correlated with a break in the Arrhenius plot. A summary of this correlation is shown in the Table. Since histidine ammonia-lyase contains 1 cysteinyl residue per subunit[11] and this –SH group has been implicated in the binding of substrate and catalysis[12] it seems reasonable to speculate that GSH and DTT may act in different ways on the enzyme. One model which is consistent with this data is that DTT acts solely to maintain the reduced form of the enzyme whereas GSH also forms a mixed disulfide accounting for the 2 bands observed on electrophoresis and the $E_{cat}^{\pm}$ value intermediate between those obtained in the presence and absence of DTT. This may be analogous to the situation reported by ROUFAGALIS et al.[4] concerning the binding of ligands to acetylcholinesterase.

The present study with histidine ammonia-lyase provides direct evidence that the concept[2] of a temperature-dependent equilibrium between 2 forms of enzyme is the best explanation for the phenomena reported in the literature concerning discontinuous Arrhenius plots and related thermodynamic and spectroscopic data. The physiological significance of such phenomena is a matter of uncertainty and contention. It is noteworthy, however, that *Pseudomonas* and other bacteria grow well over a wide range of temperature and that at 37 °C both conformers of histidine ammonia-lyase would exist. Such transitions have also been described for more complex structures such as myosin-ATPase[3] and membrane-bound yeast mitochondrial ATPase[13].

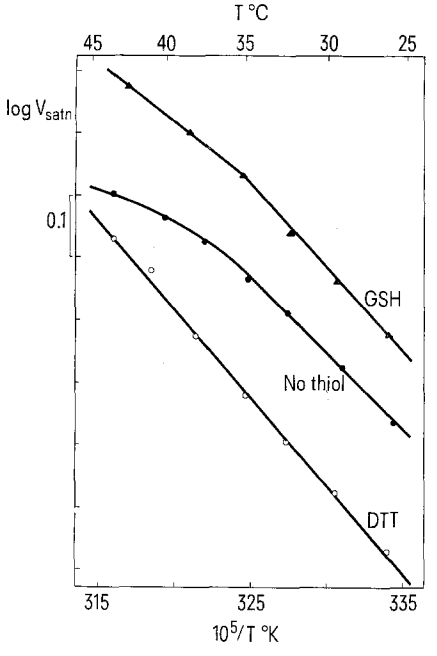

Fig. 2. Arrhenius plots of the histidine ammonia-lyase reaction. Dashes on the ordinate are spaced by 0.1 $\log_{10}$ unit. Height of curbes is merely a convenience and need not imply relative activity. Conditions are: ●, 10 DTT; ▲, 5 m*M* GSH, and ■, no thiol.

Zusammenfassung. Erstmaliger Nachweis, dass bei der *Pseudomonas* Histidin-Ammoniak-Lyase und bei Temperaturerhöhung der Enzymlösung auf 35–40° (reversibel) eine zweite Enzymspecies (Konformeres) entsteht (Disc-Gel) und dass die Umwandlung in dieser Species auch in der Arrhenius-Darstellung sichtbar ist.

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On the Nature of Unretarded Protein in a Chain Separation Method of Hemoglobin

Some years ago CLEGG *et al.*[1] introduced a new method for the separation of the α- and β-chains of human hemoglobin. This method was an improvement on former procedures and has since been used extensively by many authors[2–6].

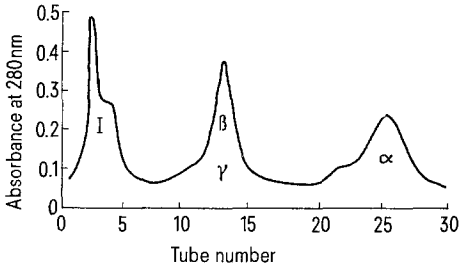

Fig. 1. Elution pattern of separation of globin chains on a carboxymethylcellulose column.

The separation is performed on a carboxymethylcellulose column in 8 *M* urea, 50 m*M* phosphate buffer pH 6.7 with 50 m*M* mercapto-ethanol. The column is eluted with a 5 to 30 m*M* Na^+-ion gradient. A representative elution pattern is shown in Figure 1. The major peaks were identified by CLEGG by the fingerprinting technique as being β- and α-chains. The small shoulders eluted just ahead of the major peaks were shown to

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consist of carbamylated products[7]. Besides these 4 fractions, a fraction of unretarded material is regularly observed (peak I). This material is discarded by most workers as being non-hemoglobin protein. In our work on the mechanism of the switch-over of the synthesis of fetal to adult hemoglobin of the cow, we also used extensively the method of CLEGG *et al.*[1] for the separation of the hemoglobin chains[8]. In view of the substantial amount of material present in peak I, it seemed necessary to characterize this material more carefully before discarding it.

Methods. Radio-active adult and fetal hemoglobin were obtained by incubating reticulocyte-rich calf blood with ^{14}C-leucine at 37 °C[8]. After incubation the cells were centrifuged and washed twice with 0.9% NaCl. The cells were lysed by adding 1 volume of water. The cell membranes were removed by centrifugation for 15 min at 12,000 $\times g$. The oxyhemoglobins were converted to the

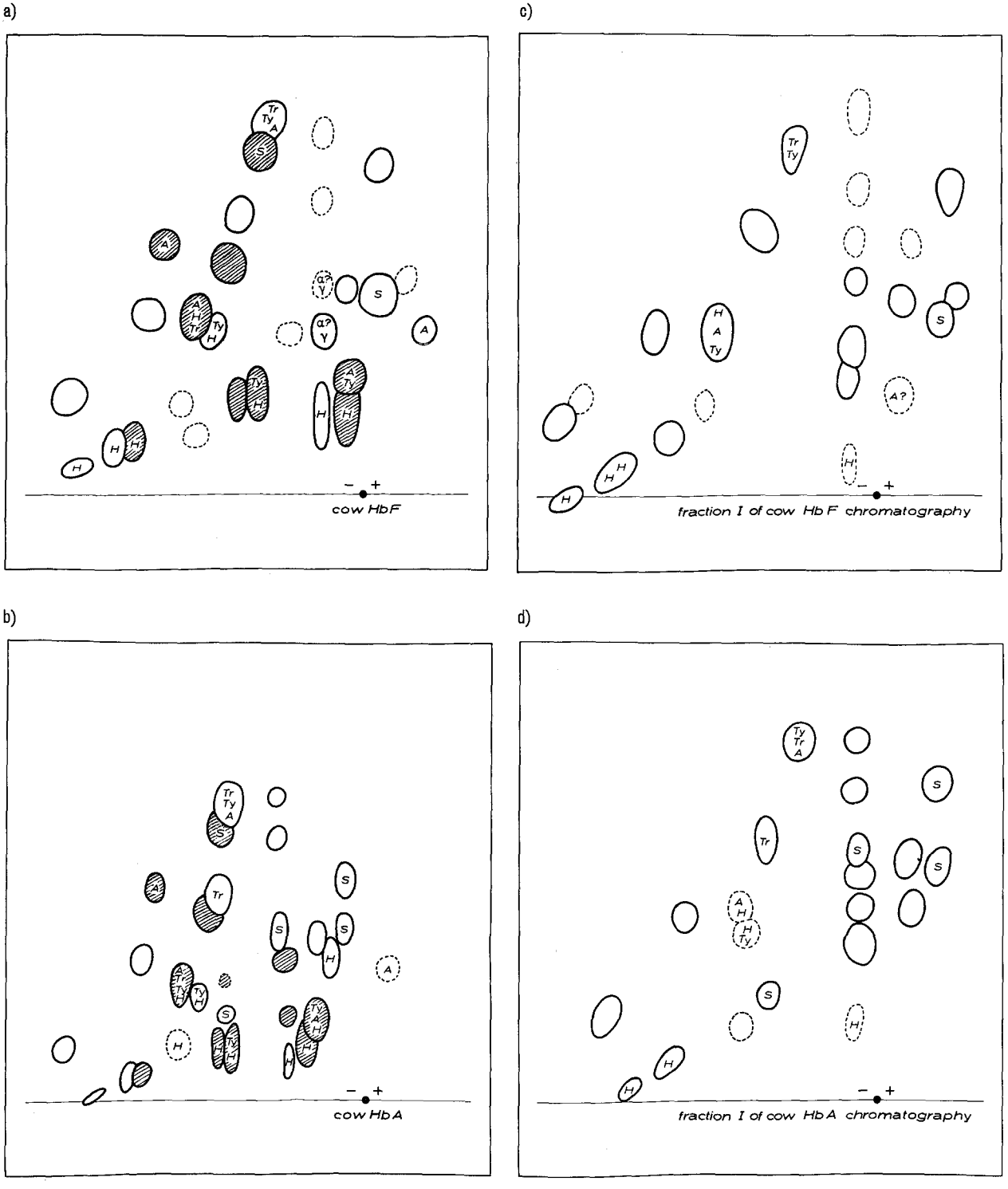

Fig. 2. a) Fingerprint of fetal globin. b) Fingerprint of adult globin. c) Fingerprint of peak I of fetal globin. d) Fingerprint of peak I of adult globin. ▨, α-chain peptides; □, β- or γ-chain peptides.

cyanomethemoglobins and, after extensive dialysis against a 0.01 *M* phosphate buffer pH 6.5, separated quantitatively on CMC with a discontinuous pH gradient (pH 6,5; pH 7.0; pH 8.6).

The non-hemoglobin proteins were eluted at pH 6.5, the HbF and HbA at pH 7.0 and at pH 8.6 respectively. Globin was prepared from the pooled CMC-fractions, by the HCl-acetone precipitation method[9]. The α- and β-chains and the α- and γ-chains were separated as described by CLEGG *et al.*[1]. In the case of fetal globin the pH of the eluting-buffer was 6.4.

Peak I and the major peak fractions were collected, dialyzed against water, freeze-dried, and the radioactivity was determined in Bray's solution with added Hyamine in a Nuclear-Chicago liquid scintillation counter. All protein fractions were digested with trypsin and the peptides were fingerprinted according to INGRAM[10]. Figures 2a and b show the fingerprints of fetal and adult globin respectively. No cross-contamination of both hemoglobins could be detected by this method.

Results and discussion. The fingerprint of peak I (see Figure 1) of fetal globin was nearly identical with that of the γ-chain. No specific α-chain peptides could be detected (Figure 2c).

Fingerprints of peak I of adult globin (Figure 2d) predominantly showed β-chain peptides. Some additional neutral peptides also appeared. No specific α-chain peptides could be observed. When peak I of fetal or adult globin was rechromatographed on the urea-CMC-columns, 2 peaks appeared at elution volumes equal to that of peak I and to that of the γ- or β-chain respectively (Figures 3a and b).

The conclusion from these experiments is that the non-absorbed material contains β-or γ-chains in any case. It remains obscure why the β- and γ-chains and not the α-chains are modified in this method. As rechromatography of the material from fraction I yields β- or γ-chains, at their normal elution volumes, its formation seems to be a reversible process,

In the Table the results of measurements of radioactivity are shown. It is remarkable that the specific activity of peak I from adult and fetal globin is about 40–45% lower than that of the corresponding chain-peaks. This demonstrates that a non- or less-labelled product contaminates the material of peak I. A part of it is heme[6]. The specific activity of the globin is lower than the average of the specific activity of the chains, and higher than that of peak I. The presumed contamination is probably not digested by trypsin, as in the case of peak I of fetal globin no additional peptides show in the fingerprint. It may form reversibly a complex with β- or γ-chains, but not with α-chains, as rechromatography of peak I yields β- or γ-chains, but no α-chains.

The results of this work show that peak I contains β- or γ-chains and has a specific radio-activity different from the major peaks. This may be a factor to be taken into account in studies on the synthesis of α- and β-, or α- and γ-chains of hemoglobins.

Specific activities of the separated chains after chromatography of adult and fetal globin

	dpm/mg		dpm/mg
Adult globin	15750	Fetal globin	11170
Peak I	10770	Peak I	7850
β-chain	19215	γ-chain	12200
α-chain	18970	α-chain	13870

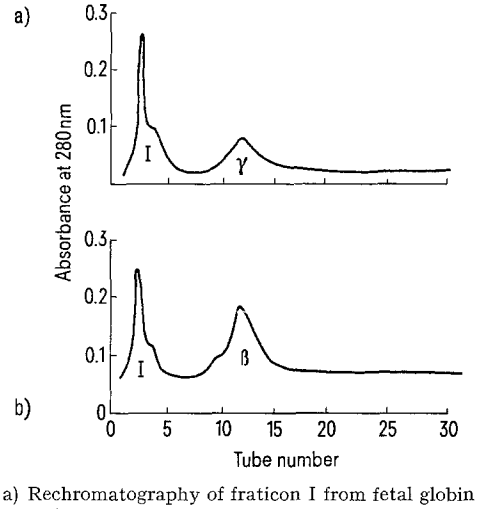

Fig. 3. a) Rechromatography of fraticon I from fetal globin on CMC (CLEGG *et al.*[1]). b) Rechromatography of fraction I from adult globin on CMC.

Zusammenfassung. Nachweis, dass bei der Trennung von α- und β- oder α- und γ-Ketten des Hämoglobins über CMC im 8 *M* Harnstoff eine nicht absorbierte Proteinfraktion vorhanden ist. Zudem konnte mit der Fingerprinttechnik bewiesen werden, dass diese Fraktion γ- oder β-, jedoch keine α-Ketten enthält.

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Inhibition of the Angiotensin I Converting Enzyme of the Lung by a Peptide Fragment of Bradykinin

The angiotensin I converting enzyme (kininase II; peptidyl dipeptide hydrolase; DH) has a dual function. It converts angiotensin I to angiotensin II by releasing the C-terminal amino acid residues His^9-Leu^{10}-OH of the decapeptide. It also inactivates bradykinin by the removal of Phe^8-Arg^9-OH[1–3]. DH cleaves substrates with the general structure of -R_1-R_2-R_3OH between R_1-R_2[3]·R_1 can be a protected amino acid or a peptide and R_3 is a free C-terminal amino acid. R_2 can be any amino acid but proline since DH does not hydrolyse substrates with