from Duner & Pernow (1958), who showed a correlation of blood level of histamine and 24 h urine volume in man given a continuous infusion of histamine. These results cast doubt on the utility of urinary histamine output as a measure of histamine turnover.

Smith and Nephew Research Ltd.,
Gilston Park,
Harlow,
Essex, U.K.

M. HORLINGTON
J. C. KOLTHAMMER
R. LAZARE

June 10, 1970

REFERENCES

ANREP, G. V., AYADI, M. S., BARSOUM, G. S., SMITH, J. R. & TALAAT, M. M. (1944). *J. Physiol, Lond.*, **103**, 155–174.

DUNER, H. & PERNOW, B. (1956). *Scand. J. clin. Lab. Invest.*, **8**, 296–303.

DUNER, H. & PERNOW, B. (1958). *Ibid.*, **10**, 390–393.

JOHNSTON, M. & KAHLSON, G. (1967). *Br. J. Pharmac. Chemother*, **30**, 274–282.

LINDELL, S. E. & WESTLING, H. (1966). *Handbook of experimental pharmacology*, Vol. XVIII/I, pp. 762–3. Berlin: Springer.

OATES, J. A., MARSH, E. & SJOERDSMA, A. (1962). *Clin. chim. Acta*, **7**, 488–497.

Macrocationic enzyme inhibition: pepsin and two polylysines

The strongly acid nature of pepsin is explained by its relatively high content of acidic amino-acids (Taylor, 1968). This property forms the principal basis of association between pepsin and its highly basic natural inhibitor which, together with miscellaneous peptides, constitute the enzymatically inactive pepsinogen (Herriott, 1962). A similar basis has been adduced for the interaction between pepsin and certain basic polyamino-acids, notably poly-L-lysine; and for one polylysine (degree of polymerization, n = 36) inhibition of peptic activity at pH 1·7 and 6 was claimed, digestion of haemoglobin and the clotting of milk being used at the two pH values respectively, to measure the activity of the pepsin (Katchalski, Berger & Neumann, 1954). These authors found that the inhibitory action of polylysine appeared to be instantaneous because pre-incubation of pepsin and polylysine for 5–30 min yielded a constant level of inhibition. It was shown later (Dellert & Stahmann, 1955) that the amount of inhibition of pepsin by a polylysine of mol. wt. 2580 rose to completion when a certain polylysine concentration range was entered, but disappeared as the macrocation concentration was increased to excess. In addition, insoluble complex formation between the polylysine and pepsin did not occur at high macrocation concentrations where inhibition was absent. This appears to be in accord both with knowledge of macroion interaction and the notion that the resulting pepsin inhibition is due to insoluble complex formation involving pepsin and the macrocation. This report deals with the inhibition of pepsin by two polylysines of different molecular weights.

The poly-L-lysines (LY102, LY115) had stated molecular weights of 12 300 and 43 870 (degree of polymerization 59 and 210 respectively), were derived from parent poly ε-carbobenzoxy-L-lysines and were purchased from Miles-Yeda Ltd., Rehovoth, Israel. They were used in solution in acetate buffer, pH 5, I = 0·05 (Long, 1961).

Twice crystallized pig pepsin (Sigma) was used at a concentration of 200 μg/ml in buffer. Gayelord-Hauser dried skim milk was reconstituted by triturating 20 g with water, adding 10 ml buffer, 5 ml 0·2M $CaCl_2$ and diluting to 100 ml with water. Solutions were mixed at 35·5° and milk clotting was carried out at this temperature.

Polylysine solution (or buffer) was mixed in equal volume with pepsin solution and at intervals of time after mixing 0·5 ml of the mixture was added to 5 ml reconstituted

milk and mixed. The time of occurrence of the first granular appearance in the film of milk on the walls, formed by gently tilting the tube, was taken as the end point. This immediately precedes coagulation. The technique is similar to that of Lee & Ryle (1967). Proteolytic units, $[P.U.]^{Ren.}_{ml}$, were calculated (Northrop, Kunitz & Herriott, 1955) and inhibition, i, calculated by expressing the difference in units between the uninhibited control and the inhibited as a percentage of the uninhibited control. Apparent absorbances of the polylysine-pepsin mixtures were read at 400 nm at the same time intervals. Inhibitions are recorded as the maximum (i_{max}) occurring during the 3 h after mixing of polylysine and pepsin and the maximum (i'_{max}) occurring during the first 0·5 h.

Fig. 1 shows that inhibition increases over 3 h particularly for LY115 and that low and high inhibitions conform to this pattern.

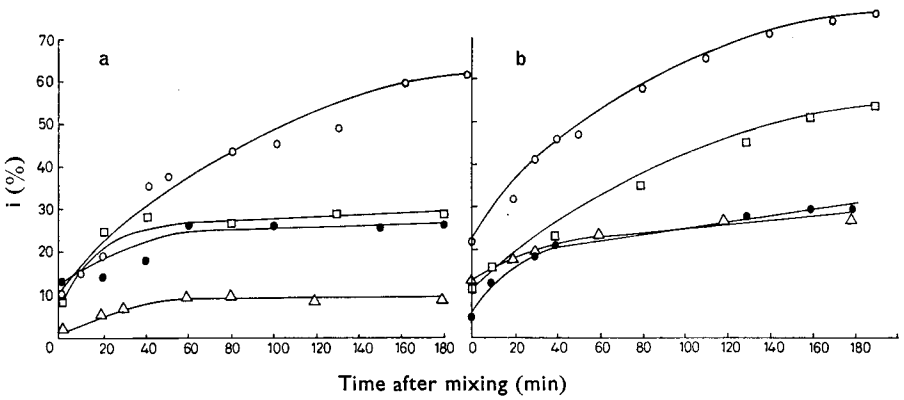

FIG. 1. Pepsin inhibition with time at varying polylsyine concentrations, a. LY102 ○, 70 μg/ml; □, 50 μg/ml; ●, 200 μg/ml; △, 400 μg/ml. b. LY115 ○, 40 μg/ml; □, 30 μg/ml; ●, 50 μg/ml; △, 800 μg/ml.

Table 1 shows that there is a polylysine concentration at which inhibition is a maximum and higher and lower concentrations result in lower, or no, inhibition. Thus for maximum inhibition for LY102 and LY115 concentrations (and polylysine: pepsin mol ratios) were 70 μg/ml (0·99) and 40 μg/ml (0·16) respectively.

Both polylysines follow similar concentration–inhibition patterns, although the higher molecular weight substance (LY115) appears twice as active on a weight basis, probably indicating an easier access to a larger number of interacting sites on the two macromolecules, pepsin and polylysine.

In only one instance was i_{max} observed to occur within 0·5 h (LY115, 40 μg/ml), although even in this instance inhibition increased by about 60% during this period of time. For other concentrations of both polylysines inhibition usually reached a maximum towards 3 h and i_{max} was usually greater than i'_{max}. Maximum absorbances did not usually occur at the same time as maximum inhibition; indeed for the highest inhibitions by both polylysines the maximum absorbance occurred within 40 min after mixing pepsin and polylysine while i_{max} itself usually occurred at around 180 min. Highest inhibition therefore appears to be associated with the most rapid rate of formation of insoluble complex.

It appears that the interaction between pepsin and these polylysines is not completed instantaneously and that some aspect of the interaction proceeds with time, involving the active sites on the pepsin molecule. Whether it is confined to these sites or is an indication of progressive involvement of the whole pepsin molecule in complexed form is not yet clear.

Table 1. *Pepsin inhibition and absorbances resulting from mixing pepsin and polylysine at pH* 5. Pepsin concentration was 200 μg/ml. Mole ratio (polylysine/pepsin) range was LY102 0·29–5·7, LY115 0·08–5·5. Inhibition measured by milk clotting. Concentrations of pepsin and polylysine are quoted before mixing.

Polylysine	Polylysine concentration μg/ml	i_{max} (%) (absorbance at i_{max})	i'_{max} (%) (absorbance at i'_{max})	Maximum absorbance and time (min) of occurrence
LY102	20	12 (0·191)	12 (0)	0·191 (130)
	40	30 (0·284)	29 (0·254)	0·284 (140)
	50	29 (0·246)	24 (0·244)	0·280 (50)
	70	67 (0·341)	41 (0·300)	0·349 (40)
	80	45 (0·345)	14 (0·185)	0·370 (110)
	120	26 (0·210)	0	0·238 (180)
	160	27 (0·209)	11 (0·05)	0·209 (180)
	200	26 (0)	13 (0·03)	0·058 (130)
	320	18 (0)	10 (0)	0
	400	9 (0)	7 (0)	0
LY115	20	15 (0·116)	15 (0·132)	0·146 (60)
	30	54 (0·511)	18 (0·476)	0·731 (60)
	40	79 (0·254)	46 (0·572)	0·366 (40)
	50	29 (0·134)	18 (0·084)	0·136 (70)
	70	24 (0·225)	5 (0·087)	0·225 (180)
	80	21 (0·160)	2 (0·150)	0·155 (120)
	120	25 (0)	3 (0)	0
	200	25 (0)	12 (0)	0
	700	25 (0)	17 (0)	0
	800	27 (0)	20 (0)	0
	1400	36 (0)	30 (0)	0

Earlier reports (Katchalski & others, 1954; Dellert & Stahmann, 1955) dealing with other polylysines have claimed or assumed instantaneous completion of interaction and attainment of maximum inhibition. It is interesting to note that some inhibition can be demonstrated in the absence of insoluble complex formation, although highest inhibitions were always accompanied by precipitation, the precipitate later aggregating and flocculating.

I thank Miss J. E. Harthill for technical assistance.

School of Pharmaceutical Sciences,
University of Strathclyde,
*Glasgow, C.*1, *U.K.*

W. ANDERSON

May 19, 1970

REFERENCES

DELLERT, E. E. & STAHMANN, M.A. (1955). *Nature, Lond.*, **176**, 1028–1029.

HERRIOTT, R. M. (1962). *J. gen. Physiol.*, **45**, 57–75.

KATCHALSKI, E., BERGER, A. & NEUMANN, H. (1954). *Ibid.*, **173**, 998–999.

LEE, D. & RYLE, A. P. (1967). *Biochem. J.*, **104**, 735–741.

LONG, C. (1961). *Biochemists' Handbook.* London: Spon.

NORTHROP, J. H., KUNITZ, M. & HERRIOTT, R. M. (1948). *Crystalline Enzymes* 2nd edn. New York: Columbia University.

TAYLOR, W. H. (1968). In *Handbook of Physiology*, Section 6, Chap. 120, pp. 2567–2587. Editors: Code, C. F. and Heide, W. Washington: American Physiological Society.