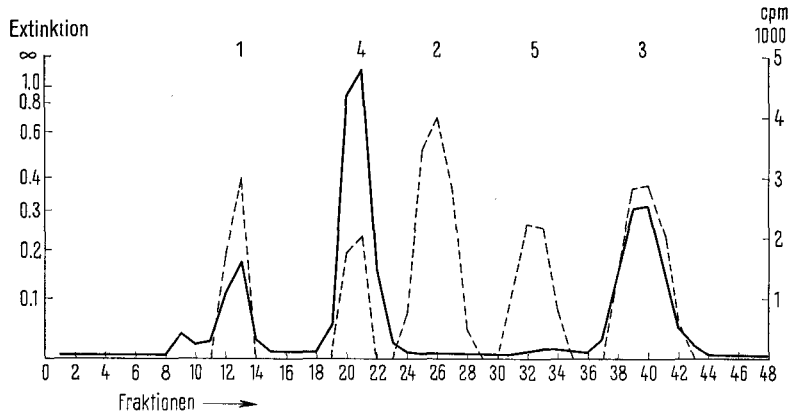

Chromatogramm des aethanolischen Pflanzenextraktes, dem die Verbindungen 1–5 zugesetzt wurden. —— = Radioaktivität aus dem Pflanzenextrakt; ----- = UV-Absorption der vorgelegten Abbauprodukte.

Chromatographische Bedingungen: Durchmesser der Säule 0,9 cm, Höhe der Säule 17,0 cm, Temperatur 60°C (Die Säule war mit einem Heizmantel versehen). Adsorptionsmittel: Dowex 50 W X4 H^+-Form. Das Säulenmaterial wurde vor dem Einfüllen, während 1 h, mit 2 *n* HCl behandelt und anschliessend neutral gewaschen. Eluiermittel: In zwei Bechergläser wurden 250 ml 0,25 *n* HCl und 250 ml 1,00 *n* HCl vorgelegt. Mit Hilfe eines U-Rohres wurden die Gefässe kommunizierend verbunden. Diese Anordnung erlaubte eine Steigerung der Acidität von 0,25 *n* in der ersten Fraktion auf 1,00 *n* in der letzten. Die Durchflussgeschwindigkeit betrug 10 ml während 16 min.

Nach 10 Tagen wurde der grüne Teil lyophilisiert. Der Rückstand wurde 3mal mit Chloroform und mehrmals mit 80prozentigem Äthanol extrahiert.

PLAISTED und THORNTON[5] beschrieben eine Kationentauschersäule, an welcher sich, aus biologischem Material, radioaktive Fraktionen, herrührend von markierten Triazinen, trennen liessen. Der äthanolische Extrakt, gewonnen aus dem Gewebe von *Coix lacryma-jobi* L. wurde, zusammen mit den folgenden möglichen Abbauprodukten[4] des Simazins, an einer Kationentauschersäule vom Typ Dowex 50 W X4 chromatographiert: (1) 2-Hydroxy-4, 6-diamino-*s*-triazin (Ammelin); (2) 2-Hydroxy-4, 6-dimethylamino-*s*-triazin; (3) 2-Hydroxy-4, 6-diaethylamino-*s*-triazin (Hydroxysimazin); (4) 2-Hydroxy-4-aethylamino-6-amino-*s*-triazin; (5) 2-Hydroxy-4-aethylamino-6-methylamino-*s*-triazin. Alle 5 Substanzen weisen, in saurer wässeriger Lösung, eine maximale UV-Absorption zwischen 235–245 mm auf.

Wie die Figur zeigt, wurden die Verbindungen 1, 3 und 4 gleichzeitig mit den radioaktiven Abbauprodukten eluiert. Nach diesem Resultat zu schliessen, vermag die Pflanze Äthylgruppen abzuspalten um so eine Inaktivierung des Wirkstoffs herbeizuführen.

Gleiche Untersuchungen mit der *Graminee Imperata cylindrica* (L) Pal., in welcher kein Benzoxazinon nachweisbar ist, sind vorgesehen[6].

Summary. Coix lacryma-jobi L., about 20 days old and grown on watercultures, were fed with ^{14}C-ring-labelled simazine. After freeze drying, the plant tissue was extracted with chloroform and 80% ethanol. The ethanol extractable activities were fractionated on a cation exchange column. The radioactive metabolites seem to be identical with 2-hydroxy-4, 6-diethylamino-*s*-triazine (hydroxysimazine), 2-hydroxy-4, 6-diamino-*s*-triazine (ammelin) and 2-hydroxy-4-ethylamino-6-amino-*s*-triazine.

J. HURTER

Eidg. Versuchsanstalt für Obst-, Wein- und Gartenbau. Sektion Pflanzenschutz, 8820 Wädenswil (Schweiz), 30. Juni 1966.

[4] ^{14}C-markiertes Simazin, sowie Vergleichssubstanzen wurden uns freundlicherweise von der Firma J. R. GEIGY AG. zur Verfügung gestellt.

[5] P. H. PLAISTED und M. L. THORNTON, Contrib. Boyce Thompson Inst. *22*, 399 (1964).

[6] Der Firma J. R. GEIGY AG, Basel möchten wir für das radioaktiv markierte Simazin, sowie für die Vergleichssubstanzen bestens danken. Unser Dank gilt ebenso Herrn Dr. P. W. MÜLLER von der Firma J. R. GEIGY AG, der uns mit seinen Ratschlägen unterstützte.

Hybrid Formation in Mixtures of Globins from Different Species

Mixtures of different hemoglobins under suitable experimental conditions are known to form hybrids, i.e. molecules which contain α and β chains of the parent hemoglobins, according to the following scheme:

$$\alpha_2^x \beta_2^x + \alpha_2' \beta_2' \rightarrow \alpha_2^x \beta_2' + \alpha_2' \beta_2^x + \alpha_2^x \beta_2^x + \alpha_2' \beta_2' .$$

The mechanism of hybrid formation is still not wholly clear, although it must obviously be associated with the tendency of hemoglobins to dissociate into subunits[1,2].

ANTONINI et al.[1] demonstrated that dissociation of hemoglobin into dimers does not lead to hybrid formation. It seems likely that the hybrids form under conditions in which hemoglobin dissociates into single chains[2].

Hemoglobins at neutral pH form hybrids only if incubated at 37 °C for a very long time[3]. Mixtures of different

[1] E. ANTONINI, J. WYMAN, E. BUCCI, C. FRONTICELLI, and R. ROSSI-FANELLI, J. molec. Biol. *4*, 368 (1962).

[2] A. ROSSI-FANELLI, E. ANTONINI, and A. CAPUTO, Adv. Protein Chem. *19*, 73 (1964).

[3] E. R. HUEHNS, G. H. BEAVEN, and B. L. STEVENS, Biochem. J. *92*, 440 (1964).

globins kept at neutral pH and at 4 °C for a few hours do not show hybrid formation.

In the present work, mixtures of different globins and hemoglobins have been kept in contact for several days at neutral pH and at 4 °C with the aim of establishing whether under these conditions hybrids are formed and whether globins and hemoglobins behave differently.

Materials and methods. Human and canine hemoglobins were prepared with toluene from freshly drawn human adult and canine blood. Globins were prepared according to the method described by ROSSI-FANELLI et al.[4]. Human and canine globins were dissolved in water at a concentration of 10 mg/ml. Aliquots of the globin solutions were mixed, in equal volumes, at 24 h intervals. The pH of the mixture was 7.3 and the ionic strength less than 10^{-3}. Mixtures of oxyhemoglobins in the same conditions were prepared at the same time. All solutions were kept at 4 °C. The starch gel electrophoreses were carried out with the discontinuous buffer system according to POULIK[5].

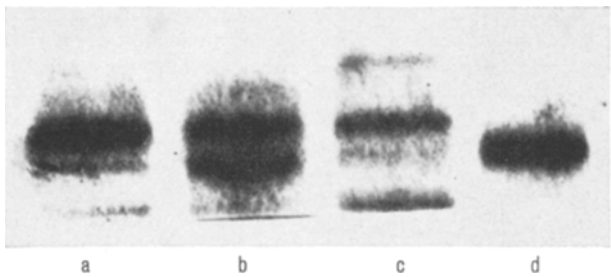

Fig. 1. Starch gel electrophoresis in the discontinuous buffer system according to POULIK[5]. Stained with nigrosine. (a) = HbA globin; (b) = HbA globin + canine Hb globin (6 h after mixing); (c) = HbA globin + canine Hb globin (5 days after mixing); (d) = Canine Hb globin.

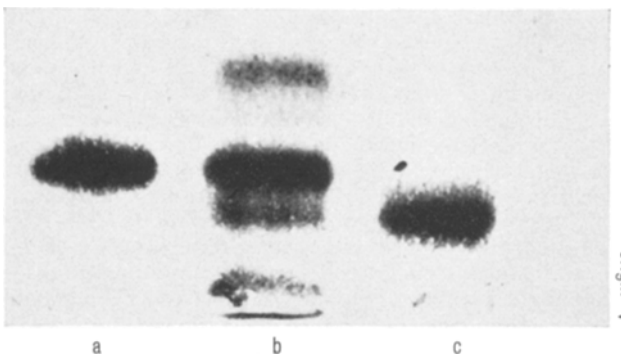

Fig. 2. Starch gel electrophoresis in the discontinuous buffer system according to POULIK[5]. Stained with nigrosine. (a) = HbA globin; (b) = HbA globin + canine Hb globin (7 days after mixing); (c) = Canine Hb globin.

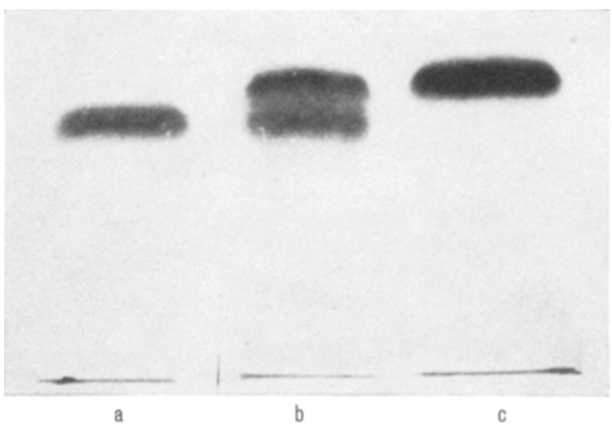

Fig. 3. Starch gel electrophoresis in the discontinuous buffer system according to POULIK[5]. Stained with nigrosine. (a) = Canine Hb; (b) = Canine Hb + HbA (7 days after mixing); (c) = HbA.

Results. The gel electrophoresis experiments show that: (1) The mixture of globins gives 2 bands corresponding to the parent globins in all the samples examined (Figures 1b, 1c, 2b). 2 more bands appear in the electrophoresis carried out 5 days after mixing (Figure 1c), a fast one with a high cathodic mobility and a slow one which remains almost at the origin. These last 2 fractions increase with time (Figure 2b) and do not correspond to any band in the parent globins (Figures 1a, 2a, 1d, 2c) nor in the freshly prepared mixture (Figure 1b). (2) The mixture of oxyhemoglobins shows only the bands corresponding to the parent hemoglobins even 7 days after mixing (Figure 3). The 2 new fractions which are formed by mixing the globins under the described experimental conditions are analogous to the fractions which are formed by hybridization of the hemoglobins and most probably represent globin hybrids.

Discussion. Globin is known to undergo, at neutral pH and low ionic strength, an association-dissociation equilibrium in which dimers (*M*w 32,000) predominate, but also monomers are present[6]. Therefore it is not surprising that hybrids are formed in a mixture of human and canine globins when these are kept together for a long time even at neutral pH and low temperature. Under identical experimental conditions hybrids are not formed in appreciable quantity in mixtures of the corresponding hemoglobins.

This result agrees with the conclusion that the respective conformations of hemoglobin and globin are very different[7,2]; in particular, it suggests that the linkages between the α and β chains are much stronger in hemoglobin than in globin.

Riassunto. Miscele di globine preparate da emoglobina umana e canina, tenute a pH neutro e a 4 °C, mostrano dopo alcuni giorni la formazione di molecole ibride. Nelle stesse condizioni miscele di emoglobine non presentano una formazione significativa di ibridi. I risultati indicano che i legami α β sono più forti nell'emoglobina che nella globina.

CARMELA IOPPOLO

Istituto di Chimica Biologica dell'Università and Centro di Biologia Molecolare del C.N.R., Roma (Italy), April 18, 1966.

[4] A. ROSSI-FANELLI, E. ANTONINI, and A. CAPUTO, Biochim. biophys. Acta *30*, 608 (1958).
[5] M. D. POULIK, Nature *180*, 1477 (1957).
[6] A. ROSSI-FANELLI, E. ANTONINI, and A. CAPUTO, J. biol. Chem. *234*, 2906 (1959).
[7] E. ANTONINI, Physiol. Rev. *45*, 1 (1965).