in mind it is tempting to suggest that the excitement phase and the aggressiveness of a rabies diseased animal might be due to a changed DA/5-HT ratio of the brain. Similarly, changes in the monoamine-balance of the type described might be pertinent for the understanding of the phases of excitement and aggressiveness often appearing in human cases of encephalitis[8].

Zusammenfassung. Mit *Herpes simplex* Virus i.c. beimpfte Mäuse entwickelten nach vorausgegangener Behandlung mit *p*-Chlorphenylalanin ein ausgesprochen aggressives Verhalten. Bei Mäusen, deren Serotoninsynthese durch Behandlung mit *p*-Chlorphenylalanin oder α-Propylacetamid ausser Funktion gesetzt war, konnte ausgeprägtes Aggressionsverhalten durch i.p. Injektion von L-DOPA induziert werden.

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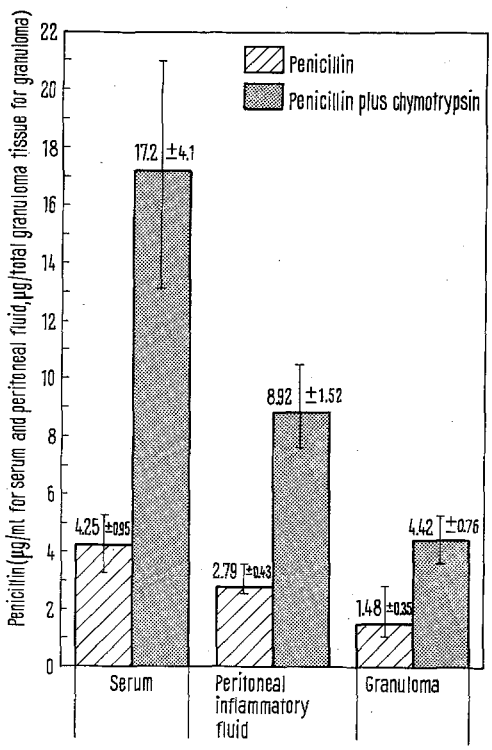
[8] We are indebted to Dr. H. Corrodi, AB Hässle, Göteborg, for having kindly provided the substances H 69/17 and H 22/54, and to Miss Gun Alfredsson, Miss Ann-Britt Björkman, Miss Anna-Karin Brorson, Mrs. Gun Lundblad and Mrs. Margareta Rosenkvist for skilful technical assistance. The study was supported by grant No. K 69-14X-2728-01A from the Swedish Medical Research Council.

Enhancement of Drug Activity by Chymotrypsin. Penicillin Penetration into Granulomatous Lesions and Inflammatory Fluids

In an earlier publication Wohlman, Syed and Ronchi[1] showed that the administration of penicillin G combined with the proteolytic enzyme chymotrypsin yields significantly higher serum, eye and brain levels of penicillin than does the administration of the antibiotic alone. It was suggested that chymotrypsin may be of considerable value in enhancing the penetration of penicillin into relatively inaccessible tissues thereby making it possible to eliminate penicillin-sensitive bacteria that have become localized in these areas. This suggestion has been experimentally tested by ascertaining the level of penicillin achieved in granulomatous and inflammatory tissues after the administration of penicillin alone and penicillin in combination with chymotrypsin.



Effect of chymotrypsin on the concentration of penicillin in serum, granuloma and inflammatory fluid.

Material and methods. The formation of granulomas in Wistar rats was accomplished by the insertion of a sterile cotton pellet into subcutaneous tissues of the axillary region. The incision was sutured and the cotton pellet allowed to remain in place for 5 days to insure complete development of granulomatous tissue surrounding the pellet. 5 days after implanting the cotton pellet the control animals received 1 ml of saline (i.p.) 30 min prior to the administration of 100 mg/kg of penicillin G (i.m.). Test animals received 10 mg/kg of pure chymotrypsin in 1 ml of saline (i.p.) 30 min before the administration of 100 mg/kg of penicillin G (i.m.) (penicillin potency: 1595 U/mg). 2 h after the administration of penicillin the rats were sacrificed. The granuloma and enveloped pellet were excised, homogenized in bovine serum albumin-phosphate buffer (pH 4.5) and centrifuged. The resultant supernatant was collected and frozen at −20 °C.

Peritoneal inflammations were induced in Wistar rats by the i.p. administration of 0.1 ml of turpentine. 15 min after the turpentine injection control animals received 1 ml of saline (i.p.) while the test animals received 10 mg/kg of pure chymotrypsin in 1 ml of saline (i.p.) 30 min after the administration of saline or chymotrypsin both control and test animals were given 100 mg/kg of penicillin G (i.m.) The rats were sacrificed 2 h after the penicillin administration and peritoneal inflammatory fluid was collected and frozen at −20 °C.

Granuloma extracts and inflammatory fluids were thawed and assayed 1 day after the experiment had been performed. The samples were appropriately diluted with bovine serum albumin-phosphate buffer (pH 4.5) in preparation for the microbiological plate assay[2]. The size of the zones of inhibition of *Staphylococcus aureus* was used as a measure of penicillin concentration. The base agar layer consisted of Difco Bacto Antibiotic Medium 2 and the seed layer consisted of Difco Bacto Antibiotic Medium 1 containing a 3% suspension of *Staphylococcus*

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Table I. The effect of chymotrypsin on the penetration of penicillin G into serum, granuloma tissue and inflammatory fluid

	Serum		Granuloma		Peritoneal inflammation	
	Penicillin	Penicillin + chymotrypsin	Penicillin	Penicillin + chymotrypsin	Penicillin	Penicillin + chymotrypsin
No. of animals	15	15	15	15	15	15
	μg/ml		μg/granuloma		μg/ml	
Mean penicillin concentration ± S.E.	4.25 ± 0.05	17.2 ± 0.35	1.48 ± 0.35	4.42 ± 0.76	2.79 ± 0.43	8.92 ± 1.52
% change		+ 305		+ 199		+ 220
Statistical significance	$P < 0.005$		$P < 0.005$		$P < 0.001$	

aureus, optical density 0.8 at a wavelength of 580 nm. The plates were incubated at 37 °C for 18 h.

In order to establish the dose-response relationships for both penicillin alone and penicillin in combination with chymotrypsin, penicillin was administered at dose levels of 25, 50, 100 and 200 mg/kg. Chymotrypsin was administered at a dose level of 10 mg/kg.

Results and discussion. The results, presented in Table I and in the Figure, clearly demonstrate that chymotrypsin significantly enhances the penetration of penicillin into serum, granuloma, and inflammatory fluid. The penicillin concentration achieved in the tissues after the administration of penicillin plus chymotrypsin is approximately four-fold greater than the antibiotic levels obtained after the administration of penicillin alone. The dose-response relationships seen in Table II indicate that the extent of enhancement is fairly constant over a wide dose range although there tends to be a more pronounced effect at the lower dose levels.

One begins to appreciate the extent of this phenomenon from calculations based on the dose-response relationships. For example, the data obtained on penicillin penetration into rat granuloma tissue reveals that in order to achieve a bactericidal level of 1 U of penicillin per milligramme of granuloma, a 70 kg human would have to receive approximately 11,600,000 U of penicillin alone or 2,800,000 U of penicillin in combination with chymotrypsin.

Table II. The penetration of penicillin into serum, granuloma tissue and inflammatory fluid as a function of the dose of penicillin both alone and in combination with chymotrypsin

	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
	Serum penicillin levels (μg/ml)			
Penicillin	2.1	3.3	6.7	12.0
Penicillin + chymotrypsin	9.1	13.3	25.9	34.5
Ratio	4.3	4.0	3.9	2.9
	Granuloma penicillin levels (μg/granuloma)			
Penicillin	0	0.4	1.1	2.3
Penicillin + chymotrypsin	0.9	1.8	4.4	6.3
Ratio	∞	4.5	4.0	2.7
	Peritoneal inflammatory fluid penicillin levels (μg/ml)			
Penicillin	1.2	1.7	4.4	6.3
Penicillin + chymotrypsin	4.8	12.1	21.3	25.6
Ratio	4.0	7.1	4.8	4.1

Preliminary investigations, designed to explore the biochemical and physiological mechanisms underlying the enzyme-induced increase in drug penetration, suggest that chymotrypsin exerts an effect on both cell membrane permeability to penicillin and the binding of penicillin to serum proteins. Evidence in support of the hypothesis that chymotrypsin increases cell permeability include the following observations: (a) chymotrypsin increases penicillin penetration into macrophages cultured in Hanks medium, and (b) after addition of chymotrypsin, isolated kidneys and eyes, being incubated in saline, become considerably more permeable to penicillin. There are however results to indicate that chymotrypsin also decreases the binding of penicillin to serum proteins. The most convincing argument in favour of decreased antibiotic-protein binding is the observation that in vitro incubation of chymotrypsin with serum containing penicillin potentiates the antibiotic activity. Since the system does not involve cells or tissues, chymotrypsin is probably releasing penicillin from its protein bound state. The details of these studies will appear in a future report.

These investigations support and extend several studies which have demonstrated that chymotrypsin enhances the absorption and distribution of various therapeutic agents[1,3–8].

Résumé. L'enzyme chymotrypsine protéolytique rehausse sensiblement la pénétration de la pénicilline G par voie intra-musculaire dans le sérum, les tissus granulomateux et le fluide péritonéal enflammé des rats.

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