of the dormant (winter) period. Phenol content was consistently less in diseased cuttings, and the difference in the phenol content between healthy and diseased stem cuttings was especially prominent in September, October and January. The phenol content in healthy stem cuttings was only marginally higher than in virus-infected stem cuttings in April, May and June, when active vegetative growth takes place.

The abscisic acid-like activity in healthy cuttings was high in November and January (the dormant period of the plant), but it decreased sharply in February before the buds resumed growth in March, and then it remained almost constant until May. The abscisic acid-like activity in diseased cuttings was also high in November and January, it decreased sharply in February and then it decreased still more in May. The abscisic acid-like activity in diseased cuttings was always less than in the healthy cuttings in all the months; but the decrease in the dormant period (November, January and February) of the plant was more than in other months, and was thus particularly significant.

It is clear from this that the amount of growth inhibitors (phenols and abscisic acid) present in diseased stem cuttings is much less than in healthy cuttings during the dormant period of the plant. This accounts for the break in bud dormancy in diseased cuttings. This conclusion is in agreement with the observation of other workers that there is an increase in growth inhibitors during the dormant period and a decrease during the growing period[7-9].

1 A. Gulati, Thesis, Panjab University, Chandigarh, India 1976.
2 C.F. Eagles and P.F. Wareing, Nature *199*, 874 (1963).
3 P.F. Wareing and P.F. Saunders, A. Rev. Pl. Physiol. *22*, 261 (1971).
4 H.G. Bray and W.V. Thorpe, in: Method of Biochemical Analysis, p. 27, Ed. D. Glick. Interscience Publ. Inc., New York 1954.
5 O. Rasmussen, Physiol. Pl. *36*, 208 (1976).
6 O.M.K. Gabr and C.G. Guttridge, Planta *78*, 305 (1968).
7 Y. Tsukamoto and H. Konoshima, Physiologia Pl. *26*, 244 (1972).
8 I.D. Railton and P.F. Wareing, Planta *112*, 65 (1973).
9 A. Alpi, N. Ceccarelli, F. Tognoni and G. Gregorini, Physiologia Pl. *36*, 362 (1976).

## Chemical characterization of human urine albumin in proteinuria[1]

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*Summary.* From the urine of a patient with proteinuria, the albumin protein component was isolated and compared with human serum albumin. By comparing the amino acid composition of the original proteins and their large cyanogen bromide fragments, peptide maps and N-terminal sequences of 33 amino acid residues, the identity of both proteins was shown.

As obvious from the published papers[2-5], the pathway of protein molecules through the blood-urine barrier is quite complicated and a large part of the proteins from the primary urine is reabsorbed by the cells of the tubuli and catabolized. In order to elucidate the mechanism of protein transfer from serum to urine at pathological states of the kidneys, our attention was primarily focussed on albumin. The determination of the complete structure[6,7] of human serum albumin (HSA), the arrangement of its disulfide bonds[8], together with progress in understanding of its molecular architecture and biosynthesis[9] have made it possible to examine the question of identity of human urine albumin (HUA) and serum albumin.

*Material and methods.* The albumin fraction was isolated from the urine of a patient with chronical glomerulonephritis. After dialysis of the urine for 24 h against distilled water at 24 °C, the dialysate was lyophylized and redissolved in a sodium chloride physiological solution (concentration of protein 5 g/l). HUA was isolated by alcohol fractionation according to Cohn[10], the comparison was made with HSA from ÚSOL (Prague, Czechoslovakia) by electrophoresis in polyacrylamide gel[11] and immunoelectrophoresis[12]. The amino acid analysis of HUA (one determination) was performed after oxidation of the sample by performic acid[13] and hydrolysis by 6M-HCl for 20 h at 110 °C by the method of Spackman et al.[14]. The N-terminal sequence of HUA was determined by stepwise degradation[15] in Beckman Model 890C Sequencer using the 'Fast Quadrol Program' recommended by the manufacturer. Using the same procedure as in previous studies[16,17], we isolated from the

Table 1. Amino acid composition of human urine albumin (HUA) and human serum albumin (HSA)

| | Amino acids | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Lys | His | Arg | Cys | Asp | Thr | Ser | Glu | Pro |
| HUA | 54.1 | 15.9 | 23.4 | 32.3 | 53.4 | 29.0 | 23.8 | 79.3 | 26.1 |
| HSA | 59 | 16 | 24 | 35 | 53 | 28 | 24 | 82 | 24 |

| | Amino acids | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Gly | Ala | Val | Met | Ile | Leu | Tyr | Phe | Trp |
| HUA | 16.5 | 56.2 | 38.3 | 6.6 | 9.7 | 61.0 | 18.6 | 31.0 | +* |
| HSA | 12 | 62 | 41 | 6 | 8 | 61 | 18 | 31 | 1 |

* Presence of tryptophan determined by a qualitative test. The analytical values for HUA are not corrected, the calculation is based on the content of 61 residues of leucine. The values for HSA show the number of the amino acid residues, determined in its complete sequence[7].

Fig. 1-4. Peptide maps of tryptic digests. In the left column (A) human serum albumin and its cyanogen bromide fragments, in the right column (B) human urine albumin and its cyanogen bromide fragments. 1A, B: Oxidized albumins; 2A, B: N-terminal fragments; 3A, B: middle fragments; 3A, B: C-terminal fragments. Horizontal direction: electrophoretic separation (pH 5.6, 30 V/cm). Vertical direction: chromatographic separation (n-butanol:pyridine:acetic acid:water 15:10:3:12, v/v).

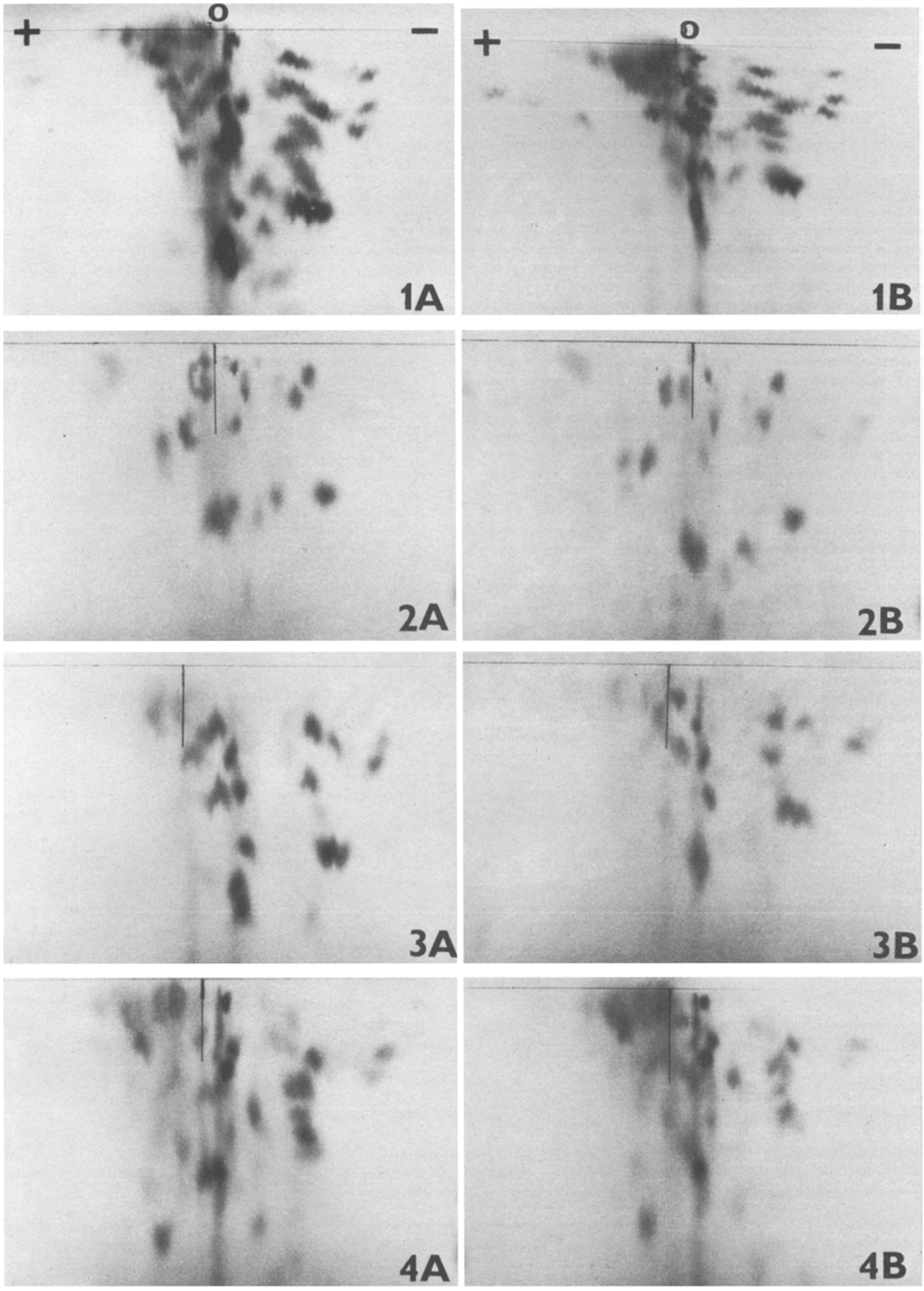
+
O
−
1A
1B
2A
2B
3A
3B
4A
4B

Table 2. Comparison of the N-terminal sequences of human serum albumin (HSA) and human urine albumin (HUA)

| | 1 | 5 | 10 | 15 | 20 | 25 |
|---|---|---|---|---|---|---|
| HSA: | Asp-Ala-His-Lys-Ser-Glu-Val-Ala-His-Arg-Phe-Lys-Asp-Leu-Gly-Glu-Glu-Asn-Phe-Lys-Ala-Leu-Val-Leu-Ile- | | | | | |
| HUA: | *Asp-Ala-His-Lys-Ser-Glu-Val-Ala-His-Arg-Phe-Lys-Asp-Leu-Gly-Glu-Glu-Asn-Phe-Lys-Ala-Leu-Val-Leu-Ile-* | | | | | |
| | (Gly)* | | | (Glu) | (Glu) | |
| | 30 | 33 | | | | |
| HSA: | Ala-Phe-Ala-Gln-Tyr-Leu-Gln-Gln | | | | | |
| HUA: | *Ala-Phe-Ala-Gln-Tyr-Leu-Gln-Gln* | | | | | |

* Data in brackets show very weak admixtures detected in the degradation steps.

were observed in the HUA preparation. The amino acid composition of HUA (table 1) was based on the presence of 61 leucine residues per mole of protein. Differences in the amino acid composition of HUA (table 1) from the values for HSA, in this case largest in the content of lysine, glycine and alanine, had been observed to a certain extent even with individual analyses of various HSA preparations. The amino acid composition of HUA is very close to that of HSA. HUA was subjected to stepwise degradation in a sequencer (table 2). Its N-terminal sequence of 33 residues does not show any differences from the N-terminal region of HSA[7]. By cyanogen bromide cleavage of the native proteins with intact disulfide bonds, in both cases 3 corresponding fragments were obtained. We marked them similarly as in the previous studies[16,17] as fragments N, M and C (N-terminal, middle and C-terminal fragment). The distribution of the fragments in the elution diagrams is identical for both proteins and reveals identity in positions of the methionine residues in both molecules. The peptide maps of both oxidized albumin preparations are practically identical (figures 1A and B) and also the tryptic peptide maps of isolated fragments are very similar (figures 2A and B, to 4A cyanogen bromide digests of native HUA and HSA, in each case 3 fragments (N, M and C), and compared their tryptic peptide maps. Peptide maps were prepared from tryptic digests (1:100, w/w; 16 h at 23 °C) of the oxidized preparations (3 mg) by paper electrophoresis[18] at pH 5.6 (30 V/cm) and chromatography in the system n-butanol:pyridine:acetic acid:water (15:10:3:12, v/v).

*Results and discussion.* The electrophoretic and immunological properties of HSA and HUA were found to be identical. As the only difference, minor additional bands and B). Also the amino acid composition of fragments N, M and C from both proteins does not show significant differences.

The results provide information that the primary structure of urine albumin is at least very close to that of serum albumin and practically exclude the possibility of any sequential differences in the N-terminal region. They permit us to draw the conclusion that albumin after passage through the glomerular barrier does not show any substantial differences in covalent structure from HSA. This conclusion is supported by the observation of Hoffsten et al.[19], that murine albumin from urine has the same mol.wt as albumin isolated from serum, and that by chromatography no fragments of albumin could be detected in the urine.

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2 T. Kavai, Clinical Aspects of the Plasma Proteins, p. 135. Springer-Verlag, Berlin, Heidelberg, New York 1973.
3 U. Ravnskov, Scand. J. Urol. Nephrol. suppl. *20*, 1 (1973).
4 R. Kinne, Naturwissenschaften *64*, 326 (1977).
5 M.G. Menefee and C.B. Meller, in: Ultrastructure of the Kidney, p. 73. Ed. A.J. Dalton and F. Hagenau. Academic Press, New York, London 1967.
6 P.W. Behrens, A.M. Spiekerman and J.R. Brown, Fed. Proc. Fed. Am. Soc. exp. Biol. *34*, 591 (1975) (abstr. 2106).
7 B. Meloun, L. Morávek and V. Kostka, FEBS Lett. *58*, 134 (1975).
8 M.A. Saber, P. Stöckbauer, L. Morávek and B. Meloun, Coll. czech. Chem. Commun. *42*, 564 (1977).
9 T. Peters, Jr, Clin. Chem. *23*, 5 (1977).
10 E.J. Cohn, L.E. Strong, W.L. Hughes, Jr, D.J. Mulford, J.N. Ashworth, M. Melin and H.L. Taylor, J. Am. chem. Soc. *68*, 459 (1946).
11 B.J. Davies, Ann. N.Y. Acad. Sci. *121*, 404 (1964).
12 J.J. Scheidegger, Int. Arch. Allergy appl. Immun. *7*, 103 (1955).
13 S. Moore, J. biol. Chem. *283*, 235 (1963).
14 D.H. Spackman, W.H. Stein and S. Moore, Analyt. Chem. *30*, 1190 (1958).
15 P. Edman and A. Henschen, in: Protein Sequence Determination, p. 232. Ed. S.B. Needleman. Springer-Verlag, Berlin, Heidelberg, New York 1975.
16 B. Meloun and J. Kušnír, Coll. czech. Chem. Commun. *37*, 2812 (1972).
17 B. Meloun, M.A. Saber and J. Kušnír, Biochim. biophys. Acta *393*, 505 (1975).
18 O. Mikeš, Coll. czech. Chem. Commun. *22*, 831 (1957).
19 P.E. Hoffsten, C. L. Hill and S. Klahr, J. Lab. clin. Med. *86*, 920 (1975).

## Stimulation of *Dugesia tigrina* auricle regeneration by exogenous putrescine, spermine or spermidine

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*Summary.* Specimens of a flatworm, *Dugesia tigrina* were decapitated and then cultured in a solution of $1 \times 10^{-4}$ M putrescine, spermine or spermidine. Subsequent observation for the reappearance of auricles indicates that the amine treatment stimulates the flatworm regeneration process.

An increased synthesis of the diamine putrescine and the polyamines, spermine and spermidine, has been shown to occur in a variety of systems undergoing growth, such as rat liver regeneration[1], embryonic development[2] and mammalian cells induced to proliferate in vitro[3]. Studies involving exogenous application of these amines indicate that they are necessary for cell proliferation. For example, Pohjanpelto and Raina[4] have presented evidence that putrescine