— O r i g i n a l —

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Changes Produced in Masugi Nephritis caused by Experimental Dysfunction of Adrenocortical Function

By

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Four different rabbit groups of Masugi nephritis were made: group with Masugi nephritis, group with Masugi nephritis previously treated with ACTH, cortisone and unilateral adrenalectomy. The picture of serum and urine protein were investigated electrophoretically and the changes of kidneys and adrenals were observed histopathologically and histochemically. From results obtained, authors concluded that the picture of serum and urine protein presented very similar findings to human nephrotic syndrome or nephronephritis in previously treated rabbits and from the histopathological changes we think to have proved that disharmony of adrenal function caused the change of glomerulitis and marked increase of pathological change in tubuli, and disharmony of pituitary-adrenocortical system participates in the genesis of nephrotic syndrome.

It is well established that allergy plays a role in the pathogenesis of glomerulonephritis. This is borne out by the fact that human acute glomerulonephritis often follows a hemolytic streptococcal infections, that infectious lesions are frequently found in nephrotic patients, and also by the results of experiments on nephrotoxic nephritis.

Masugi¹⁻³⁾ caused nephritis in rabbits with anti-rabbit-kidney duck serum, obtaining a very similar pathologic picture to human acute nephritis, and thus emphasized the allergic theory.

Masugi nephritis has a latent period as does human nephritis, and Kay⁴⁾, Maekawa⁵⁾, Takeda⁶⁾ and Fujimoto⁷⁾⁸⁾ et al. have investigated the cause of this in various ways. Sumiyoshi⁹⁾ and his co-worker pointed out that the nephritis-causing-antibody exists in γ -globulin fraction of the anti-rabbit-kidney duck serum.

Bell¹⁰⁾¹¹⁾¹²⁾ advocated the concept of membranous glomerulonephritis for nephrosis from his pathological studies. He felt that the main manifestations of nephrosis are not degeneration of the tubuli but that nephrosis is a form of glomerulo-nephritis. Addis¹³⁾ stated that nephrosis was a form of the degenerative stage of nephritis, thus neglecting the initial and latent period. Allen¹⁴⁾ and Ellis¹⁵⁾ et al. felt that nephrosis was a modified reaction to the stimulation which causes nephritis.

In Japan, nephrosis is considered as a separate entity and most now call it, the nephrotic syndrome. Since Hench and Kendall¹⁷⁾ administered cortisone for collagen diseases, pituitary-adrenocortical hormones have been used for various allergic diseases. ACTH or cortisone have been used in many instances for the treatment of the nephrotic syndrome.¹⁸⁻²³⁾ The existence of adreno-

cortical dysfunction in the patients with the nephrotic syndrome is evident by measuring urinary 17-Ketosteroids and other facts.⁽²⁴⁾⁽²⁵⁾⁽²⁶⁾

There are also reports suggesting a relationship between the cause of nephrotic syndrome and adrenocortical function.

We used large amounts of either ACTH or cortisone, or unilateral adrenalectomy for prior treatment to rabbits for the purpose of causing dysfunction of the adrenal cortex, then produced in the same rabbits Masugi nephritis. According to the clinical findings, they showed a picture similar to the human nephrotic syndrome or nephro-nephritis, and the changes in the kidneys and adrenals were investigated histopathologically and histochemically.

METHOD

Rabbits were sacrificed by blood loss, cutting the carotic artery.

A canule was inserted into the renal artery and sterile physiologic saline was used to wash out the blood. Following the decapsulation of the kidneys, they were cut into fine pieces, and ground up. A 15 per cent kidney tissue emulsion in saline was made. Approximately 20 cc of this emulsion was injected intraperitoneally in a duck.

This sensitization was repeated every 7th day to produce anti-rabbit-kidney duck serum. Blood was taken from the duck's wing vein every three weeks after sensitization, and the antibody titer of the duck serum to rabbit-kidney emulsion was measured by precipitation. After the antibody titer of the anti-rabbit-kidney duck serum reached 512X, it was inactivated at 55°C for 30 minutes and used to produce Masugi nephritis in rabbits.

Rabbits for the experiment were divided into five groups; (1) control group, (2) group with Masugi nephritis, (3) group with Masugi nephritis previously treated with ACTH, (4) group with Masugi nephritis previously treated with cortisone, and (5) group with Masugi nephritis previously treated with unilateral adrenalectomy.

Normal rabbits were used as a control group for histological comparison.

The rabbit-group with Masugi nephritis received 7 cc of anti-rabbit-kidney duck serum without any previous treatment. The third group received 100 mg of ACTH 6 hours prior to injection. The fourth group received 100 mg of cortisone 6 hours prior to injection of anti-rabbit-kidney serum.

The fifth group was treated with unilateral adrenalectomy 10 days prior to injection. Table

TABLE I

Group	Rabbit No.	Previous Treatment	Anti Rabbit Kidney Duck Serum
① (Control)	No. 41		
	" 42		
	" 43		
② (Masugi-Nephritis)	" 1		
	" 2		
	" 3		
	" 4		
	" 5		7 c. c.
	" 6		
	" 7		
	" 8		
③ Masugi-Nephritis with ACTH	" 9		
	" 10		
	" 11	ACTH 100mg 6°	
	" 12	time before in-	
	" 13	jection of anti-	7 c. c.
④ Masugi-Nephritis with Cortisone	" 14	kidney serum	
	" 15		
	" 21	Cortisone 100mg	
	" 22	6° time before in-	
	" 23	jection of anti-	7 c. c.
⑤ Masugi-Nephritis with unilateral adrenalectomy	" 24	kidney serum	
	" 25		
	" 31	Unilateral Adrenal-	
	" 32	ectomy 10 days	
	" 33	time before in-	7 c. c.
	" 34	jection of anti-	
	" 35	kidney serum	

I shows the number of animals in each group and the treatment they received.

Serum protein levels of the second, third, fourth and fifth groups were examined on the second day, the fifth day and the tenth day after the anti-rabbit-kidney serum injection. The amount of urinary protein was also measured by the electrophoretic method on the tenth day. For electrophoresis, the Schlieren-Diagonal's apparatus and Veronal-Veronal-Na Buffer (pH 8.6, ionic strength 0.1) were used. Total protein was measured by refractometer, and urine protein with low concentration was condensed by lyophilization before being measured.

Pathological study was done after fixing the kidney and adrenal tissue in formalin, then embedding the tissue in paraffine followed by Haematoxyline-Eosine and PAS staining. For the adrenals, frozen specimens were stained by Sudan III, or tested either by Plasmal reaction or Smith-Dietrich reaction.

A part of each adrenal was used for investigation of ascorbic acid content histochemically.

RESULTS

Results of serum protein determinations.

Variation of Total Protein: (Fig. 1, Table II, III, IV.)

In the second group with Masugi nephritis,

total protein showed an increase from the 2nd to the 5th day after injection in comparison with the value before injection, but decreased to 5.0–6.0 g/dl on the 10th day.

In the third group with Masugi nephritis previously treated with ACTH, total protein showed a slight increase on the 2nd day after injection, but decreased to 4.5 g/dl on the 10th day. In the fourth group with Masugi nephritis previously treated with cortisone, the total protein

decreased to about 4.0 g/dl. In the fifth group with Masugi nephritis previously treated with unilateral adrenalectomy, the total protein showed a slight increase from the 2nd to the 5th day, but went down to 4.5 g/dl on the 10th day.

Variation of Albumin Fraction: (Fig. 2, Table II, III, IV.)

The albumin fraction was about 65 % before

Fig. 1. Variations of total protein

Fig. 2. Variation of albumin

TABLE II SERUM PROTEIN IN MASUGI-NEPHRITIS

		T.P.	Alb.	α -G.	β -G.	γ -G.		T.P.	Alb.	α -G.	β -G.	γ -G.	
1	Bef.	5.6	64.1	8.3	13.6	14.0	6	Bef.	6.0	63.5	6.9	12.5	17.1
	2	5.8	58.2	13.1	13.9	14.8		2	6.2	59.6	11.8	11.9	16.7
	5	6.2	55.2	15.3	13.4	15.8		5	6.6	52.0	15.9	14.0	18.1
	10	4.9	50.5	13.9	17.4	18.2		10	5.7	46.0	13.9	20.5	19.6
2	Bef.	5.7	65.4	7.9	14.0	12.7	7	Bef.	5.9	61.9	8.7	14.2	15.2
	2	5.9	59.1	12.6	15.1	13.2		2	6.4	57.6	12.6	15.4	14.4
	5	6.3	55.3	16.2	14.8	13.7		5	6.7	54.9	15.4	14.0	15.7
	10	5.2	55.4	13.4	16.8	19.4		10	5.4	48.2	13.1	20.1	18.6
3	Bef.	5.8	63.6	7.8	14.0	14.6	8	Bef.	6.2	62.4	7.7	13.2	16.7
	2	5.9	57.6	14.4	13.6	14.4		2	6.4	57.0	11.5	14.8	17.0
	5	6.5	52.0	14.6	14.2	19.2		5	6.6	49.8	13.5	14.8	21.9
	10	5.1	48.7	11.0	18.4	21.9		10	5.5	47.4	12.8	18.6	21.2
4	Bef.	6.4	64.9	7.0	12.9	15.2	9	Bef.	6.3	64.3	7.5	12.7	15.5
	2	6.7	56.6	12.5	13.1	17.8		2	6.4	58.9	9.7	14.4	17.0
	5	7.2	51.0	14.5	14.9	19.6		5	6.8	50.4	13.1	14.2	22.3
	10	6.0	48.9	11.3	17.9	21.9		10	5.9	46.8	11.8	20.6	20.8
5	Bef.	6.2	67.0	7.0	11.8	14.2	10	Bef.	5.7	65.4	7.0	11.8	15.8
	2	6.4	60.3	12.4	13.0	14.3		2	5.9	56.2	12.0	12.5	19.3
	5	6.7	53.0	15.4	14.2	17.4		5	6.4	53.4	13.2	12.8	20.6
	10	5.5	49.2	12.0	21.0	17.8		10	5.2	47.4	12.3	22.3	18.0

TABLE III SERUM PROTEIN IN MASUGI NEPHRITIS WITH ACTH AND CORTISONE

		ACTH							Cortisone				
		T.P.	Alb.	α -G.	β -G.	γ -G.			T.P.	Alb.	α -G.	β -G.	γ -G.
11	Bef.	5.6	65.8	9.0	11.8	13.4	21	Bef.	6.0	62.5	7.0	13.9	16.6
	2	6.3	57.4	13.1	17.0	12.5		2	6.2	56.4	11.7	14.1	17.8
	5	6.0	49.8	13.9	17.6	18.7		5	6.1	52.8	14.0	19.0	14.2
	10	4.6	43.9	22.1	19.0	15.0		10	4.4	40.2	28.0	17.3	14.5
12	Bef.	5.5	64.5	7.9	12.3	13.3	22	Bef.	5.5	64.8	8.4	14.2	13.6
	2	5.9	54.6	14.2	18.4	12.8		2	5.7	57.0	12.4	14.0	16.6
	5	5.6	48.0	16.2	20.1	15.7		5	4.9	49.8	17.9	16.8	15.5
	10	4.3	37.1	23.9	22.5	16.5		10	4.0	36.1	27.2	19.0	17.5
13	Bef.	6.4	62.4	8.1	15.4	14.1	23	Bef.	5.8	62.3	7.6	13.8	16.3
	2	6.6	57.8	12.3	15.1	14.8		2	5.9	56.9	12.5	14.9	16.7
	5	5.9	52.7	13.8	17.6	16.9		5	5.6	50.1	16.6	17.9	15.4
	10	4.8	46.3	20.5	17.0	16.0		10	3.9	35.2	29.4	20.3	15.1
14	Bef.	6.3	61.6	8.2	13.2	15.0	24	Bef.	6.1	63.6	8.2	13.1	14.1
	2	6.4	54.2	13.0	15.2	17.6		2	6.0	55.3	14.2	15.1	15.4
	5	6.2	49.6	16.0	15.9	18.5		5	5.4	49.3	17.0	16.8	16.9
	10	4.6	44.2	19.8	18.2	17.8		10	4.0	37.6	26.3	21.2	14.9
15	Bef.	6.0	62.5	9.5	13.8	14.2	25	Bef.	6.2	61.9	8.4	14.9	14.8
	2	6.2	56.4	13.2	14.2	16.2		2	6.1	57.3	14.2	15.4	13.1
	5	5.9	52.0	15.3	15.2	17.5		5	5.2	50.7	18.0	16.2	15.1
	10	4.4	41.5	23.0	19.6	15.9		10	3.8	33.2	29.3	21.7	15.3

TABLE IV SERUM PROTEIN IN MASUGI-NEPHRITIS WITH UNILATERAL ADRENALECTOMY

		T.P.	Alb.	α -G.	β -G.	γ -G.			T.P.	Ald.	α -G.	β -G.	γ -G.
31	Bef. 1	5.5	64.3	5.8	12.1	17.8	34	Bef. 1	5.3	62.8	7.9	14.2	15.1
	Bef. 2	5.7	61.2	6.9	13.1	18.8		Bef. 2	5.2	61.0	8.1	15.1	15.8
	2	5.9	53.6	11.8	14.5	20.1		2	5.4	56.2	9.8	15.4	18.6
	5	6.2	48.9	14.3	13.6	23.2		5	5.4	50.8	14.8	15.3	19.6
32	10	4.8	44.2	18.4	15.9	21.5	35	10	4.2	42.2	22.6	18.2	17.0
	Bef. 1	5.8	65.8	5.2	11.4	17.6		Bef. 1	5.3	65.8	6.7	14.0	14.0
	Bef. 2	5.8	62.7	7.1	14.5	15.7		Bef. 2	5.2	63.1	7.2	14.8	14.9
	2	5.9	54.5	9.4	13.8	22.3		2	5.3	57.4	11.3	13.3	18.0
33	5	6.2	48.3	14.9	14.2	22.6		5	5.5	54.9	13.8	14.2	17.1
	10	4.7	43.2	19.6	18.2	19.0		10	4.8	46.8	18.7	15.9	18.6
	Bef. 1	5.6	66.3	6.4	13.8	13.5							
	Bef. 2	5.8	62.9	8.8	14.5	13.8							
	2	5.8	57.0	10.7	15.2	17.1							
	5	6.1	51.2	14.1	14.8	19.9							
	10	4.5	42.6	21.4	18.3	17.7							

treatment. The changes after injection were as follows;

In the second group, it decreased to 46-55 % on the 10th day. In the third group, it went down to 46-37 % on the 10th day. In the fourth group, it decreased the most, and value was 32-40% on the 10th day. In the fifth group, it decreased to 42-46 % on the 10th day.

Variation of α -Globulin Fraction: (Fig. 3, Table II, III, IV.)

The levels of α -globulin before and after injection was as follows;

In the second group, α -globulin increased, reaching a peak during the 2nd to 5th day and decreased by the 10th day. Whereas, the previously treated groups kept increasing and reached

20-24 % in the third group, 26-30 % in the fourth group and 18-22 % in the fifth group by the 10th day.

Variation of β -Globulin Fraction: (Fig. 4, Table II, III, IV.)

No particular change was observed in any group, although all groups showed an increase of β -globulin fraction after injection and the level on the 10th day was about 15-20 %.

Variation of γ -Globulin Fraction; (Fig. 5, Table II, III, IV.)

After injection, the second group showed an increase in the γ -globulin fraction. The third and fifth groups showed only a slight increase.

In the fourth group there was no increase

Fig. 3. Variation of α -globulin Fig. 5. Variation of γ -globulin Fig. 4. Variation of β -globulin

after injection.

Summarizing the above serum protein studies, the group with Masugi nephritis only showed the typical findings of acute nephritis on the 10th day, whereas those previously treated with ACTH, cortisone, or unilateral adrenalectomy show

a pattern similar to the human nephrotic syndrome or nephronephritis, since they showed a nephrotic pattern with marked decreased albumin fraction, marked increase of α -globulin and slight to no increase of the γ -globulin fraction.

Urine Protein studies; (Fig. 6, Table V, VI.)

The results of urine protein studies done on the 10th day following the anti-kidney serum injection are shown in Fig. 6, Table V, VI.

The amount of urine protein was 0.65–1.4 g/dl in the second group, 2.7–3.8 g/dl in the third group, 3.2–4.2 g/dl in the fourth group, and 1.7–3.2 g/dl in the fifth group.

In regards to urinary protein fractions, the second group (with only Masugi nephritis) showed 55.4–64.0 % albumin fraction, 8.9–14.0 % α -globulin fraction, 7.6–12.2 % β -globulin fraction and 13.3–21.6 % γ -globulin fraction. The third group (Masugi nephritis previously treated with ACTH) showed 64.8–69.1 % albumin fraction, 12.0–14.0 % α -globulin fraction, 6.7–7.6 % β -globulin fraction and 10.0–15.3 % γ -globulin fraction.

The fourth group (Masugi nephritis previously treated with cortisone) showed 68.4–72.1 % albumin fraction, 11.2–12.0 % α -globulin fraction, 7.3–10.4 % β -globulin fraction and 8.8–12.1 % γ -globulin fraction. The fifth group (Masugi nephritis previously treated with unilateral adrena-

TABLE VII CHANGES OF KIDNEYS

group.	Rabbit No.	Masugi-Nephritis.	Masugi-N. with ACTH	Masugi-N. with Cortisone	Masugi-N. with Unilateral Adrenalectomy	control
		1 2 3 4 5 6 7 8 9 10	11 12 13 14 15	21 22 23 24 25	31 32 33 34 35	41 42 43
Changes of glomerulus	enlarg. of glomerular tuft.	+	+	+	+	+
	edematous swelling	+	+	+	+	+
	enlarg. of capillaries	+	+	+	+	+
	incrustation of basement membrane	+	+	+	+	+
	anemia	+	+	+	+	+
	rich in nucleus	+	+	+	+	+
	net-like arrangement	+	+	+	+	+
	group of proliferated cells	+	+	+	+	+
	thrombus	+	+	+	+	+
	fibrosis	+	+	+	+	+
Changes of tubulus	epithelial crescent	+	+	+	+	+
	protein in capsula glomeruli (Bowman)	+	+	+	+	+
	enlarg. of space	+	+	+	+	+
	hyaline degeneration	+	+	+	+	+
	lipoid degeneration	+	+	+	+	+
	hyaline cylinder	+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+

Photo. 1. Glomerulus of Masugi-Nephritis, acid-schiff's stain.

Photo. 2. Glomerulus of Masugi-Nephritis, (crescent formation), acid-schiff's stain.

Photo. 3. Glomerulus of Masugi-Nephritis with ACTH, acid-schiff's stain.

(b) The third group with Masugi nephritis previously treated with ACTH: (Photo, 3, 4)

Glomeruli were generally swollen and there were many nuclei, but anemia was noted in every case. This increase in nuclei was caused mainly

Photo. 4. Glomerulus of Masugi-Nephritis with ACTH, (intracapsular exudation), acid-schiff's stain.

Photo. 5. Glomerulus of Masugi-Nephritis with cortisone, acid-schiff's stain.

by proliferation of the mesangial membrane cells due to the edematous swelling of the central part of the tuft and also caused by the proliferation of cells due to the net-like arrangement. The capillary wall of the tuft and mesangium membrane were markedly thickened due to the marked edematous and sclerotic changes. Narrowing or disappearance of the capillary lumen was noted, marked atrophy and fibrillosis of the glomeruli were recognized as an atrophic process. Fibrinoid thrombi were seen here and there.

The epithelial cells of the tufts showed swelling, desquamation and proliferation in every place where edematous swelling of the surrounding capillaries were seen and in the tufts where marked fibrinization occurred.

In Bowman's capsule space there was exudation and filling with protein and a few crescent formation were also seen.

Narrowing of the tubular lumen was seen and hyaline droplet degeneration of the epithelium was marked. In tubular lumen, protein and hyaline cylinders were noted in many places.

(c) The fourth group with Masugi nephritis previously treated with cortisone.: (Photo 5, 6)

Swelling of the glomeruli was slight and the number of the nuclei was slightly increased. Anemia was not marked and the architectural alteration with so-called net-like arrangement was hardly recognized.

Swelling of the glomeruli was found to be caused by edematous swelling of the capillary wall and mesangium and also due to the increase of unequal fibrillae. In those areas where the tuft wall was edematous, there were looseness, tearing and lysis of the fibrillae and capillary basal membranes.

Marked enlargement and proliferation of the

Photo. 6. Kidney of Masugi-Nephritis with cortisone, (intracapsular exudation and hyaline cylinder), acid-schiff's stain.

endothelial cells, mesangial cells and epithelial cells of the tufts were seen.

The capillary lumen were well preserved, and Bowman's capsule space was filled with dense protein. Portio principalis of the tubuli showed marked hyaline droplet degeneration, and the lumen of the Portio principalis was filled with hyaline cylinders.

(d) The fifth group with Masugi nephritis previously treated with unilateral adrenalectomy; (Photo. 7, 8)

The inflammatory process of the glomeruli, swelling of the tufts and anemia were slight, and the number of nuclear was small. In general the capillary lumens were well reserved. Enlargement and prolongation of the capillary tufts were marked. Some of them showed erythrocytes filling the lumen.

Edematous swelling of the tuft wall was only occasionally seen, but the process of fibrillar sclerosis was rather marked.

Swelling and proliferation of mesangium cells

Photo. 7. Glomerulus of Masugi-Nephritis with unilateral adrenalectomy, acid-schiff's stain.

Photo. 8. Kidney of Masugi-Nephritis with unilateral adrenalectomy, (hyaline droplet degeneration), acid-schiff's stain.

were only occasionally seen, but fibrillar sclerosis and atrophy of mesangium were marked.

Fibrillar thickening and sclerosis of the capillary wall were seen in various degree. Epithelial cells of the tuft presented slight swelling and proliferation. In Bowman's space, there was seen dense protein and erythrocytic exudation and erythrocytes filled the space. In the tubuli, hyaline droplet degeneration of the epithelial cells was marked and the tubuli were filled with many hyaline cylinders.

II. Pathological findings of the adrenal cortex ;

The changes in the adrenal cortex of each group is shown in Table VIII.

The findings of each group were as follows ;

- (a) Group II with Masugi nephritis alone ;
(Photo 12, 18)

The adrenal cortex was slightly enlarged, and enlargement of zona fasciculata and zona glomerulosa was also noted. The zona reticularis was

TABLE VIII CHANGES OF ADRENAL CORTEX

group	Rabbit No.	Masugi-Nephritis.										Masugi-N. with ACTH					Masugi-N. with Cortisone					Masugi-N. with Unilateral Adrenalectomy					control		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	21	22	23	24	25	31	32	33	34	35	41	42	43
incarceration of capsule hyperemia hypertrophy of cortex Sudan III	zona glomerulosa	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	zona fasciculata outer layer	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	zona fasciculata inner layer	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	zona reticularis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Plasma	zona glomerulosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	zona fasciculata outer layer	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	zona fasciculata inner layer	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	zona reticularis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Smith-Dietrich Vitamin C	zona glomerulosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	zona fasciculata outer layer	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	zona fasciculata inner layer	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	zona reticularis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

normal. In the zona glomerulosa, enlargement and proliferation of cells, cloudy swelling and congestion were seen. Degeneration and necrosis

of the cells beneath the capsule were seen. Sudanophilic granules were normal in number, but a slight decrease was seen in the zona reticularis and the inner layer of the zona fasciculata. Many plasmal granules were seen in the outer layer of the zona fasciculata, but slightly less was noted in the inner layer of the zona fasciculata and zona reticularis. Smith-Dietrich granules showed the same distribution as the granules. Vitamin C was slightly decreased.

(b) Group III with Masugi nephritis previously treated with ACTH; (Photo. 9, 13, 16)

Photo. 12. Adrenal cortex of Masugi-Nephritis, sudan III.

Photo. 18. Adrenal cortex of Masugi-Nephritis, Smith-Dietrich R.

Photo. 9. Adrenal cortex of Masugi-Nephritis with ACTH, (zigzag formation), hematoxylin-eosin stain.

Photo. 13. Adrenal cortex of Masugi-Nephritis with ACTH, sudan III.

Photo. 16. Adrenal cortex of Masugi-Nephritis with ACTH, Schiff's Plasmal R.

Photo. 10. Adrenal cortex of Masugi-Nephritis with cortisone, hematoxylin-eosin stain.

Photo. 17. Adrenal cortex of Masugi-Nephritis with cortisone, Schiff's Plasmal R.

Photo. 14. Adrenal cortex with cortisone, sudan III.

The cortex was moderately to markedly enlarged and was more marked in the zona fasciculata and reticularis. In the zona glomerulosa, marked proliferation of the cells of the basal layer and fascicular arrangement were noted. In the zona fasciculata, fascicular arrangement of the cells was not seen. There were some places where degenerated cell groups with eosinophilic cloudy cytoplasm showed the so-called "zigzag" formation. Congestion and hemorrhage were marked in the intercapsular surrounding fat tissue.

Many sudanophilic granules were seen in every layer, especially in the zona glomerulosa. Many plasmal granules were seen in the zona glomerulosa, but less in the outer layer of the zona fasciculata and zona reticularis.

Fewer Smith-Dietrich granules were seen in the zona reticularis and in the inner layer of the

zona fasciculata. Vitamin C was moderately decreased.

(c) Group IV with Masugi nephritis previously treated with cortisone; (Photo. 10, 14, 17, 19)

The cortex was atrophic, the zona fasciculata was narrow and the zona glomerulosa was wide. Marked cellular enlargement, proliferation and basophilia were noted, and there were some places showing fascicular arrangement.

Sudanophilic granules were increased in the zona glomerulosa, but decreased in the whole cortex. Many Plasmal granules were seen in the zona glomerulosa. There was a decrease in the outer layer of the zona fasciculata, a moderate decrease in the zona reticularis and in the inner layer of the zona fasciculata. Smith-Dietrich granules were decreased moderately in the inner layer of the zona fasciculata and zona reticularis, Vitamin C was moderately decreased.

(d) Group V with Masugi nephritis previously treated with unilateral adrenalectomy; (Photo. 11, 15, 20)

The cortex was atrophic and atrophy of the zona fasciculata and zona reticularis was marked. In the zona glomerulosa and the outer layer of the zona fasciculata, there was cloudy swelling, lysis and cell arrangements were irregular. In some parts of the zona glomerulosa, fascicular arrangement, was noted. In the zona fasciculata, there was irregular cell arrangement, breaking up of the fasciculi together with cloudy swelling.

In the outer layer of the zona fasciculata,

Photo. 19. Adrenal cortex of Masugi-Nephritis with cortisone, Smith-Dietrich R.

Photo. 11. Adrenal cortex of Masugi-Nephritis with unilateral adrenalectomy, hematoxylin-eosin stain.

Photo. 20. Adrenal cortex of Masugi-Nephritis with unilateral adrenalectomy, Smith-Dietrich R.

Photo. 15. Adrenal cortex of Masugi-Nephritis with unilateral adrenalectomy, sudan III.

glomerulus-like arrangement of cells was recognized. There were many places where the border between the zona fasciculata and glomerulosa was not clear. Sudanophilic granules were markedly decreased in the inner layer of the zona fasciculata, and considerably decreased in the zona reticularis, but in the outer layer of the zona fasciculata the decrease was slight and in the zona glomerulosa the number of granules was normal.

Plasmal granules showed a tendency to parallel the sudanophilic granules, and were markedly decreased in the zona reticularis and in the inner layer of the zona fasciculata. Smith-Dietrich granules were markedly decreased in all layers. Vitamin C was moderately to markedly decreased.

Reviewing the above findings, adrenal function showed no marked change in the group with

Masugi nephritis alone.

The stage of resistance was seen in the group with Masugi nephritis previously treated with ACTH. Moderate hypofunction to alarm reaction was seen in the group with Masugi nephritis treated previously with cortisone.

Alarm reaction to the consumed stage was seen in the group with Masugi nephritis treated previously with unilateral adrenalectomy.

DISCUSSION

Masugi nephritis in rabbit has a latent period, and is very similar to human acute nephritis in its pathology. For this reason, many investigative works have been done on its pathogenesis and pathology.

Shibata et al.³⁴⁾ reported that large amounts of cortisone and ACTH given 6 hours prior to the injection of anti-rabbit-kidney duck serum into the rabbit inhibited the appearance of Masugi nephritis almost completely.

In investigating the pathology and pathogenesis of Masugi nephritis, we administered ACTH, cortisone and DOCA under the same conditions as carried out in Shibata's experiment.

There was a decrease of glomerulitis and an increase in the pathological changes of the tubuli in the group to which cortisone was administered.

Similar changes to the nephrotic syndrome with decrease of serum pattern and increase of the urinary protein in the experiment was seen using ACTH and cortisone.

Nephritic symptoms were increased by the administration of DOCA.

Since Longsworth³⁵⁾ did electrophoretic studies on the serum and urine protein in the nephrotic syndrome, various studies have been reported on serum and urine protein in human renal diseases by Luetscher³⁶⁾, Lewis³⁷⁾, Malmors³⁸⁾, Blackman³⁹⁾, Fransworth⁴⁰⁾, Sellers⁴¹⁾, Sumiyoshi³²⁾ and others.

Pathology of the serum and urine protein in various types of experimental Masugi nephritis in rabbits, was reported in the present paper. Through this, the changes of the serum protein in the nephrotic syndrome is felt to be characteristic, and the

name "nephrotic pattern" is used at present. In the report, the serum protein patterns on the 10th day in three Masugi nephritis groups previously treated with ACTH, cortisone or unilateral adrenalectomy were found to be similar to the human serum protein patterns in the nephrotic syndrome. There was a markedly lowering of the total protein and albumin fraction, marked increase of α -globulin, and lowering of γ -globulin when compared with the group with simple Masugi nephritis.

In regards to urinary protein, the previously treated groups showed a larger amount of urinary protein, higher albumin and lower γ -globulin than those in the group with simple Masugi nephritis. These results lead us to conclude that Masugi nephritis in the rabbit produced qualitative changes in the clinical picture because of participation of pituitary-adrenocortical system, and thus produced the nephrotic syndrome or nephro-nephritis.

Concerning the etiology of the nephrotic syndrome, since Bell¹⁰⁻¹²⁾ pointed out that nephrosis is a form of glomerular nephritis, Addis¹³⁾, Allen¹⁴⁾, Ellis¹⁵⁾, and others have shown that nephrosis was caused by a qualitative variation of glomerular nephritis.

As to the factor to causing this qualitative change, investigations carried out by Epstein and Recant et al.⁴²⁻⁴³⁾ have shown that thyroid function has a relationship with the nephrotic syndrome, and Kawanami⁴⁴⁾ produced changes in the adrenal function of rats by continuous administration of thyroid extract. This report suggests that there is a close relationship between thyroid and adrenal function, and that variation in adrenocortical function can be caused by variation in thyroid function.

The fact that the pituitary-adrenocortical system plays an important part in the genesis of nephrosis, is proven.

Since Hench and Kendal⁷⁾ reported excellent results using cortisone in collagen diseases, many investigators have used ACTH, cortisone, prednisolone, etc. in the nephrotic syndrome and they were effective

in many cases.

The additional fact that urinary 17-ketosteroids decrease in the patient with nephrotic syndrome adds evidence.

These facts make us feel that the pituitary-adrenocortical system plays a part in the genesis of the nephrotic syndrome, and experimental investigations concerning this relationship were performed by Kuchmeister²⁷⁾ and the author²⁹⁾³¹⁾³²⁾ et al. Kuchmeister reported that he produced the nephrotic syndrome in dogs with nephrotoxic nephritis when they were previously treated by plasmaphoresis or exstirpation of the adrenal cortex.

Luetscher²⁸⁾ states that an increase of aldosterone causes edema in the nephrotic syndrome. Concerning function of the pituitary-adrenocortex, since Selye⁴⁵⁾⁴⁶⁾ advocated the general adaptation syndrome, histochemical and morphological investigations of the adrenals by Dempsey⁴⁷⁾⁴⁸⁾, Deane⁴⁹⁾⁵⁰⁾ etc. have shown the function of adrenal cortex.

In the present experiment, the histopathological glomerular changes showed moderate glomerulitis in the group with Masugi nephritis previously treated with ACTH but this was less when compared with the group with simple Masugi nephritis. In those groups with Masugi nephritis previously treated with cortisone or unilateral adrenalectomy glomerulitis was slight, no typical changes were recognized, and Bowman's capsule was filled with protein. Changes of the tubuli were marked in the three groups previously treated, and hyaline droplet degeneration and filling with hyaline cylinders were noted in many places. These changes were similar to that seen in human nephrosis or membranous glomerulonephritis.

The adrenal cortex showed no change in the group with simple Masugi nephritis, the resistant stage with zigzag formation in the group with Masugi nephritis previously treated with ACTH, and the hypofunction or alarm reaction (Selye) or consumptive stage in those groups with Masugi nephritis previously treated with cortisone

or unilateral adrenalectomy.

These results show that the dysfunction of the pituitary-adrenocortical system qualitatively changes the pictures in Masugi nephritis and causes the nephrotic syndrome. This also suggests that the human nephrotic syndrome may be produced by adrenocortical hypofunction.

CONCLUSION

To study Masugi nephritis under various conditions, five different groups of rabbits were used: group I control, group II with simple Masugi nephritis, group III with Masugi nephritis previously treated with ACTH, group IV with Masugi nephritis previously treated with cortisone, and group V with Masugi nephritis previously treated with unilateral adrenalectomy.

Serum and urine protein were checked electrophoretically, and the changes of the kidneys and adrenals were studied histopathologically and histochemically. Results were as follows;

(1) Serum protein on the 10th day after the injection of anti-rabbit-kidney duck serum; the three previously treated groups showed much lower total protein and albumin fractions, markedly higher α -globulin fraction, and lower γ -globulin fraction in comparison with the values found in the group without previous treatment. Urine protein on the 10th day; the previously treated groups showed a higher total protein, higher albumin fraction and lower γ -globulin fraction than those of the group with simple Masugi nephritis.

These results showed that the three previously treated groups presented very similar findings to the nephrotic syndrome or nephronephritis.

(2) Histopathological changes of the kidneys were as follows;

In the group with simple Masugi nephritis, there were edematous swelling and net-like arrangement, typical glomerulitis was seen, but changes in the tubuli were slight. In the group with Masugi nephritis previously treated with ACTH,

considerably severe glomerulitis existed, but the net-like arrangement was slight and many glomeruli showing sclerotic changes.

In Bowman's capsule there was much exudation of protein, and hyaline droplet degeneration in the tubuli was marked. In the group with Masugi nephritis previously treated with cortisone, glomerulitis was less than in the above two groups, marked protein exudation was noted in Bowman's capsule, and in the pathological changes in the tubuli, hyaline droplet degeneration and filling with hyaline cylinders were marked.

In the group with Masugi nephritis previously treated with unilateral adrenalectomy, glomerulitis was slight, but protein exudation in Bowman's capsule, hyaline droplet degeneration and tubuli filled with hyaline cylinders were marked.

In the adrenal cortex, in the group with simple Masugi nephritis no changes were noted, the group with Masugi nephritis previously treated with ACTH showed the picture of the resistant stage, and the other two groups with Masugi nephritis previously treated with cortisone or unilateral adrenalectomy showed hypofunction and alarm reaction all the way to the consumptive stage. From the above results, the author and his associates feel that the dysfunction of the adrenal caused the change of glomerulitis and the marked increase of pathological changes in the tubuli when nephritis occurs.

Dysfunction of the pituitary-adrenocortical system participates in the genesis of the nephrotic syndrome.

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