more than 90%. To confirm the nature of the derivative of phenylethylene glycol with butylboronic acid, mass spectra were carried carried out by using an LKB 9000 instrument at the following conditions: ion source temperature 290°C; ionization energy 70 eV; trap current 60 μA. Sample introduction was carried out either by direct inlet system (DIS) or by GC at the conditions described above, except for the use of helium instead of nitrogen as carrier gas. The 2 mass spectra were comparable. The enzymic activity of epoxide hydrase was expressed in nmoles of diol formed per min of incubation per mg of microsomal protein. The spontaneous hydration of styrene epoxide occurring in our conditions was lower than 5% with respect to the enzymatic and it was substracted for the calculation of enzymatic activity. Protein concentration was determined by the method of Lowry[14].

Results and discussion. The table summarizes the effects of the tricyclic drugs and their epoxides on the activity of epoxide hydrase. Only cyproheptadine epoxide had a marked inhibitory effect on the enzyme, while cylobenzaprine and its epoxide showed a slight stimulatory effect. On the other hand, cyproheptadine, carbamazepine and carbamazepine epoxide did not show any influence on the activity of the enzyme. As shown in the figure, cyproheptadine epoxide is a competitive inhibitor of epoxide hydrase; the apparent K_i-value is 0.75 mM. It may be suggested that styrene epoxide and cyproheptadine epoxide are substrates for the same enzymes, although to date there is no evidence that cyproheptadine epoxide is metabolized to the correspondant diol[15].

The significance of these in vivo findings remains to be established. However, the hypothesis may be advanced that cyproheptadine epoxide could interfere with the biotransformation process of some carcinogenic agents by affecting the degradation of their epoxides into metabolites devoid of toxic effects. Conversely, it may be suggested on the basis of data from studies in progress that tricyclic agents may also affect the formation of the epoxides of carcinogenic agents. Therefore, it seems possible to modulate the accumulation of epoxides from such agents, permitting us to assess the significance of epoxide formation in chemical carcinogenesis.

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A new approach to preparation and purification of colostrokinin from bovine colostrum*

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Summary. A simple and rapid method is reported for preparation and partial purification of colostrokinin from bovine colostral whey. The active substance obtained at the described stage of purification appears to be a basic polypeptide of low molecular weight. This peptide proved to be undestroyed by pepsin, only partially by trypsin and completely by papain.

The first report about some properties of colostrokinin dates from 1959 by Guth[1]. The crude substance was obtained by incubating kallikrein from urine or saliva with colostrum[2,3]. A method for colostrokinin preparation with a 15–20fold purification was later performed by Werle and Trautschold[4], who found some biological and biochemical differences between colostrokinin and plasmakinins, but concluded that the 2 types of kinins were chemically and pharmacologically very closely related. About 10 years later, this subject of research drew the attention of some Japanese investigators, who proposed a method for isolation and purification of colostrokinin

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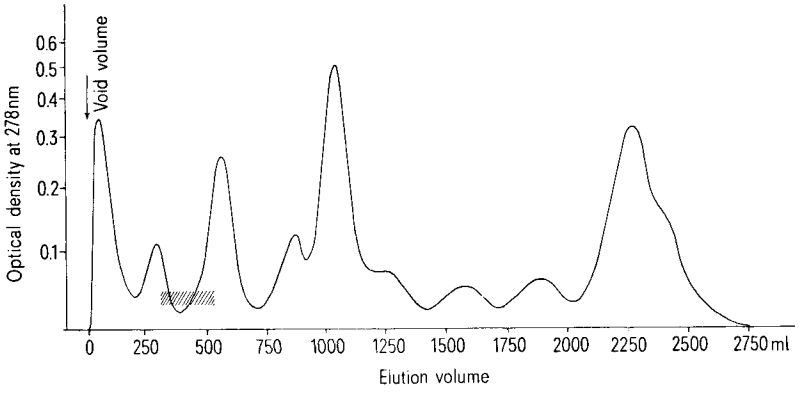

Fig. 1. Gel filtration pattern of crude colostrokinin (step 3), prepared from 2000 ml of natural bovine colostrum, on a Sephadex G-25 fine column (5.5 cm × 100 cm), equilibrated with 0.1 M ammonium acetate buffer (ph 5). Flow rate 125 ml/h. Fraction size 25 ml. Void volume measured with Blue Dextran 2000. Shaded area represents the presence of biological activity. The separation pattern was recorded at 278 nm.

Table 1. Summary of the parameters followed for colostrokinin purification[a]

Fractions obtained	Dry weight (d.w.) mg[b]	Protein content (p.c.) mg[c]	Biological activity Rat uterus			Guinea-pig ileum		
			μg as bradykinin[d]	Purification factor on d.w.	Purification factor on p.c.	μg as bradykinin[d]	Purification factor on d.w.	Purification factor on p.c.
At step 2	34,300	15,240	10.6	1	1	33.4	1	1
At step 3	832.5	344.9	9.07	35×	38×	32	39×	42×
At step 4	53	34.5	8.03	490×	335×	21.8	422×	288×
At step 5	2.2	0.397	2.88	4,236×	10,438×	1.25	583×	1,436×

[a] All data are referred to 1,000 ml of natural bovine colostrum. [b] After drying in vacuo at 100 °C for 24 h. [c] Calculated from quantitative aminoacid analysis. [d] Synthetic bradykinin kindly supplied by Sandoz Ltd.

from bovine colostrum and described some of its properties[5,6]. They showed that colostrokinin had some biological properties in common with bradykinin, but differed in having a stronger activity in increasing capillary permeability in guinea-pigs and in eliciting vasodilation in dogs. More recently Beretta et al.[7] reported that through various kinds of biological assays, colostrokinin revealed some features which clearly differentiate it from bradykinin. Since all these results have been obtained with less or more purified preparations of the kinin, the questions about its identification still remain and can be answered only with completely purified preparations of it, or better with the synthetic compound. For this reason, we have now tried a new and alternative procedure to prepare and purify colostrokinin from bovine colostral whey, the first steps of which are reported in this paper.

Materials and methods. 1. Bovine colostrum was collected within 24 h after delivery and stored at –20 °C. 2. Biological assays: ovariectomized and oestrogenized rat's uteri and guinea-pig's ilea suspended in vitro in Tyrode at 30 °C in the presence of atropine (1 μg/ml) and BOL (D-2-bromolysergic acid diethylamide; 1 μg/ml) and in Tyrode at 37 °C respectively. The biological activities of the samples have been expressed by using synthetic bradykinin (Sandoz Ltd) as a reference standard. 3. Enzymatic treatments: tryptic digestion was performed in 0.24 M phosphate buffer during a 24 h contact period at 27 °C and pH 8.3; peptic and papainic digestions during equal contact times in 0.1 M glycine-HCl buffer at 37 °C and pH 2 and respectively in 0.1 M ammonium acetate-acetic acid buffer containing 5 mM EDTA in the presence of cysteine at 37 °C and pH 6.1. The enzyme:substrate ratio was 1:10 (w/w) on the basis of the substrate's proteic content.

Results. The purification procedure was carried out by the following steps: 2000 ml of bovine colostrum were centrifuged at 11,000 × g for 30 min. The colostral whey obtained (middle layer) was brought to pH 3 with 5 N HCl, warmed at 37 °C for 5 min with stirring, adjusted to pH 5.5 with N NaOH and then dialyzed against distilled H_2O for 24 h at 2 °C. After dialysis the sample was adjusted to pH 7 with N NaOH and incubated for 5 min at 37 °C under kind stirring with kallikrein (Padutin®-lyophilized vials, Bayer, Leverkusen, BRD) 0.5 Frey Units for each ml of original colostrum. At the end of this incubation period, the solution was adjusted to pH 4.5 with 5N acetic acid, cooled and then lyophilized (step 1). The lyophilized powder was extracted with 2 N acetic acid and then lyophilized (step 2). This lyophilized material obtained was further extracted with glacial acetic acid and the acidic supernatant was brought to 89% concentration of ethyl ether. The precipitate formed during a

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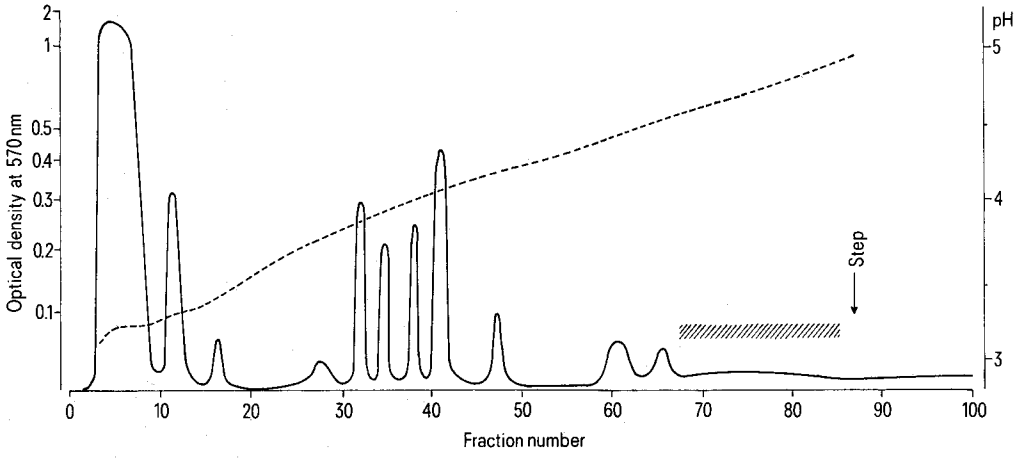

Fig. 2. Ion exchange chromatography at 50 °C of 40 mg of the fraction of step 4 on Aminex A-5. The column (1 × 25 cm) is equilibrated with 0.2 M pyridine-acetic acid buffer (ph 3.1). Gradient to pH 5 starts at tube 1 with a 2-flask-mixing apparatus (250 ml of 0.2 M pyridine-acetic acid buffer pH 3.1 + 250 ml of 2 M pyridine-acetic acid buffer pH 5.0). Step 85 ml of 2 M pyridine at tube 87. Fraction size 5.5 ml. Flow rate 30 ml/h. The separation pattern (—) was recorded at 570 nm (ninhydrin test after alkaline hydrolysis). ---, pH. Shaded area represents the presence of biological activity.

3-h-period at 20 °C was filtered on a Buchner funnel, washed twice with ethyl ether and dried. This dried material was extracted overnight with 200 ml of 70% (v/v)EtOH, and the supernatant obtained by centrifugation was evaporated to dryness under reduced pressure at 30–35 °C (step 3).

This residue was successively extracted with 33 ml of 0.1 M ammonium acetate buffer (pH5) and the supernatant collected was fractionated on a Sephadex G-25 fine column (figure 1). The active fraction (shaded area) was lyophilized (step 4). An amount of this material (corresponding to 800 ml of initial colostrum) was chromatographed on a preparative peptide-analyzer by employing Aminex A-5 (Bio-Rad Laboratories) under the conditions described in figure 2. The separation pattern (ninhydrin test after alkaline hydrolysis according to Moore and Stein[8] in order to avoid interference due to eluent buffers) was recorded at 570 nm. The active fraction was lyophilized (step 5). The fractions obtained at the points step 2, 3, 4 an 5 have been tested biologically, and the results attained listed in table 1 with the respective values of their dried weights and proteic contents. The fraction of the step 5 was moreover submitted to the enzymatic treatments described and then tested for its biological activity on rat uterus preparations. The results obtained are listed in table 2.

Table 2. Enzymatic digestion of a partially purified colostrokinin*

Sample	Per cent activity**
Control	100
Pepsin digested	100
Papain digested	2– 3
Trypsin digested	38–42

*Biological activity on rat uterus in vitro. **Per cent residual activity referred to control considered = 100.

Discussion. The steps of purification of colostrokinin now proposed only partially overlap those described by Yamazaki and Moriya[5]. The short time of enzyme-substrate contact was selected according to previous results by Beretta and Ormas[9]. The 2 successive acetic acid extractions (2 N and glacial) allow a good separation of the kinin from the high molecular weight compounds (i.e. proteins, nucleic acids, etc.) while the precipitation with an excess of ethyl ether removes the lipids still present in the menstruum. The elution Kd from the Sephadex G-25 column suggests that the active fraction is characterized by a mol.wt between 1000 and 2000. The successive purification on Aminex A-5 reveals its basic properties. This last finding is confirmed by the lack of biological activity showed by the papain-treated preparations (table 2). Since the activity of the kinin has been not damaged by pepsin and only partially by trypsin (enzymes present in the animal gastrointestinal tract), it can be suggested that when released by salivary kallikrein, colostrokinin may pass uninjured through the gastric environment and be hydrolyzed only within the duodenal tract, where, however, trypsin might exert not only a kininase-like but also a kininogenase-like activity[4]. On account of the purification factors calculated, this new procedure appears to be simple and rapid enough to allow the preparation of relatively large amounts of the kinin containing material. Studies on its chemical and biological properties are now in process in our laboratories.

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Pentobarbital: Presynaptic effect in the squid giant synapse[1]

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Summary. In concentrations that produced synaptic blockade in the squid giant synapse, sodium pentobarbital produced a dose-related, reversible decrease of the 'calcium spike' of the presynaptic terminal.

Barbiturates block synaptic transmission at lower concentrations than they block conduction along nerves[4–6]. These lower concentrations are more relevant to the general anesthetic concentrations in man, and the postsynaptic membrane is affected in this concentration range[7–9]. There has been considerable debate, however, as to whether barbiturates also have presynaptic effects. For example, a barbiturate-induced decrease in quantal content has been demonstrated in cat motoneurons[10], and barbiturates have been shown to inhibit depolarization-induced calcium uptake in rat synaptosomes[11] and in symptathetic ganglia[12]; but barbiturates cause no change or an increase in quantal content at the frog neuromuscular junction[7,13]. There have been few intracellular studies of direct presynaptic effects of barbiturates because of the difficulty of working with presynaptic terminals. The squid giant synapse is unusual in that both the pre- and the postsynaptic terminals can be

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