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

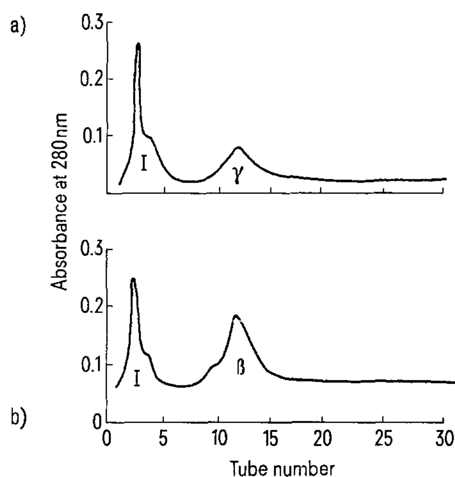

Fig. 3. a) Rechromatography of fraticon I from fetal globin on CMC (CLEGG *et al.*[1]). b) Rechromatography of fraction I from adult globin on CMC.

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

proline in penultimate position. Recently peptides which block the conversion of angiotensin I and the inactivation of bradykinin were purified from snake venoms[4-6] and subsequently synthetized. The C-terminal end of most of the potent ones is prolyproline. That makes them resistant to hydrolysis by DH. Because the N-terminal region of bradykinin also contains a prolyproline sequence (Arg^1-Pro^2-Pro^3-), we tested this tripeptide derivative of bradykinin as an inhibitor of DH.

Materials and methods. Homogenous DH was obtained by purifying the enzyme from hog lung[7]. The activity was determined in a Cary 15 recording UV spectrophotometer at 254 nm in a 0.1 M *Tris* buffer of pH 7.4 containing 0.1 NaCl[2,3]. The temperature was kept at 37 °C. The substrate was hippuryl-glycyl-glycine (HGG). In routine assay, a 1×10^{-3} M concentration was used. The inhibitor was preincubated for 5 min with the enzyme before adding the substrate.

Results and discussion. HGG was hydrolyzed by 1 mg of DH at a rate of 13 μmole per min. When the reciprocal values of velocity were plotted against the reciprocal concentrations of the substrate, and average K_m of 1.2×10^{-3} M was obtained (Figure 1).

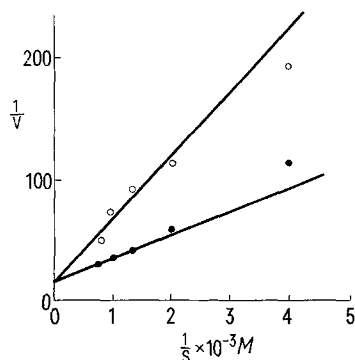

Fig. 1. Lineweaver-Burk plot of the hydrolysis of hippuryl-glycyl-glycine in presence (●—●) and absence (○—○) of 2.10^{-5} M Arg-Pro-Pro. The angiotensin I converting enzyme was purified from hog lung.

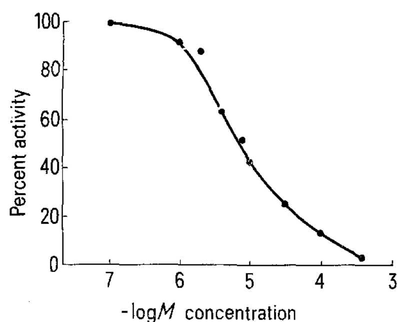

Fig. 2. Arg-Pro-Pro inhibits the hydrolysis of hippuryl-glycyl-glycine by purified angiotensin I converting enzyme of the lung.

The tripeptide Arg-Pro-Pro inhibited the reaction, giving a mean I_{50} value of 1×10^{-5} M (Figure 2). Using the same Lineweaver-Burk equation, the plotted 1/v values of the inhibited and uninhibited reactions intercepted on the ordinate. Thus, the tripeptide was a competitive inhibitor. The K_i of Arg-Pro-Pro was calculated to be 6×10^{-6} M using standard equation for estimation of K_i of competitive inhibitors.

Besides the two vasoactive peptides, DH cleaves a variety of substrates, such as the B chain of insulin[3] and several shorter optically active peptide substrates[2,3,5,7]. It was shown, however, that the ratio of the rates of cleavage of a short peptide substrate and angiotensin I stayed constant during the purification of the enzyme from lung[8,9]. Various agents inhibit DH, among them are the split products of the hydrolysis of bradykinin by DH (Phe-Arg) or that of angiotensin I (His-Leu)[1,2]. Competitive substrates such as the B chain of insulin or bradykinin inhibit the conversion of angiotensin I in the perfused lung in situ[3] and also in vitro[10].

Our experiments have shown that a fragment of bradykinin, with the same sequence as an active portion of the inhibitors derived from snake venoms, competitively inhibits DH. Thus, in addition to bradykinin which has a very short half-life in the body, its enzymatic degradation product as well, may block the conversion of angiotensin I.

Zusammenfassung. Nachweis, dass Arg-Pro-Pro, das N-terminale Tripeptid von Bradykinin, für die Hemmung der Konversion von Angiotensin I zu Angiotensin II in der Lunge verantwortlich ist.

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Ribonuclease Activity of Rat Thymus Chromatin Proteins

Today it is known that a substantial part of newly synthesized RNA in the cells of higher organisms is degraded in the nucleus[1,2]. Hybridization experiments show only about 20% of all RNA types synthesized in the nuclei come out into the cytoplasm[3,4]. Thus in the nuclei there must be an enzymatic system responsible for the strictly regulated specific degradation of certain RNA. Some data suggest that the degradation of nuclear RNA may take place directly in chromatin[5].

Many authors have tried to determine the ribonuclease activity of chromatin, mainly in order to assay the role of ribonucleases in the study of chromatin template activity