Study on the Pathogenesis of Nephritis

(With Special Reference to Autoradiography)

Part I Experimental Studies*

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The pathogenesis of nephritis was investigated in this paper, especially, by means of Autoradiograph. ^3H -Thymidine, a precursor of DNA, was given rats, and Autoradiograph was prepared in vivo. The proliferative change of kidney was parallel to numbers of labelled cellular component, because uptake of ^3H -Thymidine was premise of mitosis.

As the experimental parameter, three groups including Masugi Nephritis, heterologous serum nephritis and CCl_4 induced nephropathy of rats were used.

An attempt has been made to clarify time lag between histological findings and proliferative lesions recognized as "Labelling Index" on autoradiogram. The autoradiographic technic proves a very effective tool to detect proliferative changes of kidney, which was unable to be observed by conventional microscopic examination.

IN THE first half of the 19th Century RICHARD BRIGHT¹ first established a conception of nephropathy based on his assumption that on account of renal lesions proteinuria, anasarca or in certain cases hypertension would be caused, which he concluded from his clinical observations and autopsy findings upon 24 patients.

Following after BRIGHT, REINHARD held that such BRIGHT disease was a diffuse inflammation of kidney, and since that time various discussions and opinions were brought about. VIRCHOW understood it a parenchymatous nephritis, while TRAUBE and COHNHEIM objected to name it as one of inflammations. However, 1899, LANGHANS showed that glomerular lesions of parenchymatous nephritis was the most important factor in the pathogenesis of this disease, and first named it "glomerulonephritis". Furthermore in 1905 VOLHARD and FAHR established so-called "Volhard and Fahr Classification" in consideration of the correlation between histological findings and clinical symp-

toms.

Apart from such debate of nomenclature, on the other hand many experimental studies were carried on to find out the intrinsic nature of nephritis. Among those a success of Masugi nephritis^{2,3} developed by MASUGI in this country was an epoch-making incident. Furthermore, various researches and studies making use of fluorescent antibody technique by COONS et al.⁴ and heterologous serum nephritis introduced by DIXON^{5,6} are thoroughly investigating the detailed mechanism of nephritis. However, there are many facts unknown about mechanism of nephritis and its chronicity.

Under such circumstances the author carried on a series of experiments in order to overcome the discrepancy between the clinical course of nephritis and histological findings upon biopsy.

Since the inflammation necessarily accompanies cellular proliferation, it is of paramount importance to trace the dynamic process of the latter. Using ^3H -Thymidine⁷⁻⁹, DNA precursor-

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sor, autoradiograms were prepared to confirm DNA synthesis by labelling, which is necessary as a premise of mitosis, thus being able to investigate proliferative process on autoradiogram. That is, labelling by ^3H -Thymidine indicates that the cells will be divided once more at least. Therefore, when labelled in vivo (experimental animal group) it reveals a dynamic relation of cell proliferation and when labelled in vitro (biopsy) it indicates fissionability of the cells.

The author employed as experimental nephritis 1) the well known Masugi nephritis, 2) heterologous serum nephritis caused by administration of bovine serum albumin, and furthermore 3) carbon tetrachloride treatment mainly causing tubular damage. An attempt has been made to clarify the dynamic relation between histological findings and proliferative change of kidney, which is not easy to be detected by conventional microscopic examination.

And furthermore in Part II-clinical studies, with autoradiogram prepared, using biopsy specimens from nephritis patients in vitro, observation of clinical process in relation to the proliferative change was done.

MATERIALS AND METHODS

1) *Experimental Animal*

The animals used were male rats of the Wistar strain, weighing 100–120 grams. In consideration of the influence upon aging, every group had its control. They were raised with solid diet (pellets) (purchased from Nippon Laboratory Co. Ltd.), and water was given ad lib.

2) *Methods*

a. Masugi Nephritis Group

(i) Preparation of antiserum^{2,3,10}

A male white rat of the Wistar strain, weighing about 200 grams, was raised with solid diet (pellets) under careful observation for 2–3 weeks. Then opening the abdominal cavity under ether anesthesia, bled from abdominal aorta. Immediately after both kidneys were washed with sterile saline solution until blood was completely gone, they were excised and homogenized for 3 minutes with Waring Blender at its maximum speed and

filtered through gauze, thus 20 per cent kidney emulsion was prepared. The kidney emulsion was mixed with Freund's Incomplete Adjuvant (made by Wako Junyaku Co. Ltd.) at a ratio of 1:1 to be an antigen. For production of antiserum five rabbits were used, to which 5 ml of the above-mentioned antigen were injected intramuscularly once a week for 3 months. Two weeks after the last immunity they were bled from the carotid artery completely, and thus antiserum was made. It was inactivated at 56°C for 30 minutes and sealed in ampules, and stored in the cold.

(ii) Measurement of antibody titer

Employing the supernatant of 20 per cent kidney emulsion as antigen, antiserum was diluted in multiple, thus antibody titer was determined by ring tests. The result obtained was 64 times.

(iii) Preparation of Masugi Nephritis^{2,3,11,12,13}

To every rat of each group consisting of 6–8 rats, 0.5 ml per 100 grams of the body weight of antiserum was given intravenously from the tail without anesthetization. Investigation was carried on five groups of 2 hours, one day, 2 days, 4 days and 7 days, respectively. To the control groups 0.5 ml per 100 grams of the body weight of saline solution was injected from the tail intravenously. Having shorter testing periods of 2 hours to 7 days, the control groups for Masugi nephritis were all common to each period.

b. Bovine Serum Albumin Treatment Group^{14,15}

Two mg of Bovine Serum Albumin (herein after called B. S. A.) (produced by Daiichi Kagaku) dissolved into 2 ml of saline was injected intravenously from the tail of a rat without anesthetization 6 times a week. The results were observed on three groups of the 5-, 12- and 20-weeks group, respectively.

c. Carbon Tetrachloride Treatment Group^{16,17}

Carbon Tetrachloride (CCl_4) was diluted with olive oil to double the quantity, and 0.2 ml of which per 100 grams of the body weight was injected intramuscularly twice a week.

The results were observed on the 4-, 8- and 12-week group, respectively. Each group consisted of 7-10 white rats. The control group received intramuscular injections of olive oil only.

d. Preparation of Autoradiogram

Upon completion of the treatments mentioned above in (a), (b), and (c), $1.0 \mu\text{Ci/gm}$ of ^3H -Thymidine with a specific activity of 5.0 Ci/mM (produced by Daiichi Kagaku) was administered into the abdominal cavity. After two hours the rat was killed under etherization, and every organ was immediately fixed in 10 per cent neutral formalin. The paraffin sections with a thickness of 4μ were prepared. Using the dipping method with Kodak Nuclear Track Emulsion Type NTB-III, autoradiographs were made. After keeping in a cold and dark place for 3 weeks, they were developed with Sakura Konidol-X for 5 minutes, and fixed with Sakura Konifix for 10 minutes. Then they were

stained with Hematoxylin-Eosin (H.E.).

As per each sample counting was carried on:

1) re. kidney: numbers of ^3H -Thymidine labelled cells per every 1,000 tubular cells (hereinafter called NE-Index) as well as a percentage of glomeruli with ^3H -Thymidine labelled cells (hereinafter called GL-index), and

2) re. liver: numbers of ^3H -Thymidine labelled hepatocytes per 1,000 cells (hereinafter called L-index), respectively.

RESULTS

1. Masugi Nephritis

(a) Histological Investigation^{2,3,11,13}

(i) 2-Hours Group

Slight fibrinoid degeneration was observed on the glomerular loop. However, no abnormality was found of tubule, arteriole and interstitial tissue.

(ii) 1-Day & 2-Days Group

TABLE I MASUGI NEPHRITIS 2 HRS. AND 1 DAY GROUP

Rat No.	GL-Index	NE-Index	Rat No.	GL-Index	NE-Index
169	7	1.0	176	30	7
170	21	4.5	177	56	17
171	26	4.5	180	63	13
173	9	1.5	181	49	12
260	8	1.0	266	27	6
262	8	2.0	267	38	9
271	14	1.0	268	26	7
272	20	5.0			
Mean (N = 8)			Mean (N = 7)		
GL-Index = 14.1 ± 2.6			GL-Index = 41.3 ± 5.6		
NE-Index = 2.6 ± 0.5			NE-Index = 10.1 ± 1.5		

TABLE II MASUGI NEPHRITIS 2 DAYS AND 4 DAYS GROUP

Rat No.	GL-Index	NE-Index	Rat No.	GL-Index	NE-Index
162	70	5.5	155	53	6.5
163	63	6.5	156	40	4.5
164	60	3.5	157	46	6.0
165	81	10.0	158	37	4.0
166	72	10.0	159	67	7.5
167	44	3.0	160	50	5.0
168	70	7.5	161	32	5.0
Mean (N = 7)			Mean (N = 7)		
GL-Index = 66.9 ± 4.4			GL-Index = 46.4 ± 4.4		
NE-Index = 6.6 ± 1.1			NE-Index = 5.5 ± 0.46		

Fibrinoid degeneration became remarkable and slight infiltration of leukocyte was observed. In the tubules, cylinders and hyaline droplet degeneration as well as mitotic figures of the epithelial cells were found.

(iii) 4-Days- & 7-Days Group

Degeneration and hypertrophy of the glomerular loop became much more marked, rendering typical degenerative glomerulitis. Furthermore, slight proliferative change was partly observed. Tubular degeneration became more and even slight cellular infiltration was observed in interstitial tissue.

(b) Autoradiographical Investigation
(Tabs. I, II, III, Fig. 1)

(i) 2-Hours Group

Two-examples out of 8 indicated slight increase in GL-index, however, no significant difference was observed in comparison with the control, the GL-index being 9.9 ± 1.1 . Also no significant difference was observed statistically neither on NE-index nor L-index.

(ii) 1-Day Group

From statistical consideration GL-index was significant compared with the control being $0.005 < p < 0.01$, which, before histologically only fibrinoid degeneration of glomerular loop

TABLE III MASUGI NEPHRITIS 7 DAYS AND CONTROL GROUP

Rat No.	GL-Index	NE-Index
281	36	2
282	18	3
283	10	3
287	17	1
288	30	3
289	32	3
290	37	5

Mean (N = 7)

GL-Index = 25.6 ± 3.9

NE-Index = 2.9 ± 0.5

Rat No.	GL-Index	NE-Index
182	13	2.5
183	14	2.0
184	15	1.0
185	10	1.0
251	5	0.5
252	9	2.5
253	9	3.0
254	7	1.5
255	7	2.5

Mean (N = 9)

GL-Index = 9.9 ± 1.1

NE-Index = 1.8 ± 0.3

FIGURE 1 MASUGI NEPHRITIS

	Mean	
1 Control	9.9 ± 1.1	n = 9
2 2hrs	17.6 ± 5.2	n = 7
3 1Day	41.3 ± 5.6	n = 7
4 2 "	66.9 ± 4.4	n = 7
5 4 "	46.4 ± 4.4	n = 7
6 7 "	25.6 ± 3.9	n = 7

~ STANDARD ERROR

was observed, already indicated an increase in ^3H -Thymidine incorporation to DNA on the autoradiogram, i.e., proliferative change.

(iii) 2-Days & 4-Days Groups

As shown in Table II, GL-index was 66.9 ± 4.4 at the highest, which was significant at the level of $p=0.01$. On the autoradiogram the 2-days group revealed the highest increase in DNA synthesis. With the 4-days group, GL-index was nearly the same as that of 1-day group. When compared with the 2-days group it revealed decrease in ^3H -Thymidine up-take.

(iv) 7-Days Group

GL-index was significant at the level of $p=0.01$, which on the autoradiogram indicated that the incorporation of ^3H -Thymidine into DNA was decreasing, in spite of slight proliferative change found histologically.

2. *Bovine Serum Albumin (B.S.A.) Treatment Group*^{5,6,24,25} (Table IV)

The kidneys showed somewhat slight thickening of basement membrane, however, no other change was found at all in either of 5-, 12- and 20-weeks groups. On the autoradiogram, both GL-index and NE-index exhibited no significant difference between the experimental groups and the controls during 20 weeks.

3. *Carbon Tetrachloride (CCl₄) Treatment Group*^{17,26,27,28,29,30}

(a) Histological Investigations

(i) 4-Weeks Group

A slight thickening of basement membrane of glomeruli as well as increase in mesangial matrix were observed. The tubules revealed partly hyaline droplet degeneration, however, no change was found in arteriole and interstitial tissue. Liver showed remarkable change in-

cluding fatty degeneration, necrosis and fibrosis.

(ii) 8-Weeks Group

In kidneys thickening of glomerular basement membrane and highly increasing mesangial matrix were observed, and tubular changes were also remarkable. However, by conventional microscopic observation anything like proliferative change was not recognized. Compared with the 4-weeks group, liver showed much more changes, and some rats developed cirrhosis.

(iii) 12-Weeks Group

The glomeruli showed a remarkable increase in mesangial matrix, and the basement membrane was thickened markedly. Proliferative change was not observed in the 12-weeks group as same as the previous cases-8-weeks group. Tubule indicated renal cylinders, dilatation of tubules, hyaline droplet degeneration of epithelial cells of tubule. Liver revealed typical cirrhosis.

(b) Autoradiographical Investigation (Table V, VI, VII)

(i) 4-Weeks Group

Fairly variation was observed in GL-index, such as:

control: 10 ± 1.1

experimental group: 18.9 ± 4.0

Statistically indicating the value of $p=0.1$ L-index was shown:

control: 1.0 ± 0.26

experimental group: 12.7 ± 2.4

being significant at $0.005 < p < 0.01$

(ii) 8-Weeks Group

GL-index was shown

control group: 9.2 ± 0.6

at $p=0.01$

experimental group: 29.2 ± 4.5

TABLE IV B. S. A. TREATMENT GROUP

Group	B.S.A. 5w.						B.S.A. 12w.						B.S.A. 20 w.					
Case No.	43	44	45	46	52*	53*	47	48	49	50	56*	57*	21	22	23	24	54*	55*
GL-Index	17	10	10	12	11	12	9	16	11	15	11	9	18	12	14	10	10	9
NE-Index	1	1	1	2	2	2	2	1	1	1	1	1	0	1	1	3	0	1
L-Index	2	1	1	1	1	1	2	2	4	-	1	1	3	2	2	4	1	1

* Control

TABLE V CCL₄ INTOXICATION 4 WEEKS AND CONTROL GROUP

Rat No.	GL-Index	NE-Index	L-Index	Rat No.	GL-Index	NE-Index	L-Index
61	44	4	32	77	12	1	1
62	34	4	10	80	11	2	1
64	11	1	12	297	10	3	2
120	14	1	8	298	11	2	2
121	12	1	7	299	6	1	1
122	10	1	8	300	5	0	0
124	14	1	15	301	10	3	1
125	19	1.5	13	302	6	3	0
126	11	1	9				

Mean (N = 9)
GL-Index = 18.9 ± 4.0
NE-Index = 1.8 ± 0.6
L-Index = 12.7 ± 2.4

Mean (N = 8)
GL-Index = 8.9 ± 1.0
NE-Index = 2.0 ± 0.4
L-Index = 1.0 ± 0.3

TABLE VI CCL₄ INTOXICATION 8 WEEKS AND CONTROL GROUP

Rat No.	GL-Index	NE-Index	L-Index	Rat No.	GL-Index	NE-Index	L-Index
70	52	7	24	128	9	1.5	2
71	31	5	20	129	5	0.5	1
72	32	5	22	303	6	1	0
106	23	1	41	304	9	2	2
107	23	1.5	43	305	5	1	1
108	16	1	37	306	5	2	1
110	13	2	38	307	10	1	1
111	49	4.5	31	308	6	1	2
112	24	1.5	45	310	11	2	1

Mean (N = 9)
GL-Index = 29.2 ± 4.5
NE-Index = 3.2 ± 0.6
L-Index = 33.4 ± 2.8

Mean (N = 9)
GL-Index = 7.3 ± 0.8
NE-Index = 1.3 ± 0.2
L-Index = 1.2 ± 0.2

TABLE VII CCL₄ INTOXICATION 12 WEEKS AND CONTROL GROUP

Rat No.	GL-Index	NE-Index	L-Index	Rat No.	GL-Index	NE-Index	L-Index
189	24	8.5	206	223	9	3	2
191	15	3	222	224	10	2.5	0
193	23	4	172	225	7	3	1
198	18	1.5	98	226	8	1	0
199	21	4.5	212	227	10	2	0
201	33	5	202	229	11	1.5	0
203	14	1.5	196				
205	27	10.5	46				
206	22	2	106				

Mean (N = 9)
GL-Index = 22.0 ± 2.0
NE-Index = 4.5 ± 1.0
L-Index = 162.0 ± 19.8

Mean (N = 6)
GL-Index = 9.2 ± 0.6
NE-Index = 2.2 ± 0.3
L-Index = 0.5 ± 0.3

Thus GL-index of the experimental group was increasing significantly. That is, when histologically no change was observed, a doubling of DNA was shown, indicating that cellular proliferation occurred in the glomeruli.

(iii) 12-Weeks Group

GL-index of the experimental group was 22.0 ± 2.0 , which was rather lower than the 8-weeks group, but compared with GL-index of the control being 9.2 ± 0.6 statistically significant at the level of $p = 0.01$. It was assumed that there were not only an increase in mesangial matrix and thickening of basement membrane, but also proliferative change as in the 8-weeks group.

As to the liver, L-index was 162 ± 19.8 , being very high in value, and in comparison with the L-index of the control of 0.5 ± 0.28 , it was significant at $p < 0.005$.

Furthermore, those of 4-weeks, 8-weeks and 12-weeks groups respectively, all showed high values in hepatocytes as well as ductal and mesenchymal cells.

SUMMARY AND DISCUSSION

Since establishment of the conception of nephritis by BRIGHT, a number of studies and trials were carried on to find out the intrinsic nature of the nephritis. Among those a success of so-called Masugi nephritis by MASUGI in this country was an epoch-making one. However, since MASUGI so far satisfactory results have not always obtained, though there were so many researches and studies. The author, therefore, has tried a series of investigations on typical groups picked up from the experimental renal injuries.

(i) Masugi Nephritis

In 1931, MASUGI^{2,3} reported on experimental nephritis induced by nephrotoxin. It was an excellent study having a similarity to human glomerulonephritis with its success-ratio of 100 per cent. Masugi prepared anti-rat-kidney rabbit serum and by administering small amount of high titer antibody serum or large quantities of low titer antibody serum, recognized remarkable changes of kidneys. Several hours after injection, usually about 4 hours, he found infiltration of leukocytes, hypertrophy and proliferation of endothelial cells, eosin-

positive substance in the capillary, ischemia in loop, protein in Bowman's lumen, cylinders in tubules, hyaline droplet degeneration in the proximal convoluted tubules. Furthermore, after 24 hours he recognized proliferation of those like endothelial cells and thickening of basement membrane, while leukocytes in glomerular capillary loop were decreasing in number.

FUJIMOTO et al.^{11,12,13} followed up Masugi nephritis by means of renal biopsy and reported that the Masugi nephritis of rats caused by administration of anti-rat-kidney rabbit serum occurred in 15–20 minutes after the injection, that is, first of all, the antigen-antibody reaction between localized antinephro-antibody and glomerular capillary loop played an important role, and next the reaction of rabbit r-GI with loop wall did in the succeeding deterioration. In the case of rats there were two types found, one being degenerative glomerulitis mainly composed of fibrinoid degeneration by antinephro-antibody, and another being proliferative glomerulitis occurred after a certain incubation period by reaction of heterologous serum against rat r-GI produced therein.

Furthermore, F. MILLER et al.³² reported that, 4 days after appearance of Masugi nephritis, remarkable hypertrophy of endothelial and epithelial cells resulting in narrowed capillary lumen and ischemia were recognized.

According to SHIBATA et al.^{33,34,35} so-called Masugi nephritis could be caused not only by antinephro-serum but also by either of antipulmo-serum, antiencephalo and antitonsil sera as well as antiaorta serum, showing no organ specificity.

SEGAL et al.³⁶, using of fluorescein labelled antibody technique, traced antibody's localization and its term, thus confirmed that through the whole period of 291 days since nephritis occurred in rats, injected anti-rat-kidney rabbit serum could be found therein.

As mentioned above, numerous literatures are available referring to Masugi nephritis. There is an interesting report of LIPMAN et al. stating that 30 minutes after administration of anti-rat-kidney rabbit serum already certain proliferative change could be seen. On the other hand, FUJIMOTO et al. considered that

the characteristic feature was of degenerative glomerulitis at least for 3-4 days after the injection. On the autoradiogram, after 2 hours GL-index was not significant. However, one day and 2-days groups showed remarkable increase in GL-index, indicating proliferative lesion. Microscopically, one day and 2-days groups showed no proliferative change, only revealing degenerative glomerulitis mainly consisted of fibrinoid degeneration. In the 4-days group very slight cellular proliferation was observed. This could be assumed that in Masugi nephritis of rats caused by anti-rat-kidney rabbit serum degenerative glomerulitis was main pathology. However, on autoradiogram, it was maintained that even when proliferative change could not be seen microscopically, doubling of DNA was shown, and within 2-24 hours also proliferative change occurred in addition to degenerative glomerulitis.

(ii) Bovine Serum Albumin Treatment Group

There are many reports available stating that generally glomerulitis could be brought by giving heterologous protein as antigen intravenously, subcutaneously and intraperitoneally, etc. when sensitized for long period. DIXON et al.^{5,6} observed proliferative glomerulitis 10-14 days after administration of bovine serum albumin to rabbit intravenously, and furthermore observed arteritis and glomerulonephritis by administration of r-GI (bovine serum) and r-GI (human). Speaking on the mechanism, the immunological change did not appear yet during the incubation period and after occurrence of nephritis r-GI (rabbit) and B.S.A. were confirmed on basement membrane of glomeruli. At the same time complement fixation became positive. The term until such localization occurred would be corresponding with the incubation period needed for producing antibody after injection of antigen, such as B.S.A.

DINH et al.³⁷ observed nephrotic syndrome in rats by administration of homologous urinary protein. The author, upon administration of B.S.A. intravenously to the rats 6 days a week, found the changes of kidneys, after 5-, 12- and 20 weeks, respectively. Until 20 weeks there was no difference between experimental and control groups except for somewhat slight thickening of basement membrane in the former.

Even on the autoradiogram no significant difference could be seen. According to PARONETTO et al.²⁴ histological changes were varied due to the kinds of antigen administered, for instance, pig serum, horse serum, egg albumin, human gamma globulin, etc. and some brought a peculiar fibrosis of liver without any pathological changes of kidneys. There are only a few reports available on administration of B.S.A. to rats. Fundamentally, rats are hardly immunized and rarely get anaphylactic sensitization. LIPPMAN et al.³¹ administered B.S.A. together with complete- as well as incomplete-adjuvant, and succeeded in causing a systemic anaphylaxis. Furthermore, the rats are said to require almost 5 times or more antigen needed for immunization as a guinea pig. Two mg of B.S.A. administration carried by the author was assumed that on account of absolutely insufficient amounts and low sensitivity of rats, there was no significant change observed at all, histologically.

(iii) Carbon Tetrachloride Treatment Group²⁶⁻³⁰

There are a good number of reports available, however, almost all of them are referring to liver. Even among certain numbers of reports dealing with kidneys, most of which are taking up only tubular changes. VELV et al.³⁸ and others reported the poisoning in human, that is, histologically the kidneys showed hypertrophy and hyaline droplet degeneration of proximal tubules, granular cylinder of Henle's loop, and collecting tube, and sometimes bile pigment was found. As glomerular changes, SMETANA et al.³⁰, recognized appearance of collapse, hypertrophy of epithelial cells of Bowman's capsule, exudate in Bowman's lumen and slight thickening of basement membrane. The first study on renal disturbances, especially glomerular lesions, was reported by SAKAGUCHI²⁶ in this country. SAKAGUCHI carried a long range observation for 7 months, and in the first two months found no change microscopically. Thereafter, he found a slight thickening of basement membrane and increase in mesangial matrix. After five months, such changes became more marked, resulting in glomerulosclerosis.

In the observations on 4-, 8- and 12-weeks

groups carried out by the author, the 4-weeks group showed slight increase in mesangial matrix of glomeruli, microscopically, but proliferative change was rarely observed. However, on the autoradiogram GL-index was 18.9 ± 4.0 , which compared with the control group was slight significant at $p=0.1$. In the 8-weeks group mesangial matrix increased remarkably and indicated thickening of basement membrane, but proliferative change was hardly seen, microscopically. On the contrary, this group had GL-index of 33.4 ± 2.8 , which was significant at the level $0.005 < p < 0.01$. This apparently suggested an increase in the incorporation of ^3H -Thymidine into DNA, i.e., proliferative change. In the 12-weeks group, mesangial matrix was more increased and thickening of basement membrane, degeneration of epithelial cell of tubules, enlargement of tubular lumen, and appearance of renal cylinder became more impressive than those of 8-weeks group. On the autoradiogram GL-index was significant with $p=0.01$, compared with the control group. It is thought, however, that the reason of decreasing GL-index in this group in comparison with that of the 8-weeks group would be on account of the degenerative factors acting stronger in the former than the latter.

The author induced renal injury of rats by CCl_4 treatment and investigated it autoradiographically and microscopically. Referring to renal lesions increase in mesangial matrix, thickening basement membrane and degeneration of tubules as well as proliferative changes of glomeruli on the autoradiogram were found. That is, histological findings of kidneys caused by CCl_4 are proliferative changes of glomeruli, in addition to glomerular lesions suggested by SAKAGUCHI and tubular degeneration so far considered.

CONCLUSION

Masugi Nephritis Group caused by anti-rat-kidney rabbit serum.

B.S.A. Treatment Group.

CCl_4 Treatment Group.

From the histological and autoradiographical studies on these three groups the following results were obtained:

(i) On the autoradiogram, in the first phase of Masugi nephritis of rats, GL-index was already increasing significantly in 1-day and it arrived at the maximum in 2-days group. However, histologically no proliferative change was observed. In the 4- and 7-days groups GL-index was lowered compared with the 2-days group, in spite of slight proliferative changes observed histologically. From the fact it was known that the very early stage of degenerative glomerulitis, when no proliferative changes can be seen with conventional microscope, they were proved on the glomeruli autoradiographically.

(ii) By intravenous injection of B.S.A. to rats, there was no significant pathological changes of kidneys found either on the autoradiogram or microscopically.

(iii) In the CCl_4 treatment group, the 4-weeks showed an increase in mesangial matrix of glomeruli, and thickening of basement membrane histologically. Such change became marked in the 8-weeks and 12-weeks groups, but, microscopically, cellular proliferation was hardly observed. On the autoradiogram GL-index began to increase after 4-weeks treatment. The increase in GL-indices was definitely significant in the 8-weeks and 12-weeks groups. This also indicate that even at the time when cellular proliferation is hardly recognized with conventional method it can be detected on the autoradiogram.

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(1) Case No. 254 Kidney Control H. E. $\times 100$
Almost normal

(2) Case No. 254 Kidney Control Autoradiogram
H. E. $\times 200$
Arrow: ^3H -Thymidine labelled cell

(3) Case No. 177 Kidney Masugi Nephritis 1 Day
H. E. $\times 100$
Slight fibrinoid degeneration and tubular cast
as well found.

(4) Case No. 177 Kidney Masugi Nephritis 1 Day
Autoradiogram H. E. $\times 400$
Arrow: ^3H -Thymidine labelled cell

(5) Case No. 162 Kidney Masugi Nephritis 2 Days
H. E. $\times 100$
Slight fibrinoid degeneration and tubular cast
recognized.

(6) Case No. 162 Kidney Masugi Nephritis 2 Days
Autoradiogram H. E. $\times 400$
Arrow: ^3H -Thymidine labelled cell

(7) Case No. 155 Kidney Masugi Nephritis 4 Days
H. E. $\times 200$
Severe fibrinoid degeneration recognized.

(8) Case No. 155 Kidney Masugi Nephritis 4 Days
Autoradiogram H. E. $\times 400$
Arrow: ^3H -Thymidine labelled cell

(9) Case No. 55 B. S. A. Control 20 Weeks H. E. $\times 100$
Almost normal

(10) Case No. 24 Kidney B. S. A. 20 Weeks H. E. $\times 100$
Slight thickening of basement membrane and tubular cast recognized.

(11) Case No. 128 Kidney CCl_4 4 Weeks Control
H. E. $\times 100$ Almost normal

(12) Case No. 125 Kidney CCl_4 4 Weeks H. E. $\times 100$
Slight thickening of basement membrane and dilatation of tubules seen.

(13) Case No. 125 Kidney CCl_4 4 Weeks
Autoradiogram Hematoxylin $\times 400$
Arrow: ^3H -Thymidine labelled cell

(14) Case No. 305 Kidney CCl_4 8 Weeks Control
H. E. $\times 100$ Almost normal

(15) Case No. 107 Kidney CCl_4 8 Weeks H. E. $\times 100$
Slight thickening of basement membrane recognized. Mesangial matrix slightly increased.

(16) Case No. 107 Kidney CCl_4 8 Weeks
Autoradiogram Hematoxylin $\times 400$
Arrow: ^3H -Thymidine labelled cell

(17) Case No. 223 Kidney CCl_4 12 Weeks Control
H. E. $\times 40$ Almost normal

(18) Case No. 193 Kidney CCl_4 12 Weeks H. E. $\times 100$
Increase in mesangial matrix, thickening of basement membrane and tubular degeneration shown. The latter dilated.

(19) Case No. 193 Kidney CCl_4 12 Weeks
 Autoradiogram Hematoxylin $\times 400$
 Arrow: ^3H -Thymidine labelled cell

(20) Case No. 223 Liver CCl_4 12 Weeks Control
 H. E. $\times 100$ Almost Normal

(21) Case No. 193 Liver CCl_4 12 Weeks H. E. $\times 100$
 Typical cirrhosis indicated.

(22) Case No. 193 Liver CCl_4 13 Weeks
 Autoradiogram Hematoxylin $\times 400$
 Long arrow: hepatocytes
 Short arrow: ductal cell
 Hepatocyte revealed marked DNA
 synthesis.