LETTER TO THE EDITOR

Correspondence to "Changes in Lectin Binding Pattern During Fetal and Postnatal Development of Renal Corpuscles of the Rat Kidney as Revealed by Light and Electron Microscopy" by Imamura H. *et al.*

With intense interest, we read the paper "Changes in lectin binding pattern during fetal and postnatal development of renal corpuscles of the rat kidney as revealed by light and electron microscopy" by Imamura H., Akimoto Y., Chino I. and Hirano H. which appeared in the recent issue of this journal (6). However, we were very much disappointed that several references including the meeting abstract we presented previously (2) as well as important previous papers on lectin histochemistry of developing kidney by other authors (1, 4, 5) were neglected.

The authors used paraffin sections in their paper (6). Although we understand that paraffin sections have been widely used for lectin staining of kidney tissues (1, 5, 7), we feel it better to use cryosections than paraffin sections for detecting the localization of glycoconjugates by lectin histochemistry. In the case when the changes of lectin staining intensity in developing organs are studied, cryosections should be used, since we believe that lectin staining on paraffin sections does not show enough high sensitivity of the staining as required to detect small amounts of glycoconjugates, which may be encountered in the tissues of fetal or senescent animals. We had attempted to compare the procedures of lectin staining using cryosections and paraffin sections on the kidney tissues of rats and mice, prior to our presentation at the 32nd Annual Meeting of the Japan Society of Histochemistry and Cytochemistry in Tokyo on October 17-18, 1991 (2). Together with the histochemical staining, we performed Western blot analysis of developing kidney tissues using HRP-lectins. Lectin staining with cryosections using 16 kinds of biotinylated lectins (WGA, ConA, SSA, DSA, ABA, LCA, PHA-L₄, PHA-E₄, RCA-60, RCA-120, DBA, Lotus, MAM, PNA, SBA, UEA-I), in general,

had higher sensitivities of the staining than that with paraffin sections. Moreover, the reaction of Western blot analysis for lectins was in good accordance with those of histochemistry using cryosections in the sense that both could demonstrate the changes of glycoconjugates in developing kidney. Figs. 1 and 2 are examples to show the comparison of the lectin staining using paraffin sections and cryosections for DSA and PHA-L₄ in rat and mouse kidney tissues. The sections were stained with biotinylated lectins and ABC complex. The reaction products were visualized with DAB reaction. Please compare the staining intensity of cryosections (Figs. 1B, D, 2B, D) and paraffin sections (Figs. 1A, C, 2A, C). We can easily recognize the stronger reaction in the former, both in the rat and mouse kidney.

We would like to reproduce the previous presentation (2) again here in brief, in which lectin histochemistry using cryosections at LM level as well as lectin histochemistry at EM level was presented. The mice kidneys of six age groups: 19 days of gestation (late fetal period), 2 days (newborn period), 8 days (suckling period), 4 weeks (weaning period), 3 months (adult period), and 10 months (senescent period); five animals per age group were used. For staining with lectins at LM level, specimens were fixed in buffered 4% paraformaldehyde for 24 hr. Cryosections at 6 μ m thickness were prepared and stained with 16 kinds of biotinylated lectins (WGA, ConA, SSA, DSA, ABA, LCA, PHA-E₄, PHA-L₄, RCA-60, RCA-120, DBA, Lotus, MAM, PNA, SBA, UEA-I) in combination with the ABC and DAB method. For staining with lectins at EM level, specimens were fixed in buffered 0.1% glutaraldehyde and 4% paraformaldehyde, and embedded in Lowicry K4M at a low temperature. Ultrathin sections were stained by using 3 kinds of biotinylated lectins (WGA, Lotus, DSA) and streptavidin colloidal-gold complex (10 nm). After the staining with uranyl acetate and lead citrate, they were observed in a Hitachi H-700 electron

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FIGS. 1A-D. Light microscopic photographs of both rat and mouse kidney cortex stained with biotinylated DSA lectin and avidin-biotin-peroxidase complex. Rat kidney cortex using paraffin sections at 3 months after birth (Fig. 1A), rat kidney cortex using cryosections 3 months after birth (Fig. 1B), mouse kidney cortex using paraffin sections 3 months after birth (Fig. 1C), mouse kidney cortex using cryosections 3 months after birth (Fig. 1D). Note that the staining using cryosections (Figs. 1B and 1D) shows higher sensitivity than the staining using paraffin sections (Figs. 1A and 1C). $\times 240$ Bar = $100\ \mu\text{m}$

microscope at the accelerating voltage of 200kV. The changes of staining by lectin histochemistry were categorized into 4 patterns, #1 the staining was consistently positive, #2 the staining was positive during gestation, but became negative with aging, #3 the staining was negative in the gestational stage, but became positive with aging, #4 the staining was consistently negative. Focusing the discussion on the staining of glomeruli, our results and those by Imamura *et al.* (6) have some discrepancies. We showed the changes of staining pattern in glomeruli with WGA, ConA, DSA, RCA 120 were categorized to #1, and staining intensity increased gradually along with

development. On the other hand, Imamura *et al.* reported this to be categorized to #1, but without the change of intensity. We showed the changes of staining pattern in glomeruli with PHA-E₄, PHA-L₄ were categorized to #3, and staining intensity increased gradually along with development. On the other hand, Imamura *et al.* (6) reported this to be categorized to #1, but without the change of intensity. These differences between our data and Imamura *et al.* may be due to the species difference of materials, rat and mouse. However, we believe that another possibility exists. We are afraid that the employment of the paraffin sections could not visualize the changes of the

FIGS. 2A-D. Light microscopic photographs of both rat and mouse kidney cortex stained with biotinylated PHA-L₄ lectin and avidin-biotin-peroxidase complex (Figs. 2A-D). Rat kidney cortex using paraffin sections at 3 months after birth (Fig. 2A), rat kidney cortex using cryosections 3 months after birth (Fig. 2B), mouse kidney cortex using paraffin sections 3 months after birth (Fig. 2C), mouse kidney cortex using cryosections 3 months after birth (Fig. 2D). Note that the staining using cryosections (Figs. 2B, D) shows higher sensitivity than the staining using paraffin sections (Figs. 2A,C). $\times 240$ Bar=100 μ m

staining intensity in their paper, if the reaction on paraffin sections was not intense enough to detect the changes due to its lower sensitivity than the staining using cryosections as shown in the Figs. 1 and 2.

Considering the preliminary studies as described above and the presentation we made in the past (2), our paper entitled "Light microscopic lectin histochemistry in aging mouse kidney: Study of compositional changes in glycoconjugates" by Hanai T., Usuda N., Morita T., and Nagata T. (3) was prepared and submitted for publication in the Journal of Histochemistry and Cytochemistry in August 1992, which is now in press.

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