not significantly re-utilized for protein synthesis in the livers of adult rats[9].

The drug-induced reduction in half-life of liver proteins was not resolved into separate components of synthesis and degradation since both half-life values were reduced. The larger reduction in organ specific activity half-life values, however, suggested that drug treatment produced a greater effect on protein degradation. As a result of an increased rate of protein degradation relative to protein synthesis the toal amount of both soluble and particulate protein was reduced in the liver.

Brain protein half-life values after cyclophosphamide were reduced as calculated by the decline in protein specific activity and unchanged when measured by the decline in organ specific activity. These observations indicated that cyclophosphamide increased protein synthesis but did not affect degradation. An increased level of synthesis may be indicative of a repair process to drug induced damage. Alkylation of DNA and disruption of the cell cycle[10], alternatively, may have permitted protein synthesis to continue in an uncontrolled fashion. The failure of a 30 min pulse of ^{14}C-leucine to detect a cyclophosphamide effect on brain protein synthesis[6] may be attributed to a drug induced reduction in the specific activity of the precursor pool.

A dose of cyclophosphamide which disrupted normal postnatal development reduced the half-life of proteins from neonatal mice. A regulated balance between protein synthesis and degradation appears to be an important component of normal growth and agents which affect this balance may disrupt subsequent growth and development. The observations with cyclophosphamide, in addition, suggest that alkylating agents may render protein molecules more susceptable to degradation. Since specific proteins were not studied it was not possible to distinguish between selective degradation of alkylated proteins and general protein degradation which resulted from cell death[11].

Zusammenfassung. Untersuchung der Cyclophosphamid-Wirkung bei 1 Tag alten Mäusen. Die Halbwertszeit von Proteinen, die man während der intrauterinen Entwicklung markierte, wurde in verschiedenen Geweben bestimmt. Ermittlung des Gleichgewichts zwischen Proteinsynthese in der Diskussion der Daten über Wachstums- und Entwicklungsbeziehungen.

R. Short[12] and J. Gibson

Michigan State University, Department of Pharmacology, East Lansing (Michigan 48824), USA, 15 October 1973.

[9] I. Arias, D. Doyle and R. Schimke, J. biol. Chem. *244*, 3303 (1969).

[10] J. Roberts, T. Brent and A. Crathorn, in *The Interaction of Drugs and Subcellular Components in Animal Cells* (Ed. P. Campbell; J. & A. Churchill, London 1968), p. 5.

[11] This work was supported by Grant No. 00560 from the National Institute of Environmental Health Sciences and R. Short was supported by NIH Training Grant No. 5T01 GM 01761.

[12] Present address, Faculty of Medicine, Division of Medical Biochemistry, The University of Calgary, Calgary 44, Alberta, Canada.

Tribenoside as an Inhibitor of Chemically Induced Histamine Release

Tribenoside[1], a drug used in the treatment of venous insufficiency of the lower leg, displays a wide spectrum of pharmacological activities, including anti-inflammatory and anti-allergic effects in various test models[2–6].

In addition to suppressing non-immunological and immunological inflammatory reactions, the compound has been shown to exert antinociceptive activity in the mouse[7]. Furthermore, it affords protection against lethal wasp-venom shock in the guinea-pig[8] and against early shock reactions to lethal doses of colchicine and nitrogen mustard in the rat[9], i.e. it possesses 'antitoxic' properties. Of particular interest with regard to its main clinical use is the pronounced venotropic action of tribenoside, which can be demonstrated in various species[10–12] as well as its membrane-stabilizing effect, e.g. protection against silica-induced labilization of lipid bilayer membranes[13] or silica-induced red cell lysis[14]. In order to gain some insight into the cellular or subcellular mechanism of action of tribenoside, we have examined its effect on the histamine release reaction following exposure of rat mast cells to compound 48/80 or C44 680-Ba. The results of this study, in which various known inhibitors of immunological or non-immunological mast cell degranulation were examined comparatively, clearly demonstrate that tribenoside is one of the most potent inhibitors of chemically induced histamine release.

Histamine liberators used: C44680-Ba, a prolyl alkyl derivative of D-Ser[1], Lys[15,17,18] β-corticotrophin, which has previously been shown to be a highly potent histamine liberator[16]. Compound 48/80 (Lot. K4023) was purchased from Wellcome Res. Lab. Beckenham, England.

Methods and materials. Suspensions of unfractionated peritoneal cells were obtained as follows: 10 ml of a modified Tyrode solution (NaCl 110 mM; KCl 2.2 mM; $CaCl_2$ 1.4 mM; Na_2HPO_4 9.4 mM; NaH_2PO_4 0.3 mM; KH_2PO_4 0.3 mM; $NaHCO_3$ 4.8 mM; $MgCl_2$ 0.08 mM; glucose 4.4 mM; pH 7.2), at body temperature was administered i.p. to male rats (Ivanovas, Ra-25) weighing 180 to 200 g. After 10 min the animals were sacrificed, the peritoneal cavity was opened and the fluid containing the cells was transferred into chilled PVC tubes. Cell suspensions from 4 rats were pooled and kept in ice-water until use. Aliquots of 1 ml were mixed with 0.5 ml solution containing the potential inhibitor and pre-incubated in a metabolic shaker at 37 °C. 15 min later, 0.5 ml of a solution with the histamine-releasing agent was added to the mixture, which was further incubated for 15 min. Incubation was stopped by transferring the

[1] Active principle of Glyvenol® (ethyl-3′5′6′-tri-*O*-benzyl-D-glucofuranoside.

[2] M. di Rosa, Archs int. Pharmacodyn. *173*, 162 (1968).

[3] H. Düngemann, Praxis *57*, 643 (1968).

[4] R. Jaques and B. Schär, Schweiz. med. Wschr. *97*, 553 (1967).

[5] L. Riesterer and R. Jaques, Experientia *24*, 581 (1968).

[6] B. Urbaschek, R. Versteyl and D. Götte, Naturwissenschaften *22*, 3187 (1967).

[7] H. Helfer and R. Jaques, Helv. physiol. Acta *26*, 137 (1968).

[8] R. Jaques, Pharmacology *2*, 21 (1969).

[9] G. Egert and L. Lendle, Pharmacology *1*, 154 (1968).

[10] H. Helfer and R. Jaques, Helv. physiol. Acta *25*, 322 (1967).

[11] H. Helfer and R. Jaques, Pharmacology *5*, 23 (1971).

[12] R. Jaques, Pharmacology *4*, 193 (1970).

[13] H. Majer, Agents and Actions *2*, 33 (1971)

[14] H. Majer, personal communication.

[15] L. M. Lichtenstein and S. Margolis, Science *161*, 902 (1968).

[16] R. Jaques and M. Brugger, Pharmacology *2*, 361 (1969).

Inhibition of histamine release, ED_{50} in mM (μg/ml)

Releasing agent/Inhibitor tested	C44680-Ba [a]		Cpd 48/80 [b]	
Diethylcarbamazine hydrochloride	∅ 100	(23.600) [c]	∅ 100	(23.600)
Diethylcarbamazine citrate	3	(1.200)	3	(1.200)
Sodium citrate	15	(2.800)	10	(1.900)
Polyphloretin phosphate (PPP)	0.02 [d]	(10)	0.02	(10)
Disodium cromoglycate	30	(15.000)	10	(5.000)
Flufenamic acid	0.2	(56)	0.1	(28)
Phenylbutazone	2	(620)	2	(620)
Sodium salicylate	20	(3.200)	60	(9.600)
Norepinephrine	0.3	(51)	—	
Isoproterenol	1	(210)	—	
Theophylline	3	(520)	10	(1.740)
Nicotinamide	20	(2.400)	—	
Nikethamide (Coramin®)	3	(520)	10	(1.740)
Tribenoside (Glyvenol®)	0.02	(10)	0.02	(10)

[a] At the concentration used (0.03 μg/ml) this compound released 64.9 $\pm$ 3.2% of the total histamine (n = 35). [b] At the concentration used (0.1 μg/ml) this compound released 61.2 $\pm$ 5.9% of the total histamine (n = 25). [c] ∅, no inhibitory activity at the concentration stated. [d] Assuming that most of the compound was in the dimer form.

tubes into ice-water and subsequent centrifugation (1000 $\times g$/5 min/0 °C). The histamine was extracted (n-heptanol) from the supernatant and determined after condensation with o-phthalaldehyde (OPT) according to REDLICH and GLICK[17,18].

The capacity of a substance to inhibit the release of histamine was calculated as follows:

$$\text{Inhibition (\%)} = 1 - \frac{\text{ng histamine released on exposure to liberator and inhibitor}}{\text{ng histamine released on exposure to liberator without inhibitor}} \times 100\%$$

All histamine values were corrected for spontaneous histamine release (mean 6.4%).

Results and discussion. Of the two histamine liberators used, C44680-Ba proved some 3 to 10 times more active

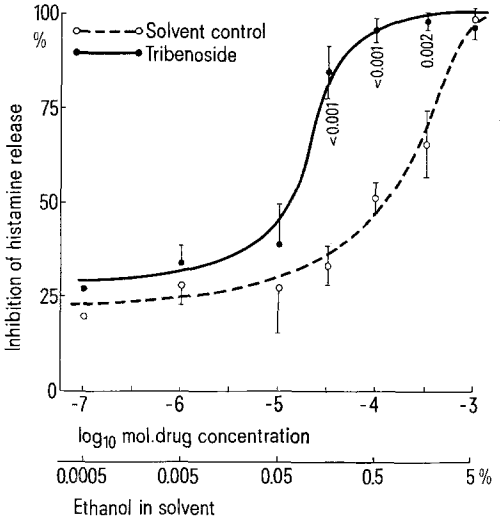

Fig. 1. Dose-dependent inhibitory effect of tribenoside on histamine-release induced by C44680-Ba in a rat peritoneal-cell system. The solid line represents the inhibitory capacity of tribenoside and the solvent, the broken line the effect of the solvent alone. The P-values of the means ($\pm$ SD) were calculated against the corresponding solvent system, the ethanol content of which is indicated.

than cpd. 48/80, thus confirming our previous finding made in a somewhat different test system[16]. The results obtained with a representative selection of compounds examined with regard to a possible inhibitory activity are listed in the Table. It is clear from the Table that anti-inflammatory drugs such as flufenamic acid, phenylbutazone and sodium salicylate, in descending order of activity, are capable of protecting rat mast cells against release of histamine induced by either cpd. 48/80 or C44680-Ba. Of the 3 compounds which have repeatedly been reported to exert inhibitory effects on reactions involving immunological or non-immunological release of mast-cell-derived histamine, only polyphloretin phosphate (PPP)[19] exerts clear-cut inhibitory activity against both liberators, while disodium cromoglycate displays only a very weak action as already reported by BAECKELAND and LECOMTE[20]. The activity displayed by PPP against 48/80-induced histamine release is in good accordance with the results published by HÖGBERG and UVNÄS[21].

Although mainly classified as agents possessing either anti-inflammatory or anti-allergic properties, all the compounds examined in this study with regard to their ability to suppress histamine release induced by compound 48/80 or C44680-Ba have been reported to inhibit various immunological reactions, e.g. PCA, Schultz-Dale reaction, Arthus phenomenon or release of mediators following antigen-antibody reactions[3,4,15,22–35].

Of special interest in this connection is our present finding that diethylcarbamazine citrate, but not diethylcarbamazine hydrochloride, inhibits histamine release elicited by compound 48/80 or C44680-Ba. It is therefore an open question whether the inhibitory action of diethylcarbamazine on immunologically caused mediator release, as observed by ORANGE et al.[31] and EYRE[23], should be regarded as an isolated phenomenon or attributed to an effect of the citrate anion rather than to that of the cation.

[17] D. VON REDLICH and D. GLICK, Analyt. Biochem. *10*, 459 (1965).
[18] D. VON REDLICH and D. GLICK, Analyt. Biochem. *29*, 167 (1969).
[19] Kindly gifted by Dr. B. HÖGBERG, Leo Laboratories, Helsingborg, Sweden.
[20] E. BAEKELAND and J. LECOMTE, C. r. Soc. Biol., Paris *166*, 218 (1972).
[21] B. HÖGBERG and B. UVNÄS, Acta physiol. scand. *41*, 345 (1957).
[22] N. CHAKRAVARTI, Acta physiol. scand. *48*, 146 (1960).

Another compound deserving interest is nikethamide (Coramin®), a respiratory stimulant, which inhibits histamine release in our test system at roughly the same concentration at which it has been reported to suppress anaphylactic histamine release in a guinea-pig mast cell or lung slice system[28]. Nicotinamide, which is an inhibitor of diphosphopyridine nucleotidase like nikethamide but lacks respiratory stimulant activity, has previously been shown to inhibit anaphylactic histamine release from various tissues[26,28,36] and is also active in our system. On the other hand, very low concentrations of the glucofuranoside derivative, tribenoside, protect rat mast cells against histamine release induced by compound 48/80 or C 44 680-Ba, the ED_{50} being 0.02 mM (10 µg/ml) in both cases (Figure 1). Besides being more active than flufenamic acid, tribenoside is also less easily washed off from treated cells. In the experiment outlined in Figure 2, the concentration of these compounds was chosen so as to produce a 50% inhibition. It is evident that after 1 washing tribenoside caused about 40% and flufenamic acid about 20% inhibition, whereas after 3 washings the inhibitory capacity of tribenoside still remained about 20% and flufenamic acid displayed no activity at all.

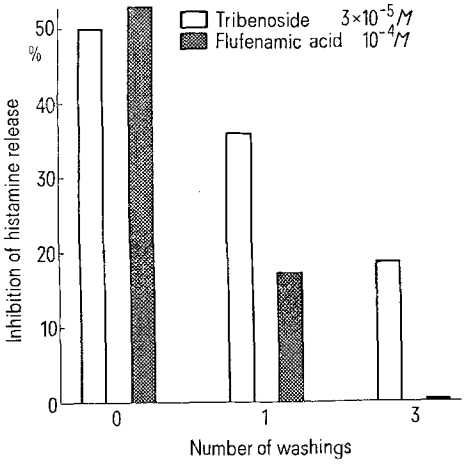

Fig. 2. Inhibition of histamine-release by tribenoside and flufenamic acid after washing-out procedures. The left-hand columns represent the inhibitory effect obtained in the presence of the inhibitors, and the middle and right-hand columns the results obtained after the cell suspensions had been washed once or 3 times, respectively. In these cases the supernatant pipetted off was replaced by fresh cell-free peritoneal fluid, diluted 1:1 with the medium in which the substances were dissolved.

Zusammenfassung. In einem Peritonealzell-System der Ratte zeigt ein Alkyl-Prolyl-Derivat von Corticotrophin (C 44 680-Ba) eine grössere Histamin-freisetzende Wirkung als Compound 48/80. Dieser Effekt der beiden Substanzen kann durch Verbindungen gehemmt werden, die anti-inflammatorische und/oder anti-allergische Eigenschaften besitzen. Tribenosid (Glyvenol®) und Polyphloretin-phosphat erweisen sich als sehr aktive Inhibitoren dieser chemisch induzierten Histamin-Freisetzung, während Diaethylcarbamazin und Dinatriumcromoglycat nur einen geringen Hemmeffekt ergeben.

M. Rüegg and R. Jaques[37]

Biological Department, Pharmaceutical Division, Ciba-Geigy Ltd., CH–4002 Basel (Switzerland),
3 October 1973.

[23] P. Eyre, Br. J. Pharmac. *43*, 302 (1971).
[24] B. Fredholm and K. Strandberg, Int. Conf. on Prostaglandins, Vienna 1972.
[25] M. C. Hines, G. F. Moss and J. S. G. Cox, Biochem. Pharmac. *21*, 171 (1972).
[26] Ch. D. May, B. B. Levine and G. Weissmann, Proc. Soc. exp. Med. *133*, 758 (1970).
[27] J. L. Mongar and H. O. Schild, J. Physiol., Lond. *135*, 301 (1957).
[28] I. Mota, W. D. Da Silva and J. F. Fernandes, Br. J. Pharmac. *15*, 405 (1960).
[29] S. Norn, Acta pharmac. toxic. *22*, 369 (1965).
[30] S. Norn, Acta pharmac. toxic. *30*, suppl. 1, 1 (1971).
[31] R. P. Orange, M. D. Valentine and K. F. Austen, Proc. Soc. exp. Biol. Med. *127*, 127 (1968).
[32] W. Smith and J. H. Humphrey, Br. J. exp. Path. *30*, 560 (1949).
[33] K. Strandberg and A. A. Mathé, Life Sci. *11*, 701 (1972).
[34] E. R. Threthewie, Aust. J. exp. biol. med. Sci. *29*, 443 (1951).
[35] E. R. Threthewie, Aust. J. exp. Biol. *35*, 541 (1957).
[36] E. Middleton and A. Devi, J. Allergy *34*, 331 (1963).
[37] The skilful technical assistance of Miss S. Thorwirth and Mr. U. Niederhauser is gratefully acknowledged.

Über den Einfluss der Kettenlänge bei C-terminalen Sequenzen der Substanz P — in Vergleich mit analogen Physalaemin- und Eledoisin-Peptiden — auf die Wirksamkeit am Meerschweinchen-Ileum

Der von Euler und Gaddum[1] in Hirn und Darm des Pferdes entdeckte und als Substanz P (SP) bezeichnete Faktor konnte von Vogler et al.[2], Boissonnas et al.[3] und Zuber[4] (auch aus anderen Spezies) isoliert und gereinigt werden. An der von Lembeck[5] gefundenen, die Speichelsekretion stimulierenden Wirkung konnten Chang und Leeman[6] die Identität mit einem aus Ratten- und Rind-Hypothalamus isolierten sialogenen Peptid nachweisen und dessen Sequenz[7] aufklären. Durch Synthese erhaltenes Material war hinsichtlich hypotensiver, sialogener Wirkung, Kontraktion an Meerschweinchen-Ileum und Ratten-Duodenum vergleichbar[8]. Studer et al.[9] fanden für SP aus Pferdedarm die gleiche Sequenz.

Das mit Eledoisin, aus der Speicheldrüse des Octopoden *Eledone moschata*[10] und Physalaemin, aus der Haut des Frosches *Physalaemus fuscumaculatus*[11] vergleichbare Wirkungsbild spiegelt sich auch in der Strukturähnlichkeit dieser drei hypotensiven Peptide wider (Figur 1).

Arg-Pro-Lys-Pro-Gln-	Phe-	Phe-	Gly-Leu-Met-NH_2	SP
pGlu-Ala-Asp-Pro-Asn-Lys-	Phe-	Tyr-	Gly-Leu-Met-NH_2	Ph
pGlu-Pro-Ser-Lys-Asp-Ala-	Phe-	Ile-	Gly-Leu-Met-NH_2	El

Fig. 1. Vergleich der Sequenzen von Substanz P (SP), Physalaemin (Ph) und Eledoisin (El)

Lübke et al.[12] fanden an C-terminalen Sequenzen des Eledoisins, dass, beginnend vom Pentapeptid, die Wirksamkeit schrittweise zunimmt und das Okta- bzw. Nonapeptid mehrfache Wirkung des Undekapeptides zeigen. Dies bestätigten Bernardi et al.[13] auch für das Ph, wobei hier das Hexapeptid 6–11 bereits volle Wirkung zeigt.