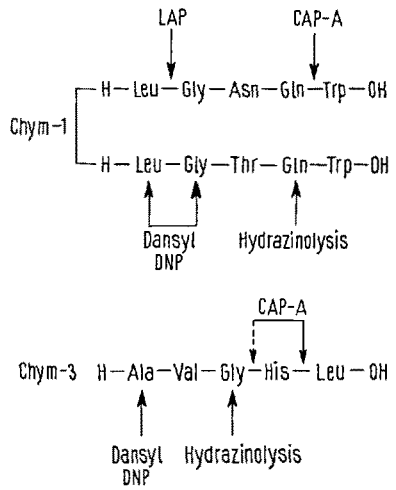
trypsin and chymotrypsin, as schematically represented in Figure 2. The enzymatic fragments were isolated by paper electrophoresis and ion exchange micro-columns.

The sequence of Tryp-I was deduced by digestion with carboxypeptidase B and hydrazinolysis of the remaining dipeptides as shown in Figure 3.

The sequences of Chym-1 and Chym-3 were deduced, as schematically represented in the Figures 4 and 5, by digestion with leucineaminopeptidase and carboxypeptidase A, dansyl and dinitrophenyl N-terminal determinations, and hydrazinolytic C-terminal determination of the full fragments and of the fragments remaining after digestion with the exopeptidases.



Synthesis has confirmed the above results. Details of this work will be published elsewhere.

Riassunto. Viene descritto l'isolamento e il chiarimento della struttura della bombesina e della alytesina due tetradecapeptidi attivi a struttura analoga presenti nella pelle fresca rispettivamente della *Bombina* e dell'*Alytes*, anfibi europei della famiglia dei discoglossidi. I due polipeptidi manifestano azioni farmacologiche similari sulla pressione del sangue, su svariati organi a muscoli lisci, sulla secrezione gastrica e sulla glicemia.

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[1] In part supported by grants from the Consiglio Nazionale delle Ricerche, Rome.

[2] V. Erspamer, G. Falconieri Erspamer and M. Inselvini, J. Pharm. Pharmac., in press.

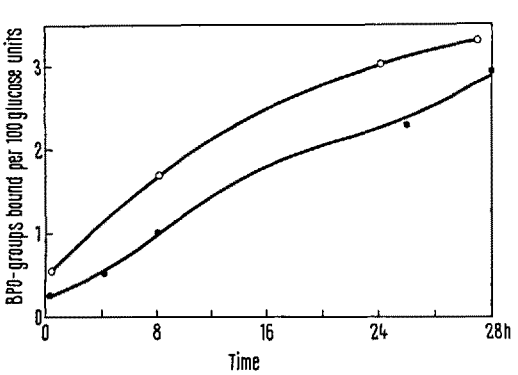
Carboxymethyl Cellulose Additives in Penicillins and the Elicitation of Anaphylactic Reactions

Penicillins react with polyoxy compounds in neutral aqueous solution thereby forming penicilloyl derivatives[1]. Since clinically used penicillins quite frequently contain carboxymethyl cellulose (CMC) as an additive, penicilloyl-CMC may accumulate in these preparations. Multivalent penicilloyl-CMC's are potentially dangerous to allergic patients since they could act as elicitors of penicilloyl-specific anaphylaxis.

We have studied the formation of penicilloyl-CMC conjugates with a low degree of penicilloyl substitution under quite innocuous conditions, namely in cold barbital buffered solution at pH 7.4 and found that within about 20 h CMC's with 2–3 benzylpenicilloyl (BPO) groups bound per 100 glucose units are easily formed (Figure). Attempts to isolate these conjugates by gel filtration or ultrafiltration failed because relatively rapid cleavage of the BPO groups from the carbohydrate occurred during processing. Therefore, a more highly penicilloylated CMC (Elmans, Grade AKV) with 10 BPO groups per 100 glucose units was prepared at pH 10 and used as starting material. It yielded a conjugate with 3–4 BPO groups per 100 glucose units after its separation from penicillin and other low molecular weight contaminants by ultrafiltration through a UM-1 membrane filter (Amicon Corporation, Lexington, Mass.). The stability of the conjugate, once isolated, is comparable to the stability of penicilloylated dextran described earlier[1] (half life at 37 °C in 0.04 *M* barbital buffer pH 7.4: 1–2 days). In acetic acid solution at pH 3 no loss of penicilloyl from the conjugate occurred during 30 h at 37 °C. Even in HCl solution at pH 1.6 very little penicilloyl cleavage was found after 6 h.

This BPO-CMC readily elicited cutaneous anaphylactic reactions in guinea-pigs passively sensitized with rabbit-anti-BPO-bovine γ-globulin-antiserum. By using appropriate controls (CMC alone and CMC with small amounts of incubated benzylpenicillin) the penicilloyl specificity of the reaction was demonstrated. It appeared that the potency of the preparation (magnitude of anaphylactic response per µmol penicilloyl determinant) was in the same range as the potency of a highly penicilloylated polylysine (BPO-poly-L-lysine, molecular weight: approximately 3000). Results are shown in the Table.

We have recently prepared penicilloylated CMC's by dialyzing solutions of 250 mg or 100 mg benzylpenicillin



Penicilloylation of CMC in 0.04 *M* barbital buffer pH 7.4 at 4 °C. Benzylpenicillin potassium salt (800 mg) and 100 mg CMC were dissolved and stored in 5 ml buffer. ●—●, CMC Elmans, Grade AKV, pharmaceutically used in liquid formulations; ○—○ CMC available from Fluka AG, Buchs, Switzerland. Penicilloylation was followed by penamaldate assay and penamaldate stability test as described previously[1–3].

Elicitation of cutaneous anaphylactic reactions in guinea-pigs after passive sensitization with rabbit-anti-BPO-bovine γ-globulin-antiserum

Elicitor	Eliciting concentration μM BPO	mg/ml CMC	Application[a] of elicitor	Sensitization[b]	Cutaneous anaphylactic reactions in mm blue zone mean of 3 animals	1 animal
BPO-CMC	1	0.004	i.d.	i.v. 1:4	9.5	
	10	0.04	i.d.	i.v. 1:4	10	
	10	0.04	i.d.	none		4
BPO-poly-L-lysine	1	–	i.d.	i.v. 1:4	9.5	
	10	–	i.d.	i.v. 1:4	11.5	
BPO-CMC	2×10^3	8	i.v.	i.d. 1:100	20	
	2×10^3	8	i.v.	none	no reaction	
BPO-poly-L-lysine	2×10^3	–	i.v.	i.d. 1:100		24
CMC alone	–	8	i.v.	i.d. 1:100	no reaction	
CMC + benzylpenicillin[c]	–	8	i.v.	i.d. 1:100	no reaction	

[a] Intradermal (i.d.) injections of elicitors in 0.1 ml were given immediately after i.v. application of 0.5 ml 1% Evans blue. Intraveneous (i.v.) injections of elicitors were in 0.5 ml, together with 0.5 ml 1% Evans blue. [b] The i.v. and i.d. application of antiserum for passive sensitization in the indicated dilutions were performed 18–20 h before elicitation. [c] Benzylpenicillin was incubated for 20 h at 4 °C in 0.04 M barbital buffer pH 7.4 and ultrafiltered in the same way as BPO-CMC. The residual solution above the filter was mixed with CMC immediately before use in the test.

potassium salt and 20 mg CMC (Elmans) in 5 ml 0.04 M barbital buffer pH 7.4 against running tap water for 5 days. The cellulose derivatives contained 0.5 and 0.2 BPO-groups per 100 glucose units respectively and were still able to elicit cutaneous anaphylactic reactions whereas control solutions, which were dialyzed without CMC but mixed with the polysaccharide before application, were negative. It is to be expected therefore that anaphylactogenic BPO-CMC's could arise even in less than 1 h (see Figure).

Our conjugate has not been tried in skin tests on patients allergic to penicillins. It may be recalled however that a number of years ago SIEGEL[4] elicited positive skin reactions with incubated mixtures of benzylpenicillin and CMC while negative tests resulted with unincubated mixtures. From the data it appears that in fact, CMC additives are a potential source for anaphylactically active penicilloyl-CMC conjugates[5].

Zusammenfassung. Penicilloylierte Carboxymethylzellulose bildet sich schon in der Kälte beim Stehen von Penicillinlösungen mit Carboxymethylzellulose. Die entstehenden Konjugate sind befähigt, penicilloylspezifische, kutane anaphylaktische Reaktionen auszulösen.

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L-DOPA Administration to Neonate Chicks: Effects on Behavior and Levels of Brain Biogenic Amines

We have observed and subsequently reported an unusual behavioral effect of L-Dopa in neonate chicks. Animals administered this amino acid in relatively large doses (100 mg/kg) exhibited immediate signs of hyperactivity and excitement identical to those obtained with amphetamine. This was followed in several minutes by catatonia, fixed, staring, and akinesia indistinguishable from that obtained after i.v. injection of dopamine[1–3]. We were able to administer the amine parenterally in earlier experiments because of the permeable blood-brain barrier to biogenic amines that exists in the neonate[4–10]. Based on these behavioral observations, we investigated the effect of L-Dopa on concentrations of adrenaline (A), noradrenaline (NA), dopamine (DA), 5-hydroxytryptamine (5-HT), and histamine (H) in whole brain of neonate chicks.

One to 3-day-old sex-linked hybrid cockerels were administered i.v. L-Dopa (100 mg/kg) dissolved in 0.9% NaCl, whereas control animals received an equal volume of 0.9% NaCl per kg. Chicks were decapitated 10 min after injection, the peak of the catatonic response, and the brain removed and analyzed simultaneously for catecholamines, 5-HT, and H[11–13].

There was a significant decrease of 14% in brain 5-HT and a very large increase in brain DA of 12 fold. No significant changes were found in the levels of brain A, NA, or H (Table).

The results indicate that both DA and 5-HT alterations in the brain may be responsible for many of the behavioral and physiological effects of L-Dopa in chicks. Recently, EVERETT and BORCHERDING[14] have observed effects of L-Dopa in mice that are similar to those of our original