90* ±21.07	29.60 7.26	55.10 ±21.04	62.75 ±31.12
Cl^- (mEq/*l*)	23.8 ±12.3	49.0 ±29.6	34.0 ±16.5	29.4 ±11.4	49.0 ±29.6	6.3* ±3.0

Values are expressed as Mean ± SD.
* (**) Significantly different from control group by Dunnett procedure $p < 0.05$ (0.01).

Table 8. Results of hematological analysis of rats administered bovine serum albumin intravenously.

Dosage group	Male			Female		
Dose (m*l*/kg/day)	Control —	Low 1.25	High 5	Control —	Low 1.25	High 5
Number of animals	5	5	5	5	5	4
RBC (10^4/mm^3)	803.0 ±29.1	805.4 ±30.6	708.8 ±88.6	697.0 ±29.0	653.0 ±9.5	565.5** ±35.0
Hematocrit (%)	45.48 1.64	44.32 ±1.72	39.78** ±3.78	40.80 ±1.90	40.02 ±0.43	34.73* ±2.30
Hemoglobin (g/d*l*)	14.88 ±0.31	14.40 ±0.53	12.52* ±1.52	13.72 ±0.61	12.94 ±0.22	10.95** ±0.85
Reticulocyte (/10^3 RBC)	15.6 ±4.3	24.2 ±14.3	36.6* ±13.8	30.6 ±7.2	44.2* ±6.2	89.8* ±41.0
WBC (10^2/mm^3)	78.2 ±23.1	81.2 ±19.9	82.6 ±16.6	96.2 ±30.8	90.2 ±21.2	134.0 ±33.4

Values are expressed as Mean ± SD.
* (**) Significantly different from control group by Dunnett procedure $p < 0.05$ (0.01).

important.

2) Hematological analysis

As shown in Table 8, the decrease in RBC counts, hematocrit and hemoglobin values and the increase in reticulocyte ratio were observed in the group of high dosage regardless

Table 9. Results of biochemical analysis of serum of rats administered bovine serum albumin intravenously.

Dosage group	Male			Female		
Dose (ml/kg/day)	Control —	Low 1.25	High 5	Control —	Low 1.25	High 5
Number of animals	5	5	5	5	5	4
GOT (I. U.)	92.2 ±12.6	80.2 ±20.1	67.2 ±10.7	93.8 ±19.4	79.2 ±8.9	55.2** ±10.3
GPT (I. U.)	17.6 0.9	12.0** ±1.0	9.4** ±1.3	13.8 ±1.9	15.8 ±5.4	10.6 ±1.7
Glucose (mg/dl)	124.8 ±16.5	137.8 ±7.0	142.2 ±7.0	105.2 ±20.3	111.6 ±23.6	125.4 ±16.3
T-Bilirubin (mg/dl)	0.14 ±0.09	0.20 ±0.00	0.26 ±0.09	0.12 ±0.04	0.26** ±0.09	0.40 ±0.19
T-Protein (g/dl)	5.82 ±0.26	6.20 ±0.20	6.52** ±0.40	6.06 ±0.35	6.56* ±0.19	6.84** ±0.31
BUN (mg/dl)	15.8 ±2.0	17.0 ±1.2	28.6* ±6.4	21.2 ±1.1	22.2 ±2.3	50.2 ±23.5
Creatinine (mg/dl)	0.50 ±0.07	0.46 ±0.05	0.56 ±0.18	0.62 ±0.04	0.60 ±0.07	0.96 ±0.42
Uric Acid (mg/dl)	1.52 ±0.34	1.26 ±0.36	1.38 ±0.29	1.30 ±0.12	1.26 ±0.18	1.66* ±0.25
Na^+ (mEq/l)	152.10 ±1.39	148.20 ±5.25	148.20* ±1.30	145.10 ±0.42	145.50 ±0.79	143.50 ±2.62
K^+ (mEq/l)	4.824 ±0.505	4.682 ±0.166	4.914 ±0.081	4.670 ±0.154	4.428 ±0.407	4.632 ±0.424
Cl^- (mEq/l)	109.6 ±3.2	107.6 ±4.9	108.2 ±0.8	107.4 ±6.3	107.8 ±2.8	104.2 ±3.2

Values are expressed as Mean ± SD.
* (**) Significantly different from control group by Dunnett procedure $p < 0.05$ (0.01).

of sex. Slight increase in reticulocyte ratio was observed in the group of low dosage regardless of sex.

3) Biochemical analysis of serum

As shown in Table 9, the increase in total protein, BUN and total bilirubin were observed in the group of high dosage regardless of sex. Though some deviations were seen in GOT, GPT, uric acid and sodium concentrations, they were not considered to be important.

4) Electrophoresis

Typical patterns of electrophoregram of urine and serum for each group were shown in Figs. 1 and 2. Two peaks derived from endogenous albumin and administered BSA were found in albumin region in all BSA treated groups. Two peaks were also found in urine in the group of high dosage regardless of sex.

5) Organ weight

As shown in Table 10, weights of kidney, liver and spleen increased remarkably in the group of high dosage regardless of sex.

6) Autopsy findings

Swelling, coarseness of surface, fading of kidney and dilatation of renal pelvis were observed in all rats in the group of high dosage irrespectively of sex. Dilatation of renal pelvis was observed in 1 male and 1 female in the

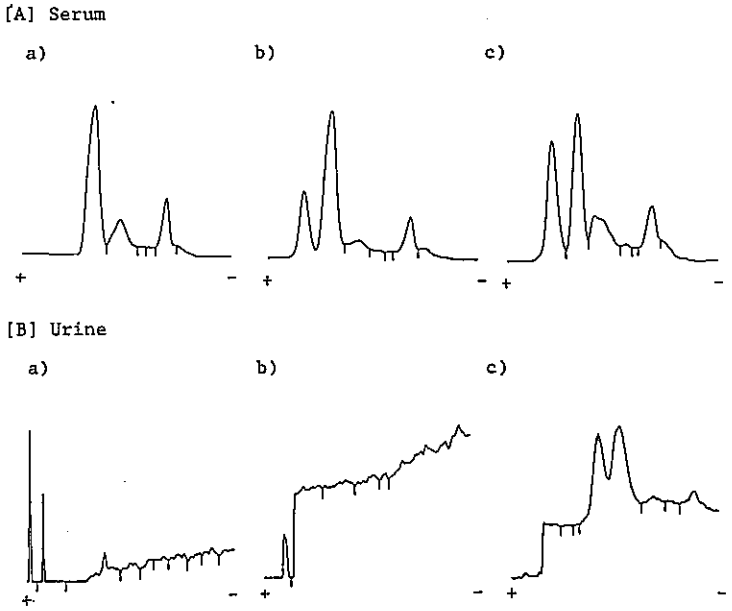

Fig. 1. Electrophoregrams of protein in male rat administered bovine serum albumin intravenously.
a) Saline control.
b) Bovine serum albumin 1.25 g/kg/day.
c) Bovine serum albumin 5 g/kg/day.

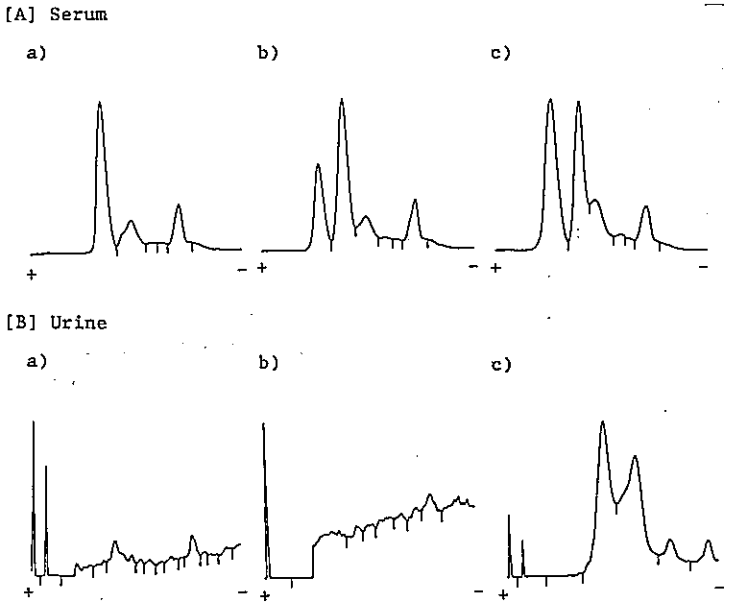

Fig. 2. Electrophoregrams of protein in female rat administered bovine serum albumin intravenously.
a) Saline control.
b) Bovine serum albumin 1.25 g/kg/day.
c) Bovine serum albumin 5 g/kg/day.

Table 10. Absolute and relative organ weights of rats administered bovine serum albumin intravenously.

Dosage group		Male			Female		
Dose (m*l*/kg/day)		Control —	Low 1.25	High 5	Control —	Low 1.25	High 5
Number or animals		5	5	5	5	5	4
Liver	(g)	9.018 ±1.041	9.820 ±0.527	10.730* ±1.098	5.943 ±0.353	6.518 ±0.543	7.898 ±0.891
	(%)	2.309 ±0.139	2.497 ±0.134	2.931** ±0.209	2.482 ±0.098	2.832* ±0.190	3.608** ±0.296
Kidney	R (g)	1.308 ±0.171	1.306 ±0.162	2.000* ±0.631	0.770 ±0.098	0.804 ±0.042	2.383* ±1.006
	(%)	0.347 ±0.035	0.345 ±0.047	0.551* ±0.182	0.321 ±0.032	0.351 ±0.024	1.082* ±0.432
	L (g)	1.250 ±0.118	1.300 ±0.180	2.018* ±0.796	0.776 ±0.043	0.770 ±0.034	2.493* ±1.042
	(%)	0.332 ±0.023	0.343 ±0.051	0.554* ±0.220	0.324 ±0.015	0.335 ±0.024	1.133* ±0.450
Spleen	(g)	0.753 ±0.125	0.758 ±0.194	0.975 ±0.007	0.540 ±0.034	0.664* ±0.074	0.785* ±0.114
	(%)	0.206 ±0.031	0.758 ±0.069	0.975 ±0.034	0.540 ±0.022	0.664* ±0.046	0.785* ±0.031

Values are expressed as Mean ± SD.
* (**) Significantly different from control group by Dunnett procedure $p < 0.05$ (0.01).

group of low dosage.

7) Histopathological findings

There were no distinctive changes in liver and spleen of all rats. As shown in Table 6, eosinophilic granules in the urinary epithelial cells (PAS-negative, Photo 3) were detected in 4 males in the group of low dosage, in 3 males and 4 females in the group of high dosage. Vacuoles in urinary epithelial cells, urinary casts, dilatation of uriniferous tubules and lymphocytic infiltration were also observed in the group of high dosage. These findings indicated chronic nephrosis (Photo 4).

Discussion

Intravenous injection of distilled water induced intravessel hemolysis and subsequent acceleration of hematopoiesis in rats. Small granules containing Fe were observed in urinary epithelial cells in these rats. They were considered to be hemosiderine, metabolite of hemoglobin. These brown granules were not stained with HE or PAS, and were different from eosinophilic bodies.

On the other hand, BSA administered intravenously was excreted into urine, accompanied with endogenous albumin. Eosinophilic granules in urinary epithelial cells of rats administered BSA remembled so-called eosinophilic bodies[1], but the former was smaller in size.

From these results, eosinophilic granules are considered to be the precursor of eosinophilic bodies, which may be formed not by intravessel hemolysis, but by hyperproteinuria.

Furthermore, increase in BUN, serum creatinine value and kidney weights, and histopathological findings showed chronic nephrosis in the rats treated with BSA. It has been reported that the high protein diet caused

various kinds of renal injury[2,3], and that BSA caused glomerulonephritis when it was injected intravenously with complete Freund's adjuvant[6]. Moreover, anemia was observed in the rats administered BSA, but granules including Fe were not found in the kidney. Accordingly, the mechanism of anemia caused by BSA may differ from intravessel hemolysis.

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ラットにおける蒸留水および牛血清アルブミンの静脈内投与による腎への影響

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摘　　要

ラットの腎尿細管上皮細胞内でしばしば認められる好酸性物質 (Eosinophilic body) の成因について検討するため，蒸留水および牛血清アルブミン (BSA) を19日間にわたり静脈内へ投与した。

蒸留水の投与では血管内溶血ならびに二次的な造血の亢進が発生し，腎尿細管上皮細胞内にヘモジデリンと思われる顆粒の沈着が認められた。

BSA の投与では，血液および尿中の蛋白質濃度の上昇，腎尿細管上皮細胞内に好酸性微細顆粒の沈着が認められ，腎重量の増加や慢性腎症も認められた。BSA 投与により生じた顆粒は染色性などについて詳細に検討した結果 Eosinophilic body と類似の性状を示すことが判明し，その前駆物質であると解された。

以上の結果からラットにおける腎尿細管上皮細胞内の Eosinophilic body の成因は血管内溶血によるものではなく，尿中蛋白質排泄の増加に伴って形成されることが示唆された。

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Explanation of Plate

Photo. 1. Fe-positive particles are seen in the urinary epithelial cells of rat administered 20 m*l*/kg/day of DW intravenously for 19 days. Berline blue staining. (X 400)

Photo. 2. Brown pigments are seen in the urinary epithelial cells of rat administered 20 m*l*/kg/day of DW intravenously for 19 days. PAS staining. (X 400)

Photo. 3. Eosinophilic granules are seen in the urinary epithelial cells of rat administered 1.25 g/kg/day of BSA intravenously for 19 days. HE staining. (X 400)

Photo. 4. Chronic nephrosis in the rat administered 5 g/kg/day of BSA intravenously for 19 days. HE staining. (X 40)

Plate