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Serum and urinary arginine-esterase activity in paediatric kidney diseases

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Serum and urinary kallikrein activities were determined from the arginine-esterase activities in various groups of kidney diseases and were compared with urinary β-glucuronidase excretion, urinary output, urinary protein content and creatinine clearance. Serum arginine-esterase activity was significantly augmented in the active stage of diffuse renal diseases but was not related to the severity of parenchymal damage. The values improved during remission; the enzyme activity in chronic uraemic patient was as low as in the control sera. There was a positive correlation between urinary output and serum arginine-esterase activity, and consequently serum kallikrein might have an enhancing effect on diuresis.

Besides other physiological and pathophysiological effects, the bradykinin released under the action of kallikrein induces vasodilation due to prostaglandin (PG) E_2 production in the kidney [8, 15] and to stimulation of renal blood circulation (RBF) [14]. This may play an important role in the improvement of flow in the ischaemic kidney [1]. Further, the PG formed in response to kallikrein stimulates the release of renin, which inhibits the effect of kallikrein [21]. Bradykinin decreases the reabsorption of sodium and water by the proximal tubules [14]. Kallen and Lee [10] observed diminished prekallikrein and kallikrein inhibitor activities in the plasma of patients with lipid nephrosis; this became normal following remission or the completion of steroid treatment. Accordingly, it was assumed that the plasma kinin generation system is activated during the disease and that this may play a part in the augmentation of glomerular capillary permeability.

The plasma and tissue (pancreas, salivary gland, intestine, kidney) kallikreins have an esterolytic effect which is directed primarily against the arginine esters. Other proteolytic substances in the serum have similar activities [3, 22]. It has, however, been shown by numerous studies that there is a close correlation between the experimentally measured non-specific esterase activity and the specific kallikrein activity, and thus arginine-esterase activity may be regarded as a measure of the kinin-forming activity of substances acting in a kallikrein-like way [14, 20, 22].

We have determined the serum and urinary arginine-esterase activity in various groups of kidney diseases in order to be able to compare the kallikrein activities. Correlations were examined between these enzyme activities, the urinary out-

put, and the urinary β-glucuronidase concentration [9], the latter being an indicator of the impairment of tubular function. Further, in the glomerulopathic cases a study was made of whether there was a correlation between the urinary protein and creatinine clearance levels and the serum and urinary arginine-esterase activities.

Patients and Methods

Examinations were made in 57 children with kidney diseases and 17 control children free from kidney disease, ranging in age from 2 to 15 years. Gastroenterological, cardiological, pulmonological, neurological and haematological diseases exerting pathological effects on the kallikrein-kinin system could be excluded in all 74 cases.

The nephrological cases were distributed as follows: glomerulopathy, 20 (nephrosis syndrome 14, chronic glomerulonephritis 6); pyelonephritis, 25 (accompanied by morphological disorder, 10; pyelonephritis occurring independently 15); chronic uraemia, 8; nephrogenic diabetes insipidus, 4. Of the children with glomerulopathies, 17 were at the beginning of the disease or of recurrence and they had not taken drugs, while in 3 chronically treated cases the therapy (furosemide, spironolactone) was suspended 4 days before determination of the esterase activity. Similarly, the other patients and the controls were not receiving drugs at the time of the examination.

In the nephrological cases, serum and urinary total protein and renal function tests (serum creatinine, urea N, uric acid, K^+, Na^+, Cl^-, creatinine clearance, urinary output) were performed, and the concentrating ability was determined with the DDAVP test [23] (with exception of the chronic uraemic patients). In the 4 uraemic cases participating in a haemodialysis programme, only the serum values were controlled. In 14 glomerulopathy patients, histologic examinations were made of renal biopsy material. Anatomical abnormalities were established by isotope and radiological examination.

Kallikrein activity was measured in terms of the arginine-esterase activity of the immediately separated serum and in the 24-hour urine towards alpha-benzoyl-L-arginine ethyl ester (BAEE, Sigma). Arginine-esterase activity was determined in barbiturate buffer pH 7.8 after incubation at 37 °C for 1 hour. The colorimetric procedure of Brown [5] was used; the concentration of substrate remaining after hydrolysis being mesured with alkaline hydroxylamine and acidic ferric chloride. Acetone activation and specific inhibitors were not applied. The results were expressed on the basis of the quantity of hydrolysed substrate, in mmol/hour for urine, and in μmol/ml sample volume/hour for serum. Serum arginine-esterase activity was determined in every case, while the urinary activity in 15 controls, 20 glomerulopathy, 15 pyelonephritis, 4 uraemia and 3 nephrogenic diabetes insipidus patients. Urinary β-glucuronidase was determined with phenolphthalein glucuronide as substrate [3] in the glomerulopathy, pyelonephritis and uraemia patients and the control children. Enzyme excretion was expressed in enzyme units/24 hours. The arginine-esterase and β-glucuronidase results for the individual groups were compared by means of the two-tailed t test. Additionally, a two-variable regression method was used to examine the correlation between the arginine-esterase activities of serum and urine taken from the patients on the same day and, in the glomerulopathy group, between these and the urinary protein and creatinine clearance levels. Statistical comparisons were also made between the serum kallikrein and urinary β-glucuronidase values.

In the 14 nephrosis syndrome patients, the changes in serum esterase activity were followed as the disease progressed and the urinary protein decreased. Tests were carried out on 4 occasions per patient on the average. The two-variable regression method was likewise employed to study the correlation between serum arginine-esterase activity and urinary output measured on the same day in 20 glomerulopathy cases, 8 pyelonephritis cases accompanied by an anatomical disorder, and 8 independent pyelonephritis cases. During urine collection, care was taken that the children's fluid intake should meet the requirements, and that overhydration and dehydration should not occur.

Results

Table I presents the serum and urinary arginine-esterase activity in the various groups. The serum levels

TABLE I

Comparison of serum and urinary arginine-esterase activity in the patient groups

	Serum arginine-esterase activity μmol/ml/h			Urinary arginine-esterase activity mmol/h		
	n	mean	±S.D.	n	mean	range
Control	17	31.2	10.2	15	Ø	
Glomerulopathy	20	67.4	15.6	20	10.3	0–78.8
Pyelonephritis	25	65.3	12.9	15	6.9	0–57.0
Nephrogenic diabetes insipidus	4	75.6	10.9	3	41.6	13.2–70.5
Uraemia	8	30.2	12.2	4	6.5	0–20.9

were practically the same in the controls and the chronic uraemia group, while the kallikrein activity of the serum in the glomerulopathy and pyelonephritis patients were significantly higher ($p < 0.001$). A statistically significant difference was not observed between the latter two groups from this respect. Nor was there a significant difference between the results for the cases of pyelonephritis accompanied by an anatomical disorder and of pyelonephritis alone. Significance calculations were not performed for the data for the nephrogenic diabetes insipidus patients because of the low number of cases, but even so it was clear that higher results than for the controls were obtained in this group, too. Urinary arginine-esterase activity, with the exception of the controls showed a wide scatter. For several patients the activity was found to be zero, but the mean for the patient groups was higher than for the controls. No significant difference was found between the various kidney disease groups. A close correlation could not be detected between serum urinary arginine-esterase activities in the patients (see Table I).

In the glomerulopathy group there was no correlation between creatinine clearance (98.2 ± 36.1 ml/min/1.7 m²) and serum and urinary arginine-esterase activity. The correlation coefficient between the activities and the urinary protein level was also low ($r = 0.2$), but considerable activity was found in the urine of 14 of the 16 proteinuria cases ($\bar{x} = 12.8$ mmole/litre/hour), whereas the urinary arginine-esterase level for the patients without proteinuria was very low or zero. At the same time, there was no essential difference between the two subgroups as regards serum arginine-esterase activity. The appearance of microscopic haematuria and the augmented urinary arginine-esterase activity were independent of each other.

Figure 1 compares the serum arginine-esterase activity and urinary glucuronidase level in the glomerulo-

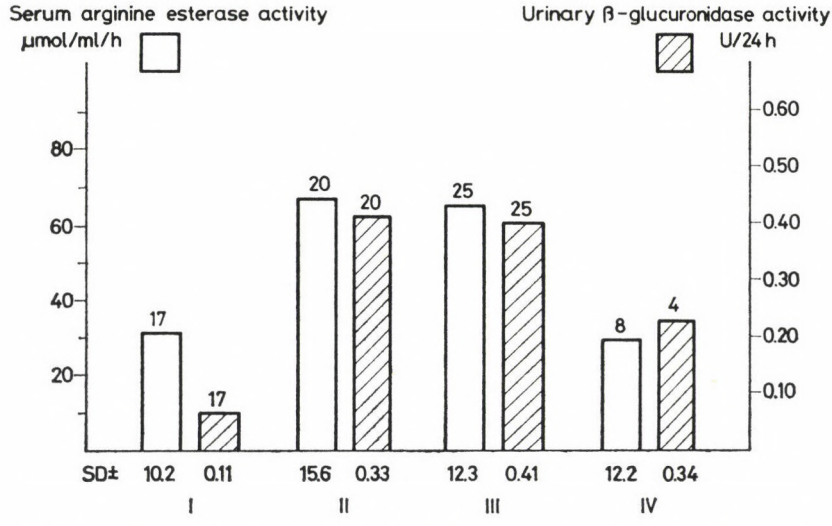

FIG. 1. Serum arginine-esterase and urinary β-glucuronidase activity in the various groups I: control; II: glomerulonephritis; III: pyelonephritis; IV: uraemia

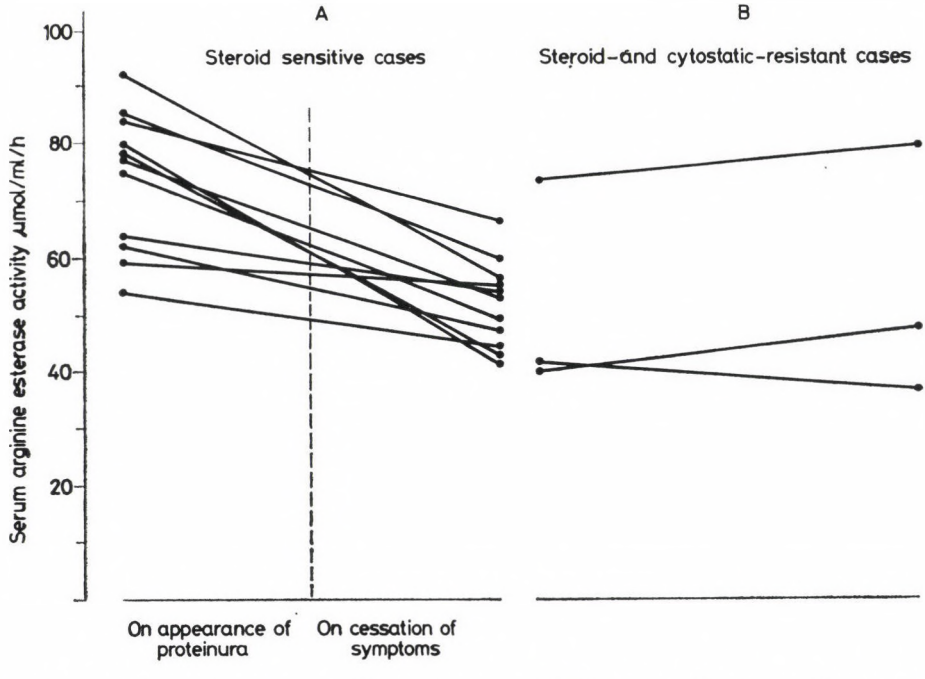

FIG. 2. Variation of serum arginine-esterase activities of nephrosis syndrome cases during the course of the disease

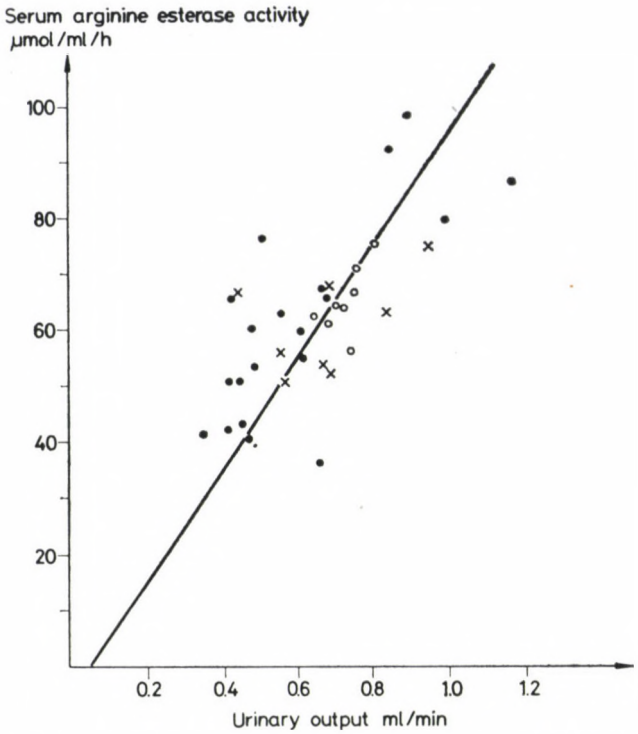

FIG. 3. Comparison of serum arginine-esterase activities and urinary outputs. Symbols: o glomerulopathy; x pyelonephritis; o pyelonephritis + anatomical disorder

pathy, pyelonephritis and chronic uraemia groups and the controls. Similarly to serum arginine-esterase activity, urinary β-glucuronidase in the glomerulopathy and pyelonephritis patients was significantly higher than in the controls ($p < 0.05$). The number of uraemia cases was too low for statistical calculations, but even so their urinary β-glucuronidase level was lower than the mean for the other patients, and higher than those for the controls. In spite of the difference between the groups, the two-variable regression did not reveal a correlation between the serum arginine-esterase and the urinary β-glucuronidase activities (Fig. 1).

Figure 2 illustrates the changes in serum arginine-esterase activity during the disease in 11 nephrosis patients who became symptom-free (2/A). The results for the same interval are also given for the serum of the 3 steroid- and cytostatic-resistant patients (2/B). It may be observed that the serum arginine-esterase activity had fallen considerably by the time the proteinuria was eliminated, though then it was still several times higher than the control level. In contrast, the activity was increased in 2 of the therapy-resistant patients, and only minimally decreased in the third case. In this latter group, the histological picture for the 2 patients with

low arginine-esterase activity corresponded to membranoproliferative glomerulonephritis (Fig. 2).

Figure 3 shows the correlation between the serum and urinary arginine-esterase activities in 20 glomerulopathy patients, 8 pyelonephritis cases accompanied by anatomical disorders, and 8 independent pyelonephritis cases. The correlation between the two was a close one ($r = 0.76$; $p < 0.001$) (Fig. 3). A similar correlation was not observed between urinary arginine-esterase and urinary output.

Discussion

Carvounis et al [7] stated that the kallikrein-kinin system is a modulator of the stimulatory effect of vasopressin on water reabsorption and salt transport. It was assumed by Carretero et al [6] that the kinins directly inhibit distal nephron sodium and water reabsorption, and also alter the peritubular Starling forces and the blood flow distribution. Nasjletti and Colina-Chourio [16] reported on a positive correlation between the rise of the blood kinin level in response to kininase II inhibitor and the enhancement of sodium excretion, the quantity of urine and the RBF. Tissue kallikrein can also be detected in the plasma [17]. Carretero et al [6] confirmed that the increase of serum kinin due to expansion of the extracellular volume has a renal origin. Besides the parallel changes in the blood kinins and the RBF, a direct correlation has been demonstrated between RBF and urinary kallikrein-esterase activity [13]. Under experimental conditions, constriction of the renal artery lowered kallikrein secretion [4, 11]. Adetuyibi and Mills [2] found a positive correlation between urinary volume and sodium and kallikrein excretion. Although this was not confirmed by other investigators [14, 24], Levy et al [12] observed an increase in kallikrein excretion when water-loading and a chronic restriction of sodium intake were applied. There are data that the increased diuresis is associated with a secondarily enhanced urinary kallikrein excretion [13]; from this it has been assumed that this was the result of a renal kallikrein wash-out effect [19].

Numerous kallikrein inhibitors are to be found in serum and urine, and in the enzyme-inhibitor complex the enzymatic activity is totally or partially eliminated [3]. Thus, the present results only permit conclusions to the activity of the spontaneously activating substances functioning similarly as kallikrein. In the measurement of arginine-esterase activity, the plasma kallikrein and that originating in the tissues may be separated by means of specific inhibitors applied in vitro [3]; this, however, was not done in the present investigations.

Spironolactone and indomethacin, together with a low sodium intake, reduce the urinary excretion of kallikrein, whereas furosemide increases its excretion. Following the remission attained by steroid treatment in nephrosis patients, the prekallikrein

and kallikrein inhibitor activities are elevated. Accordingly, in order to avoid these drug effects, our examinations were made before beginning the therapy or during its temporary suspension. Since kallikrein activity changes if the other parenchymal organs (pancreas, salivary glands, liver and brain), the intestinal mucosa, the lung (in allergic conditions) or the ischaemic heart are damaged, or if malignant haematological diseases are present and this may affect the esterolytic activity of the serum, our method cannot be regarded as specific for renal diseases. This is why the patients chosen for the examinations had to be free from gastroenterological, heart, lung, central nervous system and malignant haematological diseases.

In the nephrosis and glomerulonephritis patients, a significantly increased kallikrein activity was observed at the beginning of the disease, in accordance with the decrease in plasma prekallikrein and kallikrein inhibitor activity, demonstrated by Kallen and Lee [10]. Kallikrein activity was considerably decreased following elimination of the proteinuria and the completion of steroid treatment. In the tubulopathy and pyelonephritis patients with or without anatomical disorders the serum arginine-esterase activity was similarly high. In diffuse kidney diseases, therefore, an increased serum kallikrein activity may be detected independently of the type of lesion. An exception from this is chronic uraemia where the significantly lower values point to advanced renal damage or an augmented renin effect. The significance of renal kallikrein and especially of plasma kallikrein in raising the serum kallikrein activity is not known. As there was no correlation between serum arginine-esterase activity and the degree of glucuronidase excretion indicating a damage to the tubular system, the kallikrein detected indirectly in serum is probably not a locally released product of the inflammatory process but the result of compensation. This is indicated by the fact that the serum arginine-esterase activity was high in the nephrogenic diabetes insipidus patients, and also by the absence of a correlation between the creatinine clearance and serum esterase levels in the glomerulopathy patients. In the glomerulopathy, pyelonephritis and hydronephrosis patients, the tubular atrophy was probably a consequence of the reduced blood flow of the peritubular capillaries; this would damage the tubular epithelial cells and give rise to elevated glucuronidase excretion.

Under pathological conditions, apart from kallikrein, other enzymes with esterase effect, primarily urokinase [17] and other kininases and kinase inhibitors [18] are excreted with urine. Accordingly, in renal diseases, from the esterase assay conclusions cannot be drawn to the urinary kinin-forming capacity and kallikrein excretion. This is probably the explanation why there was such wide scatter in the results, and that no correlation could be demonstrated between urinary arginine-esterase ac-

tivity and urinary output or protein level. There is no doubt that the urine of the vast majority of the children with proteinuria exhibited a considerable arginine-esterase activity compared to the cases in remission in the glomerulopathy group, but a similar difference could also be observed in the other renal patients not excreting protein. The results of animal experiments also suggest that there is no correlation between the urinary protein level and arginine-esterase activity, even when the urinary urokinase activity too was taken into consideration, i.e. the true tissue kallikrein effect was determined after specific aprotinin inhibition [17]. In the patients with glomerulopathy, pyelonephritis, and in the group of urinary tract infections accompanied by anatomical disorders, a positive correlation was demonstrated between serum arginine-esterase activity and urinary output. This, however, probably holds only in the normally hydrated cases. The possibility arises that the higher fluid intake acting as a cause of the enhanced diuresis leads simultaneously to an elevated serum kallikrein activity. This assumption, however, is contradicted by the low arginine-esterase activity for the normally hydrated control cases. Thus, the primary role of the augmented enzyme activity appears probable in renal disease; this would improve the renal function partly by increasing the blood supply, and partly by inhibiting tubular water reabsorption [6, 7, 16]. Further work is necessary to study the interaction between the tissue and plasma kinins and to clarify the effects of drugs (steroids, diuretics) used in treatment.

The present results suggest that serum kallikrein activity is augmented in the active stage of diffuse renal diseases. In remission, the values are considerably lower, and in chronic uraemia they are low. From the correlation between urinary output and serum kallikrein activity measured at normal hydration it seems that the kallikrein measured in the serum has a diuresis stimulating effect. Comparison with renal function values indicates, however, that the increase in serum kallikrein activity is not a direct consequence of the inflammatory parenchymal damage but a result of some unknown compensatory process.

References

1. Abe K, Imai Y, Sato M, Haruyama T, Sato K, Hiwatari M, Kasai Y, Itoh S, Yasujima M, Seino M, Yoshinaga K: Exaggerated fractional sodium excretion in hypertension with advanced renal disease: the role of renal prostaglandin and kallikrein. Clin Sci 61:327, 1981
2. Adetuyibi B, Mills IH: Relation between urinary kallikrein and renal function, hypertension and excretion of sodium and water in man. Lancet 2:203, 1972
3. Bermeyer HU: Methods of Enzymatic Analysis Vol. 2 pp 930. Academic Press, New York 1974
4. Bevan DR, McFarlane NAA, Mills IH: The dependence of urinary kallikrein excretion on renal artery pressure. J. Physiol (Lond) 241:34, 1974
5. Brown ME: The colorimetric determination of arginine ester hydrolysis by human sera. J Lab Clin Med 55:616, 1960
6. Carretero OA, Scicli AG: Renal kalli-

krein: its localization and possible role in renal function. Fed Proc 35:194, 1976
7. Carvounis CP, Carvounis G, Arbeit LA: Role of the endogenous kallikrein-kinin system in modulating vasopressin-stimulated water flow and urea permeability in the toad urinary bladder. J Clin Invest 67:1792, 1981
8. Erdős EG: Commentary: the kinins, a status report. Biochem Pharmacol 25:1563, 1976
9. Gonick HC, Kramer HJ, Scharpiro AE: Urinary β glucuronidase activity in renal disease. Arch Intern Med 132: 63, 1973
10. Kallen RJ, Lee S: A study of the plasma kinin generating system in children with the minimal lesion, idiopathic nephrotic syndrome. Pediatr Res 9:705, 1975
11. Keiser HR, Andrews MJ Jr, Guyton RA: Urinary kallikrein in dogs with constriction of one renal artery. Proc Soc Exp Biol Med 151:53, 1976
12. Levy SB, Frigon RP, Stone RA: The relationship of urinary kallikrein activity to renal salt and water excretion. Clin Sci Molec Med 54:39, 1978
13. Levy SB, Lilley JJ, Frigon RP: Urinary kallikrein and plasma renin activity as determinants of renal blood flow. J Clin Invest 60:129, 1977
14. Margolius HS, Buse JB: The renal kallikrein-kinin system. In: Brenner BM, Stein JH eds: Hormonal function and the kidney. p. 115. Churchill Livingstone Edinburgh 1979
15. McGiff JC: Release of a prostaglandin E-like substance from canine kidney by bradykinin. Circ Res 31:36, 1972
16. Nasjletti A, Colina-Chourio J: Interaction of mineralocorticoids, renal prostaglandins and the renal kallikrein-kinin system. Fed Proc 35:189, 1976
17. Rabito SF: Glandular kallikrein in plasma and urine: Evaluation of a direct RIA for its determination. In: Fujii S, Moriya H, Suzuki T eds: Kinins II. Biochemistry, pathophysiology and clinical aspects, p 127. Plenum Press, New York 1978
18. Ryan JW: Components of the kallikrein-kinin system in urine. In: Fujii S, Moriya H, Suzuki T eds: Kinins II. Biochemistry, pathophysiology and clinical aspects, p 313. Plenum Press, New York 1978
19. Seeber AM, Vila SB, Catanzaro OL: The mechanism of urinary kallikrein excretion in the rat. Clin Sci 63:217, 1982
20. Shimamoto K, Chao J, Margolius S: The radioimmunoassay of human urinary kallikrein and comparison with kallikrein activity measurements. J Clin Endocrinol Metab 51:840, 1980
21. Suzuki S, Franco-Saenz R, Tan SY, Mulrow PJ: Direct action of rat urinary kallikrein on rat kidney to release renin. J Clin Invest 66:757, 1980
22. Trautschold I: Assay methods in the kinin system. In: Erdős EG ed: Bradykinin, kallidin and kallikrein. Handbuch der experimentellen Pharmakologie Vol 25, p 53. Springer Verlag, Berlin 1970
23. Turi S, Ormos J, Sztriha L: Urinary osmolality in late stage nephritis and nephrosis. In: Bulla M ed: Renal insufficiency in children. Springer Verlag, Berlin 1982
24. Zinner SH, Martin LF, Sacks F: A longitudinal study of blood pressure in children. Am J Epidemiol 100:437, 1975

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