We can conclude, so far, that fusaric acid acts in two ways on the metabolism of 5-HT: on one hand, a central effect common to most DBH inhibitors and, on the other hand, a peripheral action due to the inhibition of the binding of tryptophan to serum albumin. Studies are in progress to confirm this hypothesis and to try to dissociate the two components of the fusaric acid action.

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Effects of a water-soluble carbodiimide on the osmotic fragility and ion permeability of erythrocytes

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GODIN and Schrier[1] have shown that a progressive inactivation of ATPase activity was found when human erythrocyte ghosts are treated with the water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC), with the Mg^{2+}-dependent ATPase showing a greater susceptibility to inactivation when compared with the Na^{+}-K^{+}-Mg^{2+}-dependent ATPase. These authors conclude that carbodiimide attack on the erythrocyte membrane causes a selective structural perturbation which in turn causes a disruption of the ATP-hydrolysing system. We have previously suggested[2–7] that the Mg-ATPase located in the erythrocyte membrane could be implicated in the control of passive ion permeability and of the disc shape of this cell. Schoffeniels[8] has extended this hypothesis and regards the Ca^{2+}-stimulated Mg^{2+}-dependent ATPase as being of particular importance in the control of cation permeability. The purpose of the experiments reported in this communication was to determine whether EDAC applied to the outer surfaces of intact pig erythrocytes was able to modify their anion permeability and osmotic fragility.

Heparinized pig blood was obtained fresh from the slaughterhouse, centrifuged and the plasma and buffy coat discarded. Erythrocytes were washed three times in 0·9% NaCl (pH 7·4), and the final PCV was suspended in an equal volume of 0·9% NaCl. Three ml of suspended erythrocytes were mixed with 1 ml 0·9% NaCl which contained EDAC. The concentrations of EDAC given are the final concentration of this agent during the period of preincubation. Exposure to EDAC was for varying periods, but was usually for 30 min, either at 19° or on ice. 0·1 ml samples were then taken and added to 5 ml NaCl solution of appropriate dilution. The method of determination of osmotic resistance (fragility) followed that of Parpart *et al.*;[9] NaCl solutions (including those described above) were made by dilution of a stock NaCl–PO_4 solution to give a standard pH of 7·40. Erythrocytes and NaCl were equilibrated for 45 min in a water bath at 25° and haemolysis was stopped by the addition of the complementary solution[9] which served to return the osmotic pressure around the unhaemolysed cells to normal tonicity. Erythrocytes were then removed by centrifugation and the supernatant was read at 540 nm.

Chloride entry into erythrocytes, following exposure to EDAC, was measured using a modification of the method described by Edelberg[10]; in the presence of NH_4Cl the rate of erythrocyte swelling is dependent on the rate of exchange of external Cl^- for internal OH^-. 0·1 ml samples of washed erythrocytes were added to a mixture of 3 ml M/6 NH_4Cl plus 2 ml 0·9% NaCl and the progress of haemolysis was then followed in a spectrophotometer at 540 nm.

Cholinesterase activity in intact erythocytes, after exposure to EDAC, was measured by adding 0·3 ml suspended erythrocytes to 2·7 ml of 1 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) plus 5 mM acetylthiocholine iodide made up in 154 mM NaCl, pH 7·0 (incubation = 10 min at 25°); the reaction was stopped after 10 min by the addition of 0·5 ml 3×10^{-3} M physostigmine. Erythrocytes were removed by centrifugation and the supernatant was read at 412nm.[11]

All inorganic salts were AnalaR grade (B.D.H. Ltd, Poole, Dorset); other reagents were obtained from Sigma Chemicals Inc., St. Louis, U.S.A.

Osmotic fragility curves, showing per cent haemolysis against per cent concentrations of NaCl, were constructed for the erythrocytes after previous exposure to different concentrations of EDAC and these sigmoidal curves were transformed into straight lines by probit analysis (Fig. 1). The probit range 3·0–6·5 covers from 3 to 93 per cent of the cells not haemolysed after treatment. Previous exposure for 30 min to 6·5 mM or 10mM EDAC caused a clear increase in osmotic fragility; the concentration of NaCl required to produce 50% haemolysis changed from 0·585 to 0·600 per cent (6·5 mM EDAC; $P < 0.01$) or to 0·625 per cent (10 mM EDAC; $P < 0.001$), exposure being carried out at 19° and under the conditions defined above. 3 mM EDAC produced a significant increase in fragility, ($P < 0.01$) although the probit line lay close to that for control erythrocytes. The probit lines are parallel, showing that the majority of cells are affected to an equal extent. The effect of low concentrations of EDAC was studied by examining subsequent haemolysis in 0·600, 0·575 and 0·550% NaCl in detail. 2 mM and 1 mM EDAC consistently produced a small increase in fragility, whereas the action at 0·5 and 0·1 mM was not significant. The interaction between the EDAC and erythrocytes was time-dependent, although it was found to be almost complete within 5 min and exposure for a further 25 min produced only a small additional increase in fragility.

EDAC also accelerated the rate of entry of Cl^-. Figure 2 shows a 14 per cent decrease in the time taken for 50% haemolysis following preexposure to 10 mM EDAC. Small increases in the rate of haemolysis were also found after exposure to 5 and 3 mM EDAC.

The cholinesterase activity shown by intact erythrocytes was almost completely inhibited by 3 mM EDAC; activity fell to 12 per cent of control values at 2 mM and smaller inhibitions were also found after exposure for 30 min to 0·5 and 0·1 mM (73 and 92 per cent of normal activity respectively).

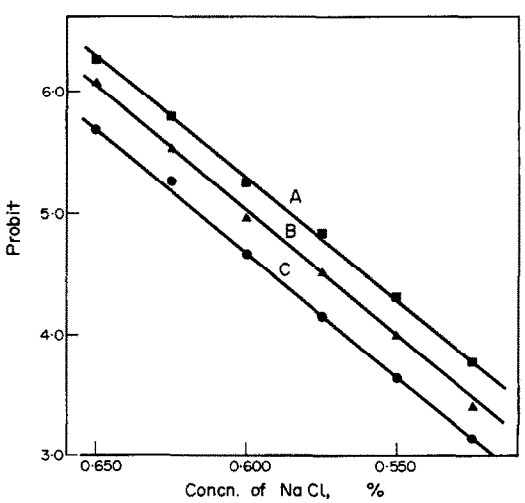

FIG. 1. Effect of different concentrations of EDAC on the osmotic fragility of pig erythrocytes. Washed erythrocytes were suspended in buffered isotonic NaCl and preincubated with EDAC solutions of appropriate concentration for 30 min at 19°. The degree of haemolysis was subsequently determined over a range of NaCl concentrations, as described in methods and Ref. 9. Line A = Control; Line B = 6·5 mM EDAC; Line C = 10 mM EDAC. Ordinate = probit of per cent cells not haemolysed after 45 min; abscissa = % concentration of NaCl.

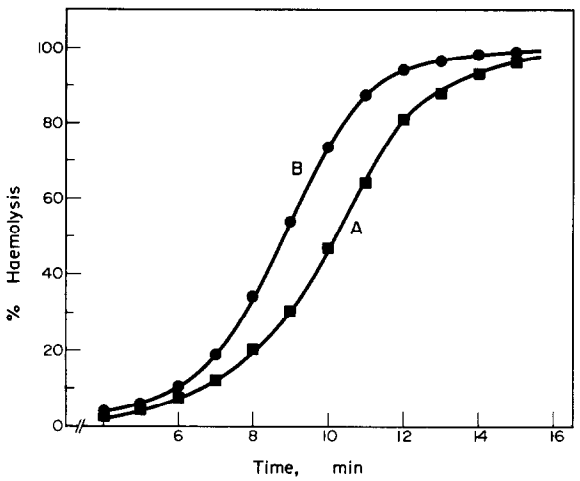

FIG. 2. Effect of 10 mM EDAC on chloride entry into pig erythrocytes. Washed erythrocytes were suspended in buffered NaCl and preincubated with 10 mM EDAC for 30 min at 19°. The % haemolysis, when the cells were subsequently exposed to NH_4Cl, was followed at 540 nm. Duplicated experimental and control tubes were followed simultaneously. Ordinate = mean % haemolysis; abscissa = time (min). A = control; B = after exposure to 10 mM EDAC.

These results demonstrate that EDAC applied to intact erythrocytes in the concentration range 3–10 mM clearly affect their osmotic fragility and anion permeability. Carbodiimides are known as inhibitors of cholinesterases, the main effect of EDAC being to inhibit the Ca^{2+} activation of purified acetylcholinesterase.[12] The present study confirms previous findings that the surface cholinesterase activity of erythrocytes is largely inhibited at the concentrations that modify membrane integrity. It is possible, therefore, that, in the intact erythrocyte, EDAC operates via an attack on the surface cholinesterase. However, a number of anticholinesterase agents at concentrations that caused complete inhibition of surface cholinesterase activity were found to have no effect on K^+ influx in monkey erythrocytes.[13] The findings of this study are also consistent with the hypothesis that Mg^{2+}-dependent ATPases are involved in the maintenance of the shape and integrity of erythrocytes and may be implicated in the control of ion-permeability.

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Demonstration of the *in vivo* action of pepstatin: effects on plasma angiotensin II concentration*

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PEPSTATIN, an *N*-acylated pentapeptide obtained from culture filtrates of actinomycetes, was originally characterized as an inhibitor of pepsin and other acid proteases.[1] Subsequently, we[2,3] and others[4] have demonstrated that pepstatin inhibits the reaction of the "neutral" protease renin with its plasma substrate. The fall in blood pressure induced by pepstatin in certain experimental conditions in the rat may be a consequence of such an inhibition. While it has been clearly demonstrated that pepstatin is a true enzymatic inhibitor of renin *in vitro*,[4]† no studies to more precisely define its mechanism of action *in vivo* have been undertaken. In the present paper, we report on experiments which show that, in the rat, pepstatin diminished the formation of angiotensin during the infusion of renin.

TABLE 1. EFFECTS OF PEPSTATIN ON PLASMA ANGIOTENSIN II CONCENTRATION IN NEPHRECTOMIZED RATS GIVEN A SINGLE INJECTION OF 0·05 DOG UNITS HOG RENIN

		Blood pressure (mm Hg)				
	n	Before renin	At 1st blood sampling	Before pepstatin or saline	After pepstatin or saline	BP
Pepstatin	10	113 ± 4	136 ± 4	118 ± 4	109 ± 4	−9* ± 31
		N.S.	N.S.	N.S.	$P < 0{\cdot}03$	$P < 0{\cdot}01$
Control	10	112 ± 5	139 ± 5	120 ± 4	120 ± 4	
		Angiotensin II plasma concentration (pg/ml)				
Pepstatin	10		190 ± 8		63 ± 7	
			N.S.		$P < 0{\cdot}0005$	
Control	10		208 ± 11		124 ± 12	

Values are means ± S.E.M.
* $P < 0{\cdot}001$ as compared with BP values before pepstatin injection, paired data.
N.S. = not significant.

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