Fixation by CDI must depend on an attack of carboxyl groups, to give an *O*-acylisourea, which can then react with a neighbouring amino group to cross-link through an amide bond[5]. Thus, gelatin is rapidly gelled at neutral pH by addition of CDI[6]. In effect, the cross-link is formed by elimination of a water molecule; no part of the CDI becomes incorporated into the protein in this reaction. Any explanation of the apparent virtues of fixation by CDI for immunohistochemistry remains partially speculative. The adoption of new fixatives must still rest heavily on empirical evaluation, although it is rational to attempt, as in the present work, to diversify the sites of chemical reaction in the tissue.

It was predicted that antibodies to hormone-carrier conjugates prepared with a CDI would bind non-specifically to tissue fixed in the same CDI, since JAFFE et al.[7] observed that such antibodies (from rabbits or goats) had affinity for groups present in the particular CDI. This effect was indeed observed in the case of rabbit antibodies, but was negligible with guinea-pig antibodies. Presumably some haptenic groups arise from CDI molecules which become integrally incorporated into the conjugate, and into the tissue, by rearrangement of unstable *O*-acylisourea to the stable acylurea[5]; this hapten is evidently far less immunogenic in guinea-pigs than in rabbits or goats. Introduction of substituted urea groups by the rearrangement just mentioned would make the tissue more basic, since carboxylate would be replaced by a positive group (one of the substituents in water-soluble CDI is basic). Surprisingly, CDI-fixed material shows little tendency to bind fluorescent globulin non-specifically, a phenomenon frequently attributed to charge interaction[1b], which increases with increasing acidophilia of the tissue.

CDI was inferior to aldehydes for preserving general structure, probably because CDI demands that carboxyl and amino groups be in close proximity for cross-links to be formed. By incorporating some bifunctional nucleophile, e.g. a suitable diamine, in the CDI fixative, cross-linking between tissue carboxyl groups should be greatly favoured. The use of CDI to preserve well both antigenicity and structure at light and electron microscope levels is the subject of current investigation[8].

Experiments performed since this manuscript was submitted suggest that EDAP-CDI may fix antigens more effectively than CME-CDI, which is a much larger molecule. Furthermore, a higher concentration (e.g. 10%) of CDI may be advantageous.

Zusammenfassung. Wasserlösliche Carbodiimide werden als Fixiermittel für immunohistochemische Untersuchungen vorgeschlagen, da sie die Verwendung von Carboxylgruppen bei der Fixierung erlauben.

P. A. KENDALL, JULIA M. POLAK and A. G. E. PEARSE

Department of Histochemistry, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 OHS (England), 16 February 1971.

[5] For a recent summary of reactions of CDI with proteins, see G. R. STARK, Adv. Protein Chem. *24*, 261 (1970).
[6] J. C. SHEEHAN and J. J. HLAVKA, J. Am. chem. Soc. *79*, 4528 (1957).
[7] B. M. JAFFE, W. T. NEWTON and J. E. MCGUIGAN, Immunochemistry *7*, 715 (1970).
[8] This work was supported by grants from the Cancer Research Campaign and the Wellcome Foundation.

CORRIGENDUM

F. PESARO und H. KOBLET: *Reinigung von F-Antigen*, Experientia *27*, p. 235 (1971). Wir verdanken R. UTZINGER und J. LINDENMANN den Hinweis, wonach Lysozym auf Sephadex verzögert eluiert werden kann[1], und zwar vermutlich aufgrund einer Affinität für β-1,4-Bindungen zwischen Hexosen. Somit besteht die Möglichkeit, dass das Molekulargewichts-Aequivalent für F-Antigen höher als 40 000 bis 45 000 ist, was die offensichtliche Diskrepanz zwischen den Bestimmungen mit Sephadex, der Polyacryamidgel-Elektrophorese und der analytischen Ultrazentrifugation zwanglos erklären würde. Molekulargewichts-Aequivalente auf Sephadex sind ohnehin vorsichtig zu interpretieren, solange nichts über die Molekülradien bekannt ist[2].

F. PESARO und H. KOBLET

Institut für Virologie der Universität Zürich, CH-8006 Zürich (Schweiz).

[1] H. M. RAUEN, *Biochemisches Taschenbuch*. 2. Auflage (Springer-Verlag, Berlin 1964), vol. 2, p. 910.
[2] D. RODBARD und A. CHRAMBACH, Proc. natn. Acad. Sci. USA *65*, 970 (1970).

CONGRESSUS

India
8th International Symposium on the Chemistry of Natural Products

in New Delhi, 6–12 February 1972

The Symposium will be devoted mainly to the following topics for which it is proposed to organize separate sections: 1. Alkaloids. 2. Polyphenolics. 3. Terpenoids and steroids. 4. Macromolecules of biological interest (proteins, peptides, nucleic acids, etc.). 5. Carbohydrates, lipids and related substances. 6. Other topics in natural products chemistry including physical methods of structure and determination.

The deadline for sending in abstracts is 1 September 1971. Further information by Prof. S. Rangaswami, Secretary, 8th IUPAC Symposium, Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi 1 (India).