freeze-dried immediately after elution; the resulting powder can be kept at freezer temperature for several months.

Purified antidiuretic hormone (vasopressin) can thus easily be obtained from commercial posterior pituitary extract by the use of preparative high voltage electrophoresis. The resulting product appears to be chemically identical to the synthetic hormone.

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Electrophoretic behaviour of copper(II) histidinate in the presence of the other copper(II) amino acid chelates

Recently[1] we studied the electrophoretic behaviour of copper(II) amino acid chelates at different pH values. We noticed that complexes of dicarboxylic amino acids and histidine behaved somewhat unusually. Splitting of the former into two distinctive Cu^{2+} positive spots may be due to the structure of these complexes, as suggested by PFEIFFER AND WERNER[2]. On the other hand at pH 11.98 copper(II) histidinate yields two spots, one of which moves to the anode and the second to the cathode. WIELAND AND FISCHER[3], in 1948, also observed the somewhat peculiar behaviour of copper(II) histidinate in the presence of copper(II) lysinate, *viz.* the formation of a third spot during the electrophoretic separation of the two complexes. They suggested that this third spot was the mixed complex copper(II) histidino–lysinate. Owing to the fact that coordination of histidine with Cu^{2+} ions is unusual (see for example references 4, 6, 8) and because the kinetic lability of copper(II) amino acid complexes in aqueous solution restricts the formation of mixed amino acid complexes, the authors have studied the electrophoretic behaviour of copper(II) histidinate in the presence of the other chelates more extensively.

Thus we found that the existence of the third spot described by WIELAND AND FISCHER is observable only in the case of copper complexes of basic amino acids (with the exception of α,β-diaminopropionic and α,γ-diaminobutyric acids owing

to their low mobilities). A similar effect with Cd^{2+}, Ni^{2+} and Zn^{2+} complexes was not observed. Nor did the third spot form when copper(II) complexes of basic amino acids were separated from each other.

Quite different results were obtained when the separation of a mixture of complexes and free amino acids was made. In the first case, when a mixture of copper(II) complexes of basic amino acid and L-histidine was separated, we detected on the electropherogram the copper complex of the amino acid and copper(II) histidinate only. On the other hand, mixtures of copper(II) histidinate and basic amino acid after separation yield a third spot as well as copper complexes of both histidine and the basic amino acid (see Table I).

TABLE I

Separation of pairs	*Mobilities ($cm^2/V \cdot cm$)*		
	Cu^{2+}-histidinate	*Third spot*	*Other part of pair*
Cu^{2+}-histidinate + Cu^{2+}-ornithinate	$0.51 \cdot 10^{-4}$	$1.11 \cdot 10^{-4}$	Cu^{2+}-ornithinate $1.66 \cdot 10^{-4}$
Cu^{2+}-histidinate + Cu^{2+}-lysinate	$0.51 \cdot 10^{-4}$	$1.06 \cdot 10^{-4}$	Cu^{2+}-lysinate $1.64 \cdot 10^{-4}$
Cu^{2+}-histidinate + Cu^{2+}-argininate	$0.50 \cdot 10^{-4}$	$0.98 \cdot 10^{-4}$	Cu^{2+}-argininate $1.44 \cdot 10^{-4}$
Cu^{2+}-histidinate + lysine	$0.51 \cdot 10^{-4}$	$1.09 \cdot 10^{-4}$	Cu^{2+}-lysinate $1.64 \cdot 10^{-4}$
Cu^{2+}-lysinate + histidine	$0.50 \cdot 10^{-4}$	—	Cu^{2+}-lysinate $1.62 \cdot 10^{-4}$

Further we found that the phenomenon described is observed only at pH 7.5 and depends on the composition of the buffer used. The concentration of the complex applied and changes in potential gradient have little effect.

In order to investigate the characteristics of the third spot, in all the cases where the formation of this third spot was observed, the latter was eluted from the paper and subjected to electrophoresis under the conditions described. On visualisation (both for Cu^{2+} ions and for the amino acids) the presence of copper(II) histidinate was always demonstrated. This result, in addition to the facts described above, *i.e.* the existence of the third spot only in the case of copper(II) complexes, its formation only in the course of separation of a mixture of copper(II) histidinate and free basic amino acids, but not when free histidine and copper complexes of basic amino acids were separated, and furthermore dependence on the pH value and composition of buffer, suggests that the third spot is more likely to be the result of interaction between copper(II) histidinate and complexes of basic amino acids, in the sense of coulombic attraction, than to be due to the formation of mixed amino acid complexes. Our conclusions are based on the fact that, in all cases, the third spot was only detected as copper(II) histidinate and further on the literature reports on histidine coordination. According to ALBERT[4], the structure of copper(II) histidinate in aqueous solution is unsymmetrical. One molecule of histidine is attached to copper normally, but the second is not attached to the Cu^{2+} through all three histidine sites[5]. The weakly coordinating donor centres (probably carboxylate oxygen, because all nitrogen donor atoms lie in the plane[6]) might be displaced by the water molecule[7]

and that may interact with the free ω-amino group of the copper(II) complex of basic amino acids. Formation of unsymmetrical copper complexes is due to the strong tendency of copper(II) ions to form square planar structures. Furthermore the dependence of the phenomenon observed on the pH value may be caused both by the composition of copper(II) histidinate in aqueous solution as such which changes with the pH value[8] giving rise to flexidentate chelation of histidine, and also by the fact that at pH 7.5 ω-amino groups of copper(II) complexes of basic amino acids have a positive charge.

The electrophoretic mobilities are given in Table I.

Experimental

Copper chelates were prepared from the L-isomers of amino acids according to the method of ABDERHALDEN AND SCHNITZLER[9].

10 μl of an 0.01 *M* solution of chelates were applied on Whatman No. 1. paper. Electrophoresis was carried out in a horizontal Tatrachema apparatus (Č.S.S.R.) under the following conditions: 0.1 *M* CH_3COONa buffer, pH 7.5, ionic strength 0.1; potential gradient 15 V/cm.

The electropherogram was sprayed with a solution of rubeanic acid and ninhydrin, respectively, to detect the spots.

All mobilities given in Table I were corrected for electroosmosis, glucose being used as zero marker.

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