Zur Darstellung der Bisselenouronium-bromide werden 0,05 Mol Selenoharnstoff, 0,0275 Mol α,ω-Dibromalkan und 50 ml absolutes Äthanol eine halbe Stunde unter Rückfluss erhitzt. Nach Abkühlen wird abfiltriert, in Dimethylformamid gelöst und mit Äther wieder ausgefällt. Nach mehrmaligem Umfällen fallen die Verbindungen analysenrein an.

Se-Carboxymethylisoselenoharnstoff erhielten wir nach der Vorschrift für S-Carboxymethylisothioharnstoff [2] und Se-(2-Carboxyäthyl)-isoselenoharnstoff nach der Vorschrift für S-(2-Carboxyäthyl)-isothioharnstoff.[3] Beide Verbindungen wurden aus Wasser umkristallisiert.

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Time Course of the Action of Pepsin on Insoluble and Soluble Collagens

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In our first paper on this subject [1] we presented a typical starch-gel electrophoretic pattern of pepsin-treated soluble collagen. The sharp resolution of the fragments could not be achieved by column chromatography on CM-cellulose, by gel filtration, or by preparative starch-gel electrophoresis. Pepsin is the most suitable proteolytic enzyme for this kind of study because the collagen fragments yield a distinct electrophoretic pattern and pepsin seems to migrate in these conditions in a direction opposite to that of the collagen fragments.

We wished to extend this procedure to "Nishihara collagens" (acid-soluble collagens obtained from insoluble collagenous fibres by treatment with enzymes, e.g. with pepsin.) Electrophoretic patterns of such preparations have not been recorded. The second purpose of this note is to present an integrated scheme of the course of the digestion of soluble collagen by pepsin in terms of gel-electrophoretic patterns.

The insoluble collagen was prepared from the skins of adult rats. The material was cleaned, homogenized (homogenizer No. 21 00 00, E. Bühler, Tübingen, West Germany), and extracted two times with 0.1 M sodium phosphate buffer of pH 7.5, once with 0.9 % (w/v) sodium chloride solution and finally five times with molar sodium chloride solution. The salts were removed from the residue by washing three times with water. The air-dried residue contained 10.3 % hydroxyproline and 12.2 % proline (w/w). This residue contained both the acid-soluble and insoluble fractions of collagen. The terms soluble and insoluble collagen are a matter of definition [2] and true dissolution may be confused with solution following slight hydrolysis at a low pH. Soluble collagen was obtained from rat-tail-tendon by extraction with 3.0 % acetic acid.

Insoluble collagen, 100 mg, was suspended in 200 ml of 0.5 % acetic acid, the pH of the suspension was brought to 2.0 with hydrochloric acid, and 1 mg of pepsin (DAB, 1:3 500) was added. Control experiments were carried out without pepsin present. The mixture was continuously shaken at room temperature for 48 h, a new 1-mg portion of pepsin was added, and the shaking continued for a further 48 h. The solution was isolated by filtration and lyophilized. About 88.5 % of the original collagen treated with pepsin was solubilized to a viscous gel. In the control experiments only 28.8 % was dissolved and a gel was not formed. Eventual complex formation between pepsin and insoluble collagen or inactivation of the pepsin were not studied.

Fig. 1 shows the gel-electrophoretic pattern of the collagen solubilized by pepsin and patterns for other collagens.
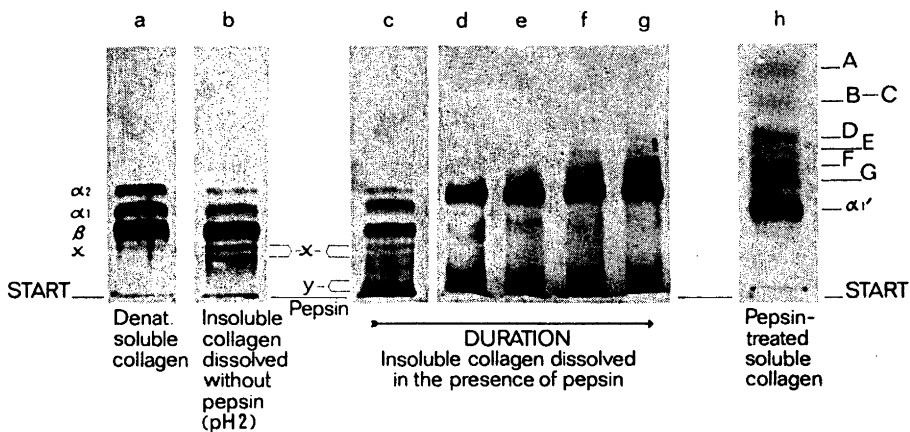
Fig. 1. Starch-gel-electrophoretic patterns [3-5] of fragments liberated by pepsin from rat-skin collagen insoluble in 1 M NaCl (*c,d,e,f,g*). For comparison are presented patterns of collagen dissolved by 3 % acetic acid from rat-tail-tendon (*a*), collagen dissolved from collagen insoluble in 1 M NaCl at pH 2 without pepsin present (*b*, "acid-soluble collagen") and pepsin-treated soluble collagen (*h*).[1]
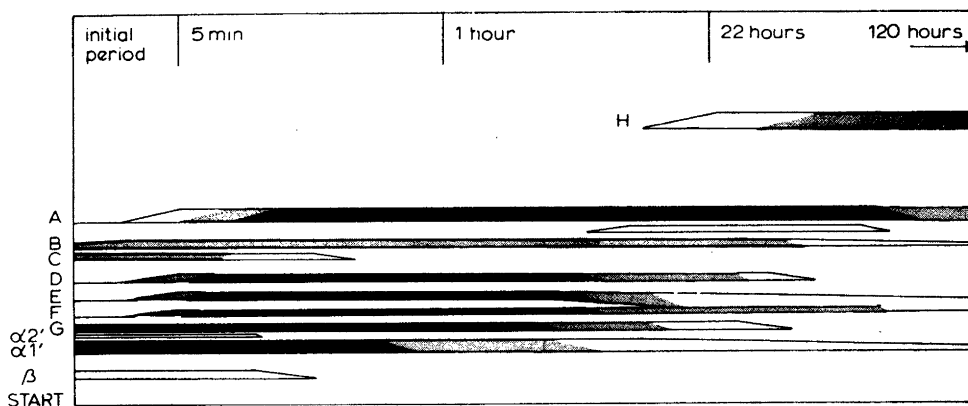

Fig. 2. Scheme showing the changes in the starch-gel-electrophoretic pattern of pepsin-treated soluble collagen [1] during digestion (pepsin:soluble collagen 1:100, pH about 2.0, temperature +40°C). The scheme is based on patterns recorded after the following digestion times: zero, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 22 h, 58 h, 80 h, 90 h, 104 h, and 120 h.

When fresh pepsin was added to the material which gave the pattern c and the mixture kept at +40°C, the pattern changed successively to d (after 30 min), e (60 min), and g (120 min). Finally a pattern A-G similar to that of soluble collagen was obtained.

The y-fractions were in some cases very large and differed clearly from the x-fractions, which correspond to the γ- and δ-fractions mentioned previously by several authors.[6] The y-fractions become less prominent after continued action of pepsin but their mobility does not change. These fractions deserve special attention because they contain the intermolecular linkages of insoluble collagen within the soluble aggregates of tropocollagen.

Fig. 2 shows schematically the progress of degradation of soluble collagen as revealed by the starch gel-electrophoretic patterns. In the denatured peptide chains there seem to be certain linkages which are broken almost immediately after the addition of pepsin. Further degradation occurs first after a definite time interval. Most interesting to us seem the fractions which are constant (A, B-C) and those which migrate close to the original components (D,E,F,G). From preliminary gel-filtration experiments we know that the particle weights of the latter fractions are between those of albumin and the α-chains of collagen. The fractions designated αl' and α2' are believed to resemble the α-chains; cf. Ref. 7.

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On the Structure of the Bromination Product of 4-Methyl-4,3-borazaroisoquinoline

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In a recent publication by Dewar and von Rosenberg [1] it is claimed that the bromination and nitration of 4-methyl-4,3-borazaroisoquinoline (I) occurs in the 8-position of the benzenic ring. On the other hand the present authors have found that the thiophene analogue of (I), 4-methyl-4,5-borazarothieno[2,3-c]pyridine (II) is brominated and nitrated in the 7-position of the boron-nitrogen containing ring.[2] Our structural proof was based on completely resolved NMR-spectral data and on chemical degradation.

I   II

III   IV
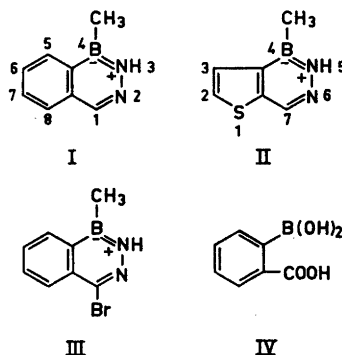
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