Study on the Pathogenesis of Nephritis
(With Special Reference to Autoradiography)
Part II  Clinical Studies*

Takanobu Ohkura

Following the previous research, the purpose of this present study is to find out some means overcoming the discrepancy between histological investigations of biopsy specimen obtained from nephritis patient and their clinical course.

³H-Thymidine was incubated with the biopsy specimen and autoradiogram was made in vitro. Proliferative change of kidneys was investigated as index of renal lesions on autoradiograph as described in the previous Part I and compared with renal function, immunological examination, biochemical study of serum as well as histological findings.

The autoradiograph in vitro opened the possibility to evaluate the extent of renal lesions and furthermore clinical course of patients.

In the first half of the 19th Century a conception of nephropathy was established by Bright, and Langhans first coined the name of glomerulonephritis. Thereafter for almost one century, the studies on nephritis have so far been progressed successfully by Masugi, Volhard, Fahr, Ellis, Bell and others. However, still many problems remain unsettled about the mechanism of occurrence and prognosis of nephritis, especially of chronic nephritis.

In spite of various investigations on renal histology, renal functions and blood biochemistry as well as immunological research, etc., the results are not always satisfactory for clinicians.

Under such circumstances, the author carried on a series of investigations to fill up the discrepancy between various examinations such as renal biopsy, etc., and the clinical course.

In Part I (Basic Studies) the author described proliferative change of kidneys under pathologic condition, which could not be determined microscopically, by giving ³H-Thymidine-a DNA precursor to rats. It is stated therein that in Masugi nephritis a progress of renal injury and cellular proliferation can be predicted and that in CCl₄ treatment group some proliferative change of glomeruli can be observed on the autoradiogram, both of which were not detected by conventional microscopical level.

Based on the experiments as stated in Part I, the author has continued the research in Part II, applying ³H-Thymidine in vitro to the biopsy specimen from nephritis patients in order to know cell fissionability and proliferation. At the same time, the clinical course, renal function, and immunological as well as biochemical investigations have been checked on the autoradiographical findings with possibility of presuming the chronicity of renal diseases.

Materials and Methods
1. Materials

Nephritis patients in the 3rd Department of Internal Medicine, the Nagoya University Hospital, as well as those in the Social Insurance Chukyo Hospital were chosen for this

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Nagoya University School of Medicine, II 3rd Department of Internal Medicine, Nagoya (Chief Prof. Dr. Kozo Yamada)
study since April 1967. As the control, some patients of movable kidney received nephropexy in the Chukyo Hospital were employed. Sex and age were stated in Table I. Twenty-seven nephritis and 16 controls totalling 43 patients were examined.

2. Methods

For 2–3 weeks of admission, general observations together with renal function tests\(^8\)–\(^11\) (Fishberg, P.S.P., \(C_{\text{Ubio}}\), \(C_{\text{PSup}}\)), excretion pyelography, blood and urinary biochemical tests, etc., were done. When proteinuria, urinary sediment and blood pressure became stable, renal biopsy was performed.

a) Method of Renal Biopsy

Silvermans’ needles with 2 mm of outer diameter and 9 cm or 12 cm long of Tohoku University or Keio University type were used. As to pyelography, 1–2 ampules of 76 per cent urograin were injected intravenously. Under TV video, renal pelvis and kidney picture were confirmed. The inferior pole of kidney (the left kidney as usual) was punctured. In the control groups, an open biopsy was carried out during the nephropexy operation with the above-mentioned puncture needle.

b) Method of Autoradiogram in vitro\(^12\)–\(^17\)

After 2 hours of \(O_2\) gas perfusion, the test material was put into a culture solution consisting of 4.5 ml Ringer’s solution, 0.5 ml serum of the patient, 10,000 unit penicillin, and 15 \(\mu\)Ci of \(^3\)H-Thymidine, and shaken for one hour.
under a room temperature. Immediately after washing several times with 10 per cent neutral formalin, the specimen was fixed and paraffined. Then paraffin sections of 4μ thick were prepared for autoradiogram (9-12 pieces of serial sections to be contained in a slide glass) as well as for HE, PAS, Azan, and silver stains. The autoradiograph was made using Kodak Nuclear Track Emulsion Type NPB-III. It was developed with Sakura Koniodol X for 5 minutes after exposure for 3 weeks at 4°C in the dark and fixed with Konifix for 10 minutes. Then routine HE stain was performed. The counting on autoradiogram was carried out in the same way as described before (Part I). 

(c) Method of Immunological Investigation

Measurement of immunoglobulin (rG, rM, rA) as well as immunohistological study using the fluorescent technique were done. For the former, partigen of Boehringer Werke, and for the latter, anti-serum labelled with fluorescent isothiocyanate of the same company were used. Nikon’s fluoromicroscope was employed for observation.

Results

1. Histological Investigation

(Table I, Table II)

After Volhard1 and Fähr, Fishberg, Christian, Bell2, Allen2 as well as many others have tried the histological classification of nephritis. However, opinions are still equivocal. The author adopted the Kinoshita16-19 classification, which would be at present the most appropriate one in considering clinicopathological correlationship.

In Table I histological classification of 27 nephritis patients was shown including 22 cases of subchronic nephritis and 5 cases of chronic nephritis, which are further subdivided as follows—D-1:2, D-2:4, D-3:15, D-4:1, E-1:5, E-2:0 (Nos. of case). In this study, there was no opportunity observing patients of any acute and subacute nephritis in the hospital. Tissues taken as the control from patients with movable kidneys showed partly somewhat slight thickening of basement membrane and increase in mesangial matrix, otherwise they could be considered almost normal renal tissues and furthermore all patients revealed normal renal functions with negative urinary findings.

2. Autoradiogram

(a) The Control Group (Table III)

Autoradiograms were made in 16 cases. GL-index was all less than 20 per cent and NE-index indicated 0-1 per cent except for one case of 3 per cent.

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<tr>
<th>Case No.</th>
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Japanese Circulation Journal Vol. 34, November 1970
The control group consisted of 6 males and 10 females, the age ranging from 17 to 50 years old, but there was no difference of GL-index found per sex and age. As stated in Part I, GL-index of the control rats was on average around 10 per cent, which was not due to aging. Even in consideration of a difference between in vivo and in vitro, or difference in species of animals, it would be possible to assume GL-index in the human control group being less than 20 per cent.

(b) Nephritis Group (Fig. 1)

GL-index of 27 nephritis patients varied from normal ranges (0–20 per cent) to very high values (40–50). Among those with slight clinical and histological findings, two cases of D-1 and 4 cases of D-2, showed GL-index less than 20 per cent. On the contrary in D-3 group, which had rather deteriorated histology and clinical course, 9 cases out of 15 showed GL-index more than 20 per cent. D-4 was of a type so-called recurrent nephritis, among which one case showed a normal value. In E-1 group, except one case out of 5, each exhibited the higher

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value. GL-indices of either group, in which renal function was normal or impaired, varied from normal to very high values except for E-1 group.

3. Renal Functions and Blood Biochemistry (Table IV, Fig. 2)

As shown in Table III subchronic nephritis according to KINOSHITA criteria indicated no abnormal blood biochemistry at all. Referring to renal functions, especially G.F.R. (ml/min.), D-1 group was perfectly normal and in D-2 and D-3 groups slight abnormality was observed. In the E-1 group of chronic nephritis, renal functions were apparently lowered in G.F.R. being a range of 30.0–47.5 ml/min., and blood biochemistry revealed slight abnormality in B.U.N. and creatinine. Electrolyte was almost in the normal range. Total protein and albumin were decreased while cholesterol was high in the cases of nephrotic syndrome (Case No. 3, 19 and 27).

4. Immunological Investigation\(^{20-24}\) (Table V)

With 15 patients of nephritis, rG showed higher values in two cases of No. 17 and No.

![Graph](image1.png)

**Fig. 1. Relation between GL-Index and P.S.P. Value**

![Graph](image2.png)

**Fig. 2. Nephritis Patient Group GL-Index and G.F.R.**

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*Japanese Circulation Journal  Vol. 34, November 1970*
23, however, others were all within normal limit. Gamma M (rM) value revealed normal range in all cases including nephrotic syndrome clinically. Gamma A (rA) value too did not show any abnormality.

Immunohistochemically, there was no significant difference found between experimental group and blocking test. No specific fluorescence positive material was recognized in the basement membrane.

5. Clinical Course and Autoradiogram

(a) Control Group
All the cases showed no abnormal findings in urine, neither in blood pressure nor renal functions. However, lumbar back pain was complained by a half of patients besides general lassitude.

On the autoradiogram, GL-index was less than 20 per cent as stated above.

(b) Nephritis Group (Table VI)

(i) Proteinuria
In all cases except D-4 group, proteinuria was found consistently. In D-4 group it was

TABLE VI  Nephritis Patient Group
Histologic Diagnosis and Clinical Findings

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so severe less than 0.5 g/day tested by 20 per cent sulfosalicylic acid\textsuperscript{25-28}, being (±)-(+-), and in D-2 group (+)-((+)) and 0.5-1.0 g/day, respectively. GL-indices were all less than 20 per cent both in D-1 and D-2 groups. Proteinuria in D-3 group varied with clinical progress, and in cases with nephrotic syndrome a big amount of proteinuria was found. However, others showed 1-3 g/day of proteinuria. There was no correlation between the amount of protein and GL-index.

D-4 group (Case No. 3) was a type of so-called recurrent nephritis with preceding pharyngitis, showing a typical nephrotic symptom clinically. The amount of protein reached to 8-12 g/day. The renal biopsy was carried out twice. The first one revealed slight thickening of basement membrane and increase in mesangium. By administration of steroid hormone, proteinuria became negative and renal functions as well as blood biochemistry returned perfectly to normal. Thus the patient was discharged. On the second admission the patient was infected with pharyngitis and again nephrotic syndrome forced him hospitalization. The biopsy showed slight fibrosis and more thickening of basement membrane as well as increase in mesangial cells, while the second autoradiogram (GL-index) showed 0 per cent. This would indicate that the biopsy was carried upon normalization of renal functions, urine findings and blood biochemistry, etc.

There was no case of E-1 group showing massive proteinuria, that is, a rang of (+)-(++) by sulfosalicylic acid test and 1-3 g/day in amount. Correlation between the amount of protein and GL-index was hardly recognized, but except one case, GL-index indicated more than 20 per cent in every case, which was consistent with chronic nephritis after Kinoshita classification.

(ii) Hematuria (Microhematuria)

Hematuria is one of the most remarkable findings in nephritis, and its amounts correlated with the clinical course. Ten ml of urine was centrifuged at 1,500 rpm for 3 minutes\textsuperscript{25-28}, and sediment was examined at 400X, microscopically. When 1-3 red cells or more were observed in a visual field, it was considered to be abnormal. In D-1 group there was no microscop ic hematuria observed at all.

Four cases of 5 in D-2 group indicated slight microscopic hematuria.

In D-3 group microscopic hematuria became severer than D-1 and D-2 groups, and 12 cases out of 15 showed red cells from a few to numerous in a visual field.

In D-4 group, only one case revealed microscopic hematuria at recurrent period, however, by administration of steroid, the symptom was improved with disappearance of hematuria.

Microscopic hematuria was observed in all cases of E-1 group. There were also epithelial cells as well as cylinders, which indicated active chronic glomerulonephritis. They exhibited the highest value of GL-index.

(iii) Blood Pressure

It was accounted for hypertension when either of systole pressure or of diastole was over 150 mmHg and 90 mmHg, respectively.

Case No. 7 in D-1 group showed hypertension with 150-170 mmHg/70-80 mmHg. Renoscinogram, renogram, renoangiography and hormonal investigations carried out with no abnormality. The patient had a family history of hypertension and seemed to fall into the category of so-called essential hypertension.

In D-2 group, all 5 cases indicated the normal blood pressure.

Two cases (Case No. 9 and 25) out of 15 in D-3 group showed hypertension.

One case of D-4 group was apparently hypertensive at the time of recurrence, but blood pressure returned to normal during remission.

Of 5 cases in E-1 group, 3 showed hypertension.

The GL-indices were more than 20 per cent, while in D-3 group, it was high in only one case.

Almost all the cases of chronic nephritis showed generally hypertension during the course, and at the so-called latent period (sub-chronic nephritis might also be included), the blood pressure remained for a long time within normal. In nephrosis too, many exhibited hypertension. Therefore, rise of blood pressure was assumed to indicated progress of nephritis, the higher the pressure of being fixed, the more it would progress rapidly. It is very interesting that all of three cases in E-1 group showed

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high values of GL-index.

(iv) Edema

Edema is said to appear on two third of all the patients suffering from nephritis. However, according to the author's data, there were only 6 cases out of 27, being less than expected.

It was considered that the cases were consisted of mainly subchronic nephritis with mild clinical findings. The six cases were: 3 in D-3 group (Case No. 9, 15, and 19), 1 in D-4 group (Case No. 3), and 2 in E-1 group (Case No. 18 and 23).

(v) Decreasing Ratio of G. F. R. and Clinical Course (Fig. 3)

For following up clinical course of patients, various examinations, such as renal biopsy, blood biochemistry, renal functions (Fishberg, P.S.P., Clearance, etc.) and urinary findings, were carried out, however, renal biopsy was not performed repeatedly. The biochemical observation would be worthwhile only when renal functions are deteriorated very much with 30.0 ml/min. or less of G.F.R., resulting in retention of urea nitrogen and creatinine, etc. The urinary findings were not in proportion with extent of nephritis although it helped to estimate activities of nephritis to some degree. Among renal function tests, sodium thiosulfate clearance (C_{S,thio})^{8,11,25} is rather easier to handle for measurement and higher reliability. It is considered to reflect fairly the extent of renal damage. The author applied sodium the thio-
sulfate clearance (C_{S,thio}) and para-amino hippuric acid clearance (C_{PAH}) at the same time, and studied on correlation with GL-index and clinical course. C_{S,thio} and C_{PAH} showed deterioration of renal functions only when they went down to less than 20 per cent as normal, in consideration of measurement to the value was adopted as decreasing ratio of G. F. R.

In D-1 group, 2 cases showed no decrease in G. F. R.

Five cases in D-2 group indicated decreasing ratio of G. F. R. less than 20 per cent, and had no difference with the controls, GL-index being 0–20 per cent.

In D-3 group 16 cases exhibited various values and varied in renal function together with clinical course.

Case No. 18 showed a decrease as follows:

March 1969: G. F. R. 87.0 ml/min.
April 1969: Renal biopsy.
May 1969: G. F. R. 77.0 ml/min.
October 1969: G. F. R. 30.0 ml/min.

During such period no provoked stress such as laryngitis, saprodenia and sinuitis, which seemed to have some connections with deterioration of nephritis. GL-index of this case was 50.0 per cent, and decreasing ratio of G. F. R. was 65.5 per cent, respectively. Case No. 3 showed GL-index of 15 per cent being in the normal range. The patient, however, caught a cold in October 1969 and revealed worse urinary findings, thus being forced to readmit. In December 1969 the second renal biopsy revealed progressive findings with fibrosis and hyalinization compared with the first one made in January 1969. Histologically, it showed transitional picture between D-3 and E-1, decreasing ratio of G. F. R. being 22 per cent. Except the above-mentioned two cases which were followed up, other 11 cases indicated a decreasing ratio of G. F. R. less than 10 per cent.

One case (Case No. 4) in D-4 group was readmitted due to recurrence, and again renal biopsy was performed. Histologically it revealed recrudescence picture added to D-2. G. F. R. was tested after improvement of edema, showing normal.

Five cases in E-1 group, except Case No. 10, indicated all high GL-indices.

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Case No. 10 got renal biopsies performed three times. The histological picture showed thickening of basement membrane, cellular proliferation, interstitial fibrosis and infiltration of round cells along with hyaline droplet degeneration of tubules.

In the second renal biopsy (November 1969), there was less extent of proliferation. Interesting enough GL-index was 0 per cent on autoradiogram, confirming the histological findings.

Case No. 19 showed 17.6 per cent decrease in G.F.R. Case No. 5, 17 and 23 indicated more than 20 per cent decrease in G.F.R. ratio.

In E-1 group, apparently those with higher GL-index showed lower G.F.R.

**SUMMARY AND DISCUSSION**

As stated in Introduction, referring to the intrinsic nature of nephritis, especially chronic nephritis, there are still many problems unsettled. On the mechanism of chronic nephritis as well as its development, opinions are divergent in spite of such good numbers of studies so far carried.

The trouble is for the clinicians how to decide the course whether progressive or not. It is not easy to give an accurate determination of chronicity, using various kinds of examination histologically, immunologically and blood biochemically as well as in view of renal functions. Therefore, the author has carried out a series of investigations to fill the discrepancy between determination of prognosis by means of conventional methods and the actual clinical course.

As to the histological classification of nephritis, Volhard’s and Fähr’s method is considered to be rational. Meanwhile, Addis, Christian et al. classified the same in their own way without employing the term “nephrosis”. If nephrosis is considered to be an inflammation, it should be a glomerulonephritis, while if it means degeneration, it can be a glomerular degeneration. In either way, at present, there would not be objection to considering it as a glomerular disease. Bell adopted a term of “membranous glomerulonephritis” and Leitter used the term “glomerulonephrosis”. Even in classification, they never agree with each other at present. At this point Kinoshita’s classification which is based on the pathohistological way and clinical progress as well is thought the most appropriate one ever seen. So far, uncured nephritis was taken as chronic one. However, even such case might be cured several years later. From this point Kinoshita established a category named subchronic nephritis, which is originally an non-progressive nephritis, in consideration of histology and clinical course. However, even with this classification it still contains some active type, making it quite hard to determine whether it is histologically progressive or not. As one of the solutions of such problem the author paid attention to the proliferative change of kidneys which can not be determined on the microscopical level, and adding GL-index to the conventional biopsy findings as well as various clinical tests, finally has found out that such vacancy between biopsy and clinical course could be filled to a certain extent by taking up the autoradiogram in vitro.

As a prerequisite to mitosis, DNA synthesis should occur. The nuclear labelling on autoradiogram indicates that the cell should be divided once more at least. Therefore, when labelled in vitro, it reveals fissionability of the cell itself. There are only a few studies on nucleic acid in kidney, such as RNA metabolism in rabbit’s kidney by Foster and Riad and incorporation of $^3$H-Thymidine of rats with hypertension by Crano and Dutta. Referring to studies in vitro with human being, there are some reports available by using biopsy materials from bladder tumor, breast cancer, hepatitis, cirrhosis of liver etc., however, research for human renal disease in vitro has not appeared yet. The author prepared an autoradiogram in vitro with the materials obtained from the nephritis patients and as the control, from those with movable kidney who had no abnormal symptom including renal functions, blood biochemistry, urinary findings and blood pressure. The results obtained were significant, i.e., in the control group incorporation of $^3$H-Thymidine into glomeruli, namely GL-index remained all within a range of 0–20 per cent, while GL-index of nephritis group showed variable values within 0–50 per cent. The fact indicates that the mechanisms of cellular pro-
liferation in the control and nephritis groups, respectively, are entirely different.

In the latter, renal functions, blood pressure, and urine as well as blood biochemistry were checked up in every 3–6 months. The renal biopsies were done twice in 4 cases, i.e., Case Nos. 2, 3, 10, and 22.

In D-1 and D-2 groups, GL-index, renal functions and blood biochemistry showed no abnormality at all.

Seven cases indicated higher GL-indices among 15 cases of D-3 group. Out of them, 2 cases, namely, No. 2 and No. 18 showed deterioration of renal functions more than 20 per cent in decreasing ratio of G.F.R. However, other cases with nomal GL-indices showed no abnormality of renal functions and blood biochemistry at all during the period of observations for 3–12 months.

In D-4 group, a slight increase in fibrosis was observed at the second biopsy, otherwise no abnormality was seen on renal functions and GL-index.

Five cases in E-1 group, except Case No. 10, revealed higher GL-indices as stated above, as well as deterioration of renal functions. In 3 cases, namely Case Nos. 5, 17 and 19, abnormalities of blood biochemistry were observed.

From the above results it was known that impairment of renal functions as well as blood biochemical and histological deterioration reflect increase in DNA synthesis of glomeruli, i.e., higher GL-index, except exasperation apparently due to exciting causes, such as tonsilitis, bronchitis, saprodontia and other suppurative diseases. Such a study, employing biopsy material from nephritis patients, was so far never seen, although there is one interesting report by LEEVY et al. on autoradiogram using biopsy specimen of liver.

As to the kinds of proliferating cells of glomeruli in nephritis, there are many equivocal opinions, and even now some doubt the existence of mesangium. There are also many authors, such as VOLHARD supporting endothelial cells, FUJIMOTO assuming from tracing-of of Masugi nephritis that they are endothelioid, YAJIMA assuming mesangium cells in the basement membrane due to breaking down of mesangium and JOHNS et al. stating that glomerulitis is a sort of pericapilleritis and that proliferating cells are originated from interstitial cells.

The epithelial cells of glomerular loops as well as those of Bowman's capsule are quite few, and deterioration of the labelling of endothelial and mesangium cells on the autoradiogram carried out by the author is extremely hard with a 4 μ section, being a number of cells undifferentiated. However, referring to the cells relatively differentiated, most of them may be considered as mesangium cells.

Observed from the report of immunoglobulin, rG was high in Case No. 17 (D-2) and Case No. 23 (E-1) among 15 cases, and all others were in the normal range. Gamma M (rM) and rA did not show any abnormality in all the cases, including Case Nos. 3, 9, 13 and 15 of nephrotic type there were reports that they took the lowest value within normal limit, however from the author's data no such tendency was observed. There was also no significant relation between GL-index and immunoglobulins. Therefore, the interpretation of change in immunoglobulins is very difficult, from a view of autoimmunity, which concerns with chronicity of nephritis. Immunohistochemically, there are many reports stating fluorescence positive materials found in the part corresponding to the basement membrane in acute nephritis, S.L.E. and membranous glomerulonephritis. The author, using the staining by anti-r-Gl with F.I.T.C., could not find any significant fluorescence on the basement membrane, even in the Case Nos. 17 and 23 showing high rG values. From this fact, it is understood how difficult it is to trace immunity in subchronic and chronic nephritis.

Azotemia, usually occurs when G.F.R. is decreased less than 20–30 per cent. It is due to increase in urea nitrogen along with creatinine and uric acid. The concentration of urea nitrogen and creatinine in blood are determined by production in the body and excretion from kidneys. Almost all the patients whom the author observed were of subchronic nephritis. They had no abnormalities biochemically. Case Nos. 18 and 23 showed only slight increase in creatinine and urea nitrogen among E-1 group accompanying remarkable decrease in G.F.R.

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In Case Nos. 3, 9, 13, 15 and 19 of nephrotic syndrome were decreased in total protein and albumin with increase in $\alpha_2$-gl and $\beta$-gl.

The author has prepared autoradiograms in vitro using biopsy material in addition to the conventional histological investigation, renal function test, immunological and biochemical studies in order to overcome the discrepancies between histological findings and clinical course, and found that GL-index was very useful to predict prognosis.

CONCLUSION

Twenty-seven nephritis patients and 16 controls totaling 43 cases were investigated with the conventional methods including immunological, immunohistochemical, blood biochemical studies as well as renal function tests.

In addition, autoradiogram in vitro was applied.

(1) There are no relationships the clinical course and immunological as well as immunohistochemical investigation in nephritis patients.

(2) In subchronic nephritis cases of D-1, D-2, and D-4 groups after KINOSHITA classification, GL-index and renal functions do not show any abnormality. Nor are exasperating tendencies of clinical courses.

Half of cases of subchronic nephritis D-3 group, in which histological deterioration is severe in extent, reveals high value of GL-index. They are considered active chronic from of nephritis. In chronic nephritis E-1 group, GL-index is apparently high, reflecting progressive course with impairment of renal function.

(3) GL-index on autoradiogram in vitro using biopsy material from nephritis and clinical progress are in close relationship with each other.

That is nephritis patients with high GL-index may take active and progressive course.

Acknowledgment

The author gratefully acknowledges constant interest and guidance of Prof. Yamada, K. and the help of Dr. Masuko, K.; Department of Medicine and Dr. Ito, N.; Department of Pathology, for preparing the autoradiographs, in this investigation.

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REFERENCES

Control Group

1. Case No. 3 H. E. ×40
   Slight dilatation of tubules

2. Case No. 5 H. E. ×40
   Almost normal

Nephritis Patient Group

3. Case No. 6 H. E. ×40
   Slight proliferation of mesangial cell

4. Case No. 6 H. E. ×100

5. Case No. 7 H. E. ×40
   Slight proliferation of mesangial cell

6. Case No. 7 H. E. ×100

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7. Case No. 16 H. E. ×40
Mesangial cell increase and thickening of basement membrane

8. Case No. 16 PAS ×40

9. Case No. 16 H. E. ×400 Autoradiogram
Arrow: mesangial cell (³H-Thymidine labelled)

10. Case No. 4 H. E. ×40
Mesangial cell increase and thickening of basement membrane with round cell infiltration in interstitial tissue found

11. Case No. 4 H. E. ×400 Autoradiogram
Arrow: tubular cell (³H-Thymidine labelled)

12. Case No. 2 H. E. ×40 2nd. Biopsy
Kinoshita Classification: D3~E1 (transitional stage)

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13. Case No. 2 PAS ×40

14. Case No. 2 H.E. ×100

15. Case No. 2 H.E. ×400 Autoradiogram
   Arrow: epithelial cell of Bowman's Capsel
   (³H-Thymidine labelled)

16. Case No. 3 H.E. ×40 1st. Biopsy
   Mesangial cell increase and round cell
   infiltration in interstitial tissue

17. Case No. 3 H.E. ×100

18. Case No. 19 H.E. ×40
   Mesangial and epithelial cell proliferation.
   Basement membrane thickening, slight fibrosis
   of interstitial tissue and intimal thickening
   of small artery

*Note: The images and captions are placeholders and do not represent the actual content of the document.*

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19. Case No. 19 H. E. ×400 Autoradiogram
   Long arrow: endothelial cell (^H-Thymidine labelled)
   Short arrow: mesangial cell

20. Case No. 19 H. E. ×200 Autoradiogram
   Arrow: tubular cell (^H-Thymidine labelled)

21. Case No. 5 Azan ×40

22. Case No. 5 H. E. ×400 Autoradiogram
   Arrow: mesangial cell

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