Amino Acids (1997) 12: 353–361

Amino
Acids

Change of serum L-tryptophan levels following the development and recovery of acute puromycin aminonucleoside nephrosis in rats

E. Sasaki[1], **Y. Ohta**[1], **R. Shinohara**[2], and **I. Ishiguro**[1]

[1]Department of Biochemistry, School of Medicine and [2]Department of Biochemistry, School of Health Sciences, Fujita Health University, Toyoake, Japan

Accepted September 20, 1996

Summary. It is known that total L-tryptophan (Trp) levels decrease with a decrease in albumin-bound Trp levels and an increase in free Trp levels in the plasma or serum of nephrotic children. We, therefore, examined the change of serum Trp levels following the development and recovery of acute nephrosis in 6-week-old male Wistar rats injected once with puromycin aminonucleoside (100 mg/kg body weight) and checked the levels of 16 amino acids including Trp in the serum and the levels of Trp in the liver, kidney, and urine under nephrotic conditions. In this study, the development and recovery of nephrosis were checked by the changes of levels of urinary protein and serum protein and albumin. Total serum Trp and albumin-bound serum Trp levels decreased with the development of nephrosis and these decreased levels returned to the normal level with its recovery. In contrast, free serum Trp levels increased with the development of nephrosis and this increased level returned to the normal level with its recovery. In the serum of nephrotic rats, the decrease of albumin-bound Trp levels and the increase of free Trp levels were well consistent with a decrease in albumin levels and an increase in the level of non-esterified fatty acids which are known to weaken the binding of Trp to albumin and among 16 amino acids studied, only Trp showed a significant change in its levels. Trp levels increased in the liver and kidney but not in the urine under nephrotic conditions. These results indicate that the change of serum Trp levels should be closely related to the condition of nephrosis and that although serum Trp is lost under nephrotic conditions, the lost serum Trp is accumulated in the liver and kidney.

Keywords: Amino acids – L-Tryptophan – Albumin – Non-esterified fatty acids – Puromycin aminonucleoside – Experimental nephrosis (rat)

Introduction

L-Tryptophan (Trp) is one of the nutritionally essential amino acids. Under physiological conditions, more than 90% of total plasma Trp is known to be

converted to kynurenine and subsequently to niacin and NAD in the liver through the kynureine pathway in which tryptophan 2,3-dioxygenase takes part (Bender, 1982). The remaining Trp is available for protein synthesis in a variety of tissues and for serotonin synthesis in the brain (Bender, 1982). Unlike other amino acids, 80–90% of total Trp present in the plasma or serum of humans and animals normally occurs in an albumin-bound form, while the remainder (ca. 10μM) circulates in a free form, i.e., an albumin-unbound form (McMenamy et al., 1957; McMenamy et al., 1958; Fuller et al., 1973; Saito et al., 1986; Sasaki et al., 1993a). Recently, we studied the effect of albumin on the disappearance of Trp from the perfusate into isolated perfused rat livers, and suggested that under physiological conditions (ca. 100μM Trp and 4% albumin), albumin contributes to the maintenance of the total serum Trp concentration and to the constant supply of serum Trp to the liver by lowering changes in serum Trp concentrations through its binding to the amino acid (Sasaki et al., 1993a). It is known that besides Trp, endogenous substances such as long-chain fatty acids, unconjugated bilirubin, and bile acids bind to albumin (Peter, 1985). In addition, it has been demonstrated *in vitro* and *in vivo* that long-chain fatty acids weaken the binding of Trp to albumin (Curzon et al., 1973; Curzon et al., 1974; Brodersen et al., 1989; Sasaki et al., 1993b).

Pirazzoli et al. (1983) have reported that in children with nephrotic syndrome, a decrease in total plasma Trp levels occurs with an increase in free plasma Trp levels and that there are a positive correlation between albumin and albumin-bound Trp concentrations and a negative correlation between albumin and free Trp concentrations in the plasma. In addition, the same authors have shown that there is no increase in urinary Trp levels in the nephrotic children (Pirazozoli et al., 1983). Fydryk et al. (1984) also have reported that in the serum of children with refractory nephrotic syndrome, a decrease in total Trp concentration occurs with a decrease in the concentration of Trp bound to albumin fraction, and have supposed that children with refractory nephrotic syndrome and persistent hypoalbuminemia are potentially exposed to Trp deficiency. However, it is still unclear how Trp levels in the plasma or serum change with the development and recovery of nephrosis and where of the body Trp lost in the plasma or serum is accumulated and/or metabolized under nephrotic conditions.

We, therefore, examined the change of levels of serum Trp (total, free, and albumin-bound) following the development and recovery of acute nephrosis in rats injected once with puromycin aminonucleoside (PAN) which is well known to induce nephrosis resembling minimal change nephropathy with focal segemental glomerulosclerosis seen in humans (Frenk et al., 1955; Diamond and Karnovsky, 1986). In addition, the levels of 16 amino acids including Trp in the serum and the levels of Trp in the liver, kidney, and urine were checked in the nephrotic rats.

Materials and methods

Male Wistar rats aged six weeks, which were purchased from SLC Co. (Hamamatsu, Japan), were used in the present study. The animals were once injected intraperitoneally

with PAN (Sigma Chemical Co., St. Louis, MO, U.S.A.) dissolved in 0.85% NaCl at a dose of 100 mg/kg body weight. The control rats were injected intraperitoneally with an equal volume of 0.85% NaCl. During the whole experiment, rats received normal rat chow, Oriental MF (Oriental Yeast Co., Tokyo, Japan) and tap water *ad libitum*. The collection of 24-hour urine was conducted one day before PAN injection and 4, 6, 9, and 13 days after the injection. The urine was collected in a glass flask containing 5 ml of toluene and 5 ml of 0.1 M potassium phosphate buffer (pH 7.5). During the urine collection, rats were maintained in individual metabolism cages. After the urine collection, rats were sacrificed between 9:00–10:00 AM under ether anesthesia at which time blood was collected from the vena cava caudalis. The collected blood was separated into serum. The liver and kidney were removed after collecting the blood. Before removement of both tissues, ice-cold 0.15 M KCl was infused from the portal vein for 5 min to remove blood remaining within the tissues, especially the liver. The urine, serum, liver, and kidney were stored at −80°C until use.

Protein in the serum was measured by the biuret method (Gornal et al., 1949). Albumin and non-esterified fatty acids (NEFA) in the serum were measured using commercial kits of Albumin B Test Wako based on the bromcresol green method (Doumas et al., 1971) (Wako Pure Chemical Ind., Ltd., Osaka, Japan), and NEFA KAINOS (Kainos Co., Tokyo, Japan), respectively. Amino acids in the serum were determined in a Hitachi L-8500 amino acid analyzer (Hitachi Co., Tokyo, Japan) as described previously (Sasaki et al., 1991). Protein in the urine was measured by the method of Lowry et al. (1951). The urine sample for this measurement was prepared as follows: a part of the collected urine was mixed with trichloroacetic acid (a final concentration of 5%) and the mixture was placed on ice for 10 min. Then, the mixture was centrifuged at 10,000 × g for 10 min at 4°C to collect proteins. The precipitated proteins were dissolved with 1.0 M NaOH and the resulting solution was used for protein measurement. Trp in the serum, liver, and kidney was determined using high-performance liquid chromatography (HPLC) with electrochemical detection as described in our previous reports (Saito et al., 1986; Sasaki et al., 1991; Sasaki et al., 1993a; Sasaki et al., 1993b). A Yanagimoto L-500 pump (Yanaco Co., Kyoto, Japan), a Yanapak ODS-T (4 ϕ-250 mm, Yanaco Co.), and a volutametry detector VMO-101A (Yanaco Co.) were used in this HPLC system. The chromatography was conducted at 40°C and a voltage of 900 mV with a solution of 0.1 M citric acid-0.1 M sodium acetate and methanol (4:1 v/v) used as an elution solution, and the flow rate was 1.0 ml/min. Samples for determinations of total Trp and free Trp in the serum were prepared as follows: ten min after mixing the serum with an equal volumes of ice-cold 0.4 M perchloric acid, the mixture was centrifuged at 10,000 × g for 10 min at 4°C. The resulting supernatant was used for determination of total Trp. After centrifugation of another aliquot of serum at 1,500 × g for 30 min at 4°C in a Centriflo membrane cone CF 25A (Amicon Grace Co., Tokyo, Japan), the resulting filtrate was used for determination of free Trp. The concentration of albumin-bound Trp was estimated from the difference between total Trp and free Trp concentrations determined. Samples for determinations of liver and kidney Trp were prepared as follows: the liver and kidney were homogenized in 4 volumes of ice-cold 0.15 M KCl and then each homogenate was mixed with an equal vol. of ice-cold 0.4 M perchloric acid. Ten min later, the mixtures were centrifuged at 10,000 × g for 10 min at 4°C. Each resultant supernatant was used for determination of its corresponding tissue Trp. Trp in the 24-hour urine was measured by the fluorometric method of Denckla and Dewey (1967).

All values obtained are expressed as means ± SD. Results were statistically analyzed by Student's *t*-test if necessary. The level of significance was set at $p < 0.05$.

Results

When the levels of urinary protein and serum protein, albumin, and NEFA were examined in rats over a 14 day period after a single injection of PAN

(100 mg/kg body weight), these levels changed as shown in Fig. 1. The level of urinary protein tended to increase at 5 days after the PAN treatment and a significant increase in the level occurred at 7 days; urinary protein content in the PAN-treated group was 14-fold more than that in the control group at 7 days (Fig. 1A). This increased level returned to the control level thereafter and completely recovered at 14 days (Fig. 1A). In the PAN-treated rats, serum protein and albumin levels tended to decrease at 5 days after the PAN treatment and significantly decreased at 7 days (Fig. 1B and C). Serum protein and albumin concentrations in the PAN-treated group were lower than those in the control group by 32 and 37%, respectively, at 7 days after the PAN treatment (Fig. 1B and C). These decreased levels returned to the control level thereafter and completely recovered at 14 days (Fig. 1B and C). In the PAN-treated rats, serum NEFA levels tended to increase at 5 days after the PAN treatment and significantly increased at 7 days; serum NEFA concentration in the PAN-treated group was 2.7-fold higher than that in the control group at 7 days (Fig. 1D). This increased level returned to the control level thereafter and almost completely recovered at 14 days after the PAN treatment (Fig. 1D).

When total Trp, free Trp, and albumin-bound Trp levels were examined in the serum of rats over a 14 day period after a single injection of PAN, these levels changed as shown in Fig. 2. There were no significant changes in total

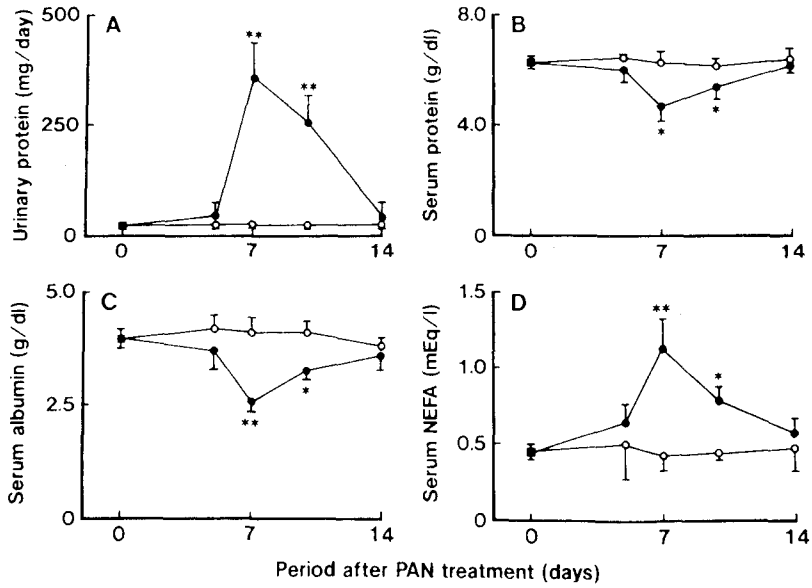

Fig. 1. Changes in levels of urinary protein (**A**) and serum protein (**B**), albumin (**C**), and non-esterified fatty acids (*NEFA*) (**D**) in rats after a single injection of puromycin aminonucleoside (*PAN*). Rats were intraperitoneally injected with PAN (100 mg/kg body weight) dissolved in 0.85% NaCl. The control rats were given intraperitoneally with an equal volume of 0.85% NaCl. Urinary protein and serum protein, albumin, and NEFA were determined at 0, 5, 7, 10, and 14 days after the PAN treatment as described in Materials and methods. Open circle, control rats; closed circle, PAN-treated rats. Each value is a mean ± SD (n = 4–6). *, $p < 0.01$; **, $p < 0.001$ (vs. control rats)

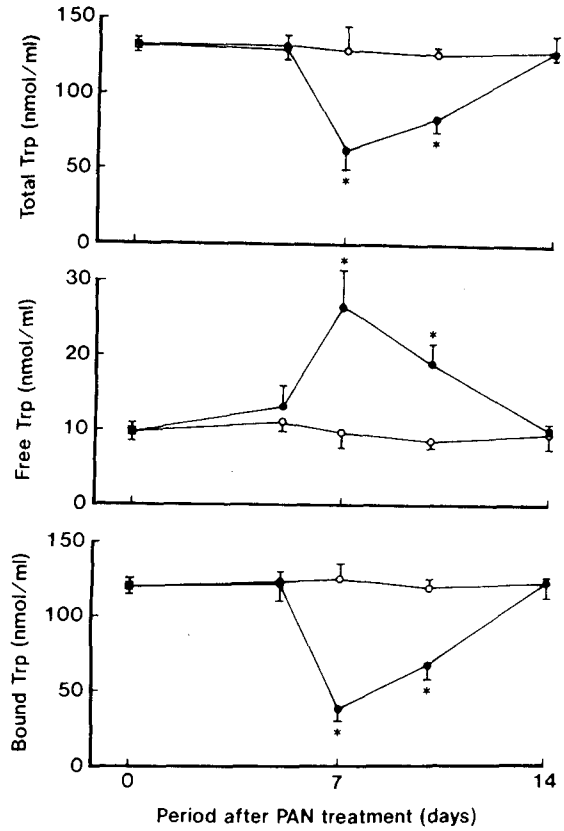

Fig. 2. Changes in levels of total L-tryptophan (*Trp*), free Trp, and albumin-bound Trp in the serum of rats after a single injection of puromycin aminonucleoside (*PAN*). Rats were intraperitoneally injected with PAN (100 mg/kg body weight) dissolved in 0.85% NaCl. The control rats were given intraperitoneally with an equal volume of 0.85% NaCl. Total Trp, free Trp, and albumin-bound Trp in the serum were determined at 0, 5, 7, 10, and 14 days after the PAN treatment as described in Materials and methods. Open circle, control rats; closed circle, PAN-treated rats. Each value is a mean ± SD (n = 4–6). *, $p < 0.001$ (vs. control rats)

Trp, free Trp, and albumin-bound Trp concentrations in the serum at 5 days after the PAN treatment. At 7 days after the PAN treatment, total serum Trp and albumin-bound serum Trp concentrations significantly decreased, while free serum Trp concentration significantly increased. The increase of total serum Trp and albumin-bound serum Trp concentrations and the decrease of free serum Trp concentration returned to the control level thereafter. At 14 days after the PAN treatment, the decreased total serum Trp and albumin-bound serum Trp concentrations and the increased free serum Trp concentration completely recovered up to the control level.

At 7 days after a single injection of PAN at which time an apparent nephrotic syndrome was observed, the levels of 16 serum amino acids including Trp, tyrosine (Try), phenylalanine (Phe), leucine (Leu), isoleucine (Ile), valine (Val), and so on were compared between the PAN-treated and control rats. As shown in Fig. 3, there were no significant differences in the concentra-

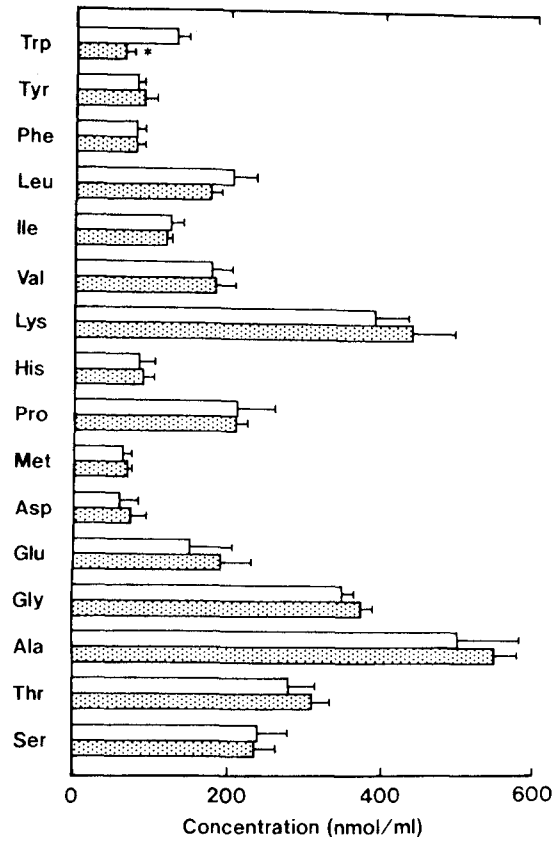

Fig. 3. Levels of serum amino acids in rats with and without nephrotic syndrome induced by puromycin aminonucleoside. At 7 days after a single injection of PAN (100 mg/kg body weight) at which time an apparent nephrotic syndrome was observed, 16 amino acids were determined in the serum of PAN-treated and control rats as described in Materials and methods. Open bar, control rats; dotted bar, PAN-treated rats. Each value is a mean ± SD (n = 4–6). *, $p < 0.001$ (vs. control rats)

Table 1. L-Tryptophan levels in the liver, kidney, and urine of rats with and without nephrotic syndrome at 7 days after a single injection of puromycin aminonucleoside (*PAN*)

Group	Liver (nmol/g tissue)	Kidney (nmol/g tissue)	Urine (nmol/day)
Control rats	45.5 ± 4.4	72.4 ± 13.9	782.5 ± 204.5
PAN-treated rats	68.4 ± 5.1**	107.9 ± 12.2*	732.7 ± 214.7

Trp in the liver, kidney, and urine of rats with and without nephrotic syndrome was determined at 7 days after a single injection of PAN as described under Materials and methods.
Each value is a mean ± SD (n = 5–6). *, $p < 0.01$; **, $p < 0.001$ (vs. control rats).

tions of these serum amino acids between both groups except for Trp. Among serum amino acids studied, only Trp showed a significant decrease in its levels in the PAN-treated rats.

When Trp levels were checked in the liver, kidney, and urine of rats at 7 days after a single injection of PAN at which time an apparent nephrotic syndrome was observed, the levels in the liver, kidney, and urine were as shown in Table 1. The liver and kidney of the PAN-treated group contained significantly more Trp than those of the control group; liver and kidney Trp contents in the former group were 1.5- and 1.3-fold, respectively, more than those in the latter group. There was no difference in urinary Trp levels between the PAN-treated and control groups.

Discussion

In the present study, rats with a single injection of PAN showed an apparent nephrotic syndrome, judging from the levels of urinary protein and serum protein and albumin. In the PAN-treated rats, the levels of total Trp, free Trp, and albumin-bound Trp in the serum were found to change with the development and recovery of nephrosis. Namely, the concentrations of total serum Trp and albumin-bound serum Trp decreased with the development of nephrosis and these decreased levels returned to the normal level with its recovery. In contrast, the concentration of free serum Trp increased with the development of nephrosis and this increased level returned to the normal level with its recovery. Thus, the concentration of total serum Trp decreased with a decrease in the concentration of albumin-bound serum Trp as well as with an increase in the concentration of free serum Trp under nephrotic conditions. Such results were well consistent with the results examined in children with nephrotic syndrome which have been reported by Pirazzoli et al. (1983) and Fydryk et al. (1984). In addition, when the levels of 16 serum amino acids including Trp, Try, Phe, Leu, Ile, Val, and so on were compared between the nephrotic and control rats, only Trp levels were found to change under nephrotic conditions. From these results, it is indicated that the change of serum Trp levels should be closely related to the condition of nephrosis. As to the decrease of albumin-bound serum Trp concentration and the increase of free serum Trp concentration under nephrotic conditions, these changes seem to be due to not only the decrease of serum albumin levels but also the increase of serum NEFA levels. It has been shown *in vitro* and *in vivo* that long-chain fatty acids weaken the binding of Trp to albumin (Curzon et al., 1973; Curzon et al., 1974; Brodersen et al., 1989; Sasaki et al., 1993b). In the PAN-treated rats, serum NEFA levels changed in parallel with the changes of albumin-bound serum Trp and free serum Trp levels. However, Pirazzoli et al. (1983) have reported that plasma NEFA should not be responsible to the increase of free plasma Trp concentration found in children with nephrotic syndrome because there is no significant change in plasma NEFA levels in the nephrotic children.

We have reported that in Nagase analbuminemic rats which are genetically lacking in plasma albumin, Trp present in the serum is taken up by the liver more rapidly than by other tissues such as the kidney, muscle, spleen, and

brain (Sasaki et al., 1991). Trp is known to be the limiting amino acid for synthesis of albumin under some circumstances (Rothschild et al., 1969). It has been demonstrated that in children with nephrotic syndrome, the rate of synthesis of albumin is at the upper limit of the normal range (Gitlin et al., 1959). It has been shown that in rats with PAN nephrosis, plasma albumin catabolism occurs in the kidney (Sellers et al., 1961; Katz et al., 1963; Katz et al., 1964). Pirazzoli et al. (1983) have reported that in children with nephrotic syndrome, Trp levels do not increase in the urine. Therefore, Trp levels were examined in the liver, kidney, and urine of rats with and without PAN nephrosis in order to clarify the fate of Trp lost in the serum under nephrotic conditions. An increase in Trp levels was found in the liver and kidney of the nephrotic rats, but there was no difference in urinary Trp levels between the rats with and without nephrosis, indicating that under nephrotic conditions, Trp lost in the serum is accumulated in the liver and kidney. This accumulation of Trp in the liver under nephrotic conditions seems to be due to the above-mentioned rapid uptake of Trp by the tissue under hypoalbuminemia and to be useful to maintain albumin synthesis in the tissue under nephrotic conditions. However, further investigation is required to elucidate how Trp lost in the serum is accumulated in the liver under nephrotic conditions. The accumulation of Trp in the kidney without its abnormal excretion into the urine under nephrotic conditions suggests that under nephrotic conditions, all of Trp released from albumin excreted into the urine and broken in the kidney is reabsorbed by the glomerulus and that at least a part of the released Trp is retained within the kidney tissue. From this finding, it seems that Trp deficiency hardly occurs under nephrotic conditions, although Fydryk et al. (1984) have supposed that children with refractory nephrotic syndrome and persistent hypoalbuminemia are potentially exposed to Trp deficiency. Thus, one can think that under nephrotic conditions, Trp lost in the serum is retained and utilized within the tissues without its excretion into the urine.

In conclusion, the present results indicate that serum Trp levels change in response to the condition of nephrosis in rats injected once with PAN, that total serum Trp levels decrease with a decrease in albumin-bound serum Trp levels as well as with an increase in free serum Trp levels under nephrotic conditions, and that Trp lost in the serum is accumulated in the liver and kidney without its excretion into the urine under nephrotic conditions.

References

Bender DA (1982) Biochemistry of tryptophan in health and disease. Mol Aspect Med 6: 101–197

Brodersen R, Vorum H, Skribver E, Pederson AO (1989) Serum albumin binding of palmitate and stearate. Multiple binding theory for insoluble ligands. Eur J Biochem 182: 19–25

Curzon G, Friedel J, Knott PJ (1973) The effect of fatty acids on the binding of tryptophan to plasma protein. Nature 242: 198–200

Curzon G, Friedel J, Katamaneni BD, Greenwood MH, Lader MH (1974) Unesterified fatty acids and the binding of tryptophan in human plasma. Clin Sci Mol Med 47: 415–424

Denckla WD, Dewey HK (1967) The determination of tryptophan in plasma, liver, and urine. J Lab Clin Med 69: 160–169
Diamond JR, Karnovsky MJ (1986) Focal and segemental glomerulosclerosis following a single intravenous dose of puromycin aminonucleoside. Am J Pathol 122: 481–487
Doumas BT, Watson A, Biggs HG (1971) Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta 31: 87–96
Frenk S, Antonowicz I, Craig JM, Metcoff J (1955) Experimental nephrosis syndrome induced in rats by aminonucleoside. Renal lesions and body electrolyte composition. Proc Soc Exp Biol Med 89: 424–427
Fuller RW, Roush BW (1973) Binding of tryptophan to plasma proteins in several species. Comp Biochem Physiol 46B: 273–276
Fydryk J, Kurzawska O, Szuba W (1984) Serum tryptophan (Try) level and its binding capacity to albumin in nephrotic syndrome (SN). Int J Pediat Nephrol 5: 124
Gitlin D, Janeway CA, Farr LE (1959) Studies on the metabolism of plasma proteins in the nephrotic syndrome. I. Albumin, γ-globulin and iron-binding globulin. J Clin Invest 35: 44–56
Gornal AG, Bardawill CS, David MM (1949) Determination of serum protein by means of the biuret reaction. J Biol Chem 177: 751–766
Katz J, Bonorris G, Sellers AL (1963) Albumin metabolism in aminonucleoside nephrotic rats. J Lab Clin Med 62: 910–934
Katz J, Sellers AL, Bonorris G (1964) Effect of nephrectomy on plasma albumin catabolism in experimental nephrosis. J Lab Clin Med 63: 680–686
Lowry OH, Rosebrough MJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275
McMenamy RH, Lund CC, Oncley JL (1957) Unbound amino acid concentration in human blood plasmas. J Clin Invest 36: 1672–1679
McMenamy RH, Oncley JL (1958) The specific binding of L-tryptophan to serum albumin. J Biol Chem 233: 1436–1447
Peter T Jr (1985) Serum albumin. Adv Protein Chem 37: 161–245
Pirazzoli G, Casadio L, Rosini R, Gualandi S, Tazzari R, Corsini F (1983) Studio delle correlazioni fra triptofano, NEFA e albumina nella sindrome nefrosica. Boll Soc Ital Biol Sper 59: 1–7
Rothschild MA, Oratz M, Mongelli J, Fishman L, Schreibner SS (1969) Amino acid regulation of albumin synthesis. J Nutr 98: 395–403
Saito K, Sasaki E, Ohta Y, Nagamura Y, Ishiguro I (1986) Mode of L-tryptophan uptake into rat hepatocytes via trypsin-sensitive high-affinity transport system. Biochem Int 13: 873–883
Sasaki E, Saito K, Ohta Y, Ishiguro I, Nagamura Y, Shinohara R, Takahashi H, Tagaya O (1991) Specific binding of L-tryptophan to serum albumin and its function in vivo. Adv Exp Med Biol 294: 611–614
Sasaki E, Ohta Y, Shinohara R, Ishiguro I (1993a) Effect of albumin on the disappearance of L-tryptophan from the perfusate into isolated perfused rat livers. J Clin Biochem Nutr 15: 185–194
Sasaki E, Ohta Y, Shinohara R, Ishiguro I (1993b) Effect of serum fatty acid levels on disappearance of blood L-tryptophan in rats fed lard. J Jpn Soc Nutr Food Sci (Nippon Eiyo Shokuryo Gakkaishi) 46: 487–493
Sellers AL, Katz J, Rosenfeld S (1961) Plasma albumin catabolism in experimental nephrosis. Nature 192: 562–563

Authors' address: Y. Ohta, Ph.D., Department of Biochemistry, School of Medicine, Fujita Health University, Toyoake, Aichi 470-11, Japan.

Received May 10, 1996