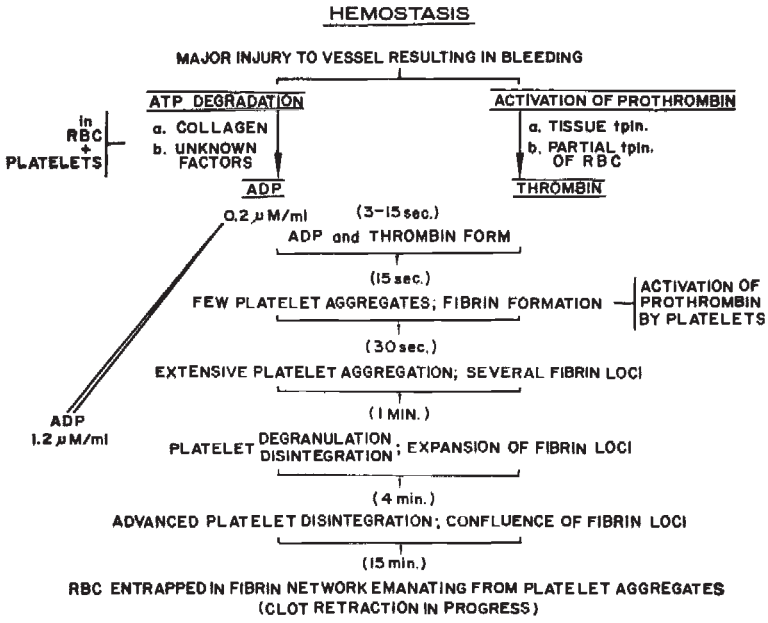

Fig. 5. A schema of the mechanisms of haemostasis after major injury to vessel wall resulting in bleeding

degradation is measured in clotting whole blood, a very complicated pattern emerges, involving formation of adenosine monophosphate and perhaps adenosine after certain critical levels of ADP are reached. The amount of residual coagulation components in shed blood is not known, but the appearance of fibrin does prove that the coagulation mechanisms are initiated.

The stimulus for degradation of ATP in shed blood must certainly come from the damaged vessel wall, even though detectable amounts of adenosine nucleotides are not released into the blood as it passes the injured cells (Table 2). Hellem[1] has stated that ADP release from red blood cells is brought about by contact with a foreign surface. The stimulus may be from the contact with the air, with collagen, or tissue thromboplastin from the injured vessel wall or some as yet undescribed component. The injured vessel wall must change very rapidly, for aggregated platelets building up to form a haemostatic plug must alter the characteristics of the orifice through which the blood is escaping. The concentrations of ADP formed in whole shed blood are much higher than those necessary to initiate platelet aggregation in platelet-rich plasma in either rabbit or human material.

The role of red blood cells in the arrest of bleeding should not be disregarded. Since few platelets were seen in our electron micrographs after 15 sec of bleeding and 20 times as many red blood cells are present in circulating blood as platelets, the ADP we measured probably comes in large part from the red blood cells. The close proximity of fibrin to red blood cells suggests that the partial thromboplastin of red blood cells described as thromboplastic cell component by Shinowara[14] may account for the activation of prothrombin to thrombin, thus converting fibrinogen to fibrin.

Fig. 5 summarizes our data on haemostatic plug formation when major injury to a vessel wall resulting in bleeding has occurred.

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These results show that thrombin and ADP form a few seconds after blood is shed, and both are available to bring about platelet aggregation before the necessary platelets are available. Our schema of the mechanisms of haemostasis (Fig. 5) attempts to incorporate both activation of prothrombin and degradation of ATP. The interrelation of these two mechanisms remains to be elucidated.

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PROTECTION OFFERED TO POLIOVIRUS-INFECTED TISSUE-CULTURE CELLS BY METHOXY- AND HYDROXY-METHYL COMPOUNDS RELATED TO 2-BENZYLBENZIMIDAZOLE

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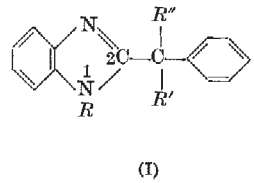
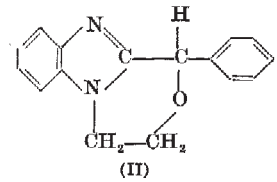
THE high degree of protection conferred by 1-alkyl-2-(α-hydroxybenzyl)-benzimidazoles (I; R = alkyl, R' = H, R'' = OH) (ref. 1) on rabbit-embryo kidney cells, infected with types 1, 2 and 3 poliovirus, makes derivatives with modified alkyl substituents at position 1 of particular interest. We have found 1-(β-methoxyethyl)-2-(α-hydroxybenzyl)-benzimidazole (I; R = $-CH_2CH_2OMe$, R' = H, R'' = OH) (ref. 2) (m.p. 135°–136°) to be less toxic than the simple 1-alkyl derivatives towards rabbit-embryo kidney cells, the maximum tolerated concentration of the ether being 300 μmoles/l. (μM).


(I)


(II)

This compound, at 150 μM concentration, affords rabbit-embryo kidney cells considerable protection against the cytopathic consequences of infection with types 1, 2

Table 1. PROTECTION GIVEN BY 1-BUTYL-, 1-β-METHOXYETHYL- AND 1-PENTYL-HBB TO CELLS INFECTED WITH TYPES 1, 2 AND 3 POLIOVIRUS

Virus		Mean delay (days) between infection with virus and degeneration of half the cell population						
			0·25 M.T.C.			0·5 M.T.C.		
Type	Conc.	Control*	1-Bu (15 μM)	1-MeOCH$_2$CH$_2$- (75 μM)	Control*	1-Bu (30 μM)	1-MeOCH$_2$CH$_2$- (150 μM)	1-Pentyl (50 μM)
1	10^{-1}	1·0	1·5	1·25	1·0	4·75	1·5	1·25
	10^{-2}	1·75	3·75	2·5	1·75	> 5·0	> 5·0	2·25
	10^{-3}	2·0	6·0	4·5	2·25	> 5·0	> 5·0	3·5
3	10^{-1}	0·75	1·25	1·0	0·75	1·5	1·25	1·0
	10^{-2}	1·25	2·0	1·5	1·0	2·75	2·0	1·75
	10^{-3}	1·75	2·75	2·25	1·5	3·75	3·0	2·25
					1-Pentyl (25 μM)			
2	10^{-1}	1·25	4·25	2·75	2·25			
	10^{-2}	1·75	6·25	4·25	4·0			
	10^{-3}	2·25	7·5	> 5·0	> 5·0			

Times are quoted to the nearest quarter of a day.
Approximate initial virus concentration (before dilution): $10^{7\cdot2}$ TCD_{50} units per ml. (types 1 and 2 virus) and $10^{7\cdot5}$ TCD_{50} units per ml. (type 3 virus).
* Infected control containing no protective agent. Uninfected and untreated controls always survived more than 5 days. Examination of culture tubes was usually stopped on the fifth day.
M.T.C., maximum tolerated concentration.

and 3 poliovirus. The length and shape of the β-methoxyethyl- are similar to those of the butyl-substituent. Table 1 shows that the protection given by the 1-β-methoxyethyl derivative (I; $R = -CH_2CH_2OMe$, $R' = H$, $R'' = OH$) lies between that given by the 1-butyl (ref. 1) and 1-pentyl (ref. 3) derivatives (all tested in tissue-culture by the method previously outlined[1,4]).

An attempt to prepare 1-(β-hydroxyethyl)-2-(α-hydroxybenzyl)-benzimidazole by demethylation of the 1-β-methoxyethyl compound gave instead the cyclic ether (II) (ref. 2) (m.p. 161°–162°). This ether (maximum tolerated concentration 600 μM) is much less toxic to rabbit-embryo kidney cells than other benzimidazoles we have tested and, at 300 μM, has selective protective action (activity/toxicity ratio) against the 3 poliovirus types which, although high, is less than that of the 1-β-methoxyethyl derivative (Tables 1 and 2). It is slightly less effective than the 1-pentyl compound against the type 2 virus, but more effective than the 1-pentyl compound against the types 1 and 3 viruses. The two ethers, (I; $R = -CH_2CH_2OMe$, $R' = H$, $R'' = OH$) and (II), offer particularly good protection against the cytopathic effects of the types 1 and 2 viruses (Tables 1 and 2). The activity of the cyclic ether (II) makes it necessary to reconsider the role of the α-hydroxy group.

Table 2. PROTECTION GIVEN BY CYCLIC ETHER (II) AND BY 2-(α-METHOXYBENZYL)-BENZIMIDAZOLE TO CELLS INFECTED WITH TYPES 1, 2 AND 3 POLIOVIRUS

Virus		Mean delay (days) between infection with virus and degeneration of half the cell population			
Type	Conc.	Control	Ether (II) (300 μM)	α-Methoxy-HBB (150 μM)	HBB (100 μM)
1	10^{-1}	1·0	3·5	2·0	2·0
	10^{-2}	1·5	4·75	3·0	3·0
	10^{-3}	2·0	6·75	3·75	3·5
3	10^{-1}	0·75	1·5	1·0	1·0
	10^{-2}	1·0	2·0	1·75	1·5
	10^{-3}	1·75	3·0	2·0	2·0
			(150 μM)*		
2	10^{-1}	1·0	2·0	1·75	2·0
	10^{-2}	1·5	3·0	3·0	3·25
	10^{-3}	2·0	4·5	4·5	5·0

* Half M.T.C. was used except when the cyclic ether was tested with the type 2 virus. (Strictly the value for HBB itself should be 105 μM), but 100 μM HBB has been taken as the standard. Untreated-uninfected control cells began to die from over-crowding during the seventh day. Otherwise the footnotes to Table 1 apply.

2-Benzylbenzimidazole (I; $R = R' = R'' = H$) has very low selective activity against type 2 poliovirus, while its α-hydroxy derivative HBB (I; $R = R' = H$, $R'' = OH$) has high selective activity[5]. Replacement of the α-hydroxy group by an *o*-hydroxy group, similarly sited in space but substituted in the side-chain phenyl group giving structure (III; $R = OH$), slightly reduces the protective activity[6]. In contrast, 2-(*p*-hydroxybenzyl)-benzimidazole has low activity[6]. We have now examined 2-[α-(hydroxymethyl)-benzyl]-benzimidazole (I; $R = R' = H$, $R'' = -CH_2OH$) (ref. 2) (m.p. 150°–151°; maximum tolerated concentration 200 μM) and find that at 80 μM it gives protection to cells infected with type 2 poliovirus similar to that given by 50 μM HBB (Table 3). Against the cytopathogenicities of types 1 and 3 poliovirus, the hydroxymethyl compound at 80 μM is inferior to 50 μM HBB and it provides just detectable protection, of doubtful significance, under the condition of our assays.

Table 3. PROTECTION GIVEN BY 2-[α-(HYDROXYMETHYL)-BENZYL]-BENZIMIDAZOLE, THE HYDROCHLORIDE OF GLYCERIC ACID (IV) AND HBB TO CELLS INFECTED WITH TYPE 2 POLIOVIRUS

Virus	Mean delay (days) before onset of cytopathic effects			
conc.	Control	α-Hydroxymethyl compound (80 μM)	Glyceric acid (IV) hydrochloride (60 μM)	HBB (50 μM)
10^{-1}	1·5	1·75	1·5	1·75
10^{-2}	2·0	2·75	2·25	2·75
10^{-3}	3·0	4·0	3·25	3·75

Initial virus concentration (before dilution) ~ $10^{6\cdot2}$ TCD_{50} units per ml.
Otherwise the footnotes to Table 1 apply.

In order to determine whether an alkyl group at position 1 would increase the activities, as is the case with HBB (ref. 1), 1-ethyl-2-[α-(hydroxymethyl)-benzyl]-benzimidazole (I; $R = Et$, $R' = H$, $R'' = -CH_2OH$) was prepared by condensing *N*-ethyl-*o*-phenylenediamine with tropic acid by the method described previously[1]. White needles were obtained from aqueous methanol (yield 7 per cent from tropic acid), m.p. 158°–159° (found: C, 77·1; H, 7·0; N, 10·6; $C_{17}H_{18}N_2O$ requires C, 76·7; H, 6·8; N, 10·5 per cent). Introduction of the ethyl group results in considerable increase in protective action. At half maximum tolerated concentration, the 1-ethyl derivative (I; $R = Et$, $R' = H$, $R'' = -CH_2OH$) is superior to HBB (100 μM) against type 2 poliovirus and is similar to HBB (100 μM) against the type 1 and type 3 viruses (Table 4).

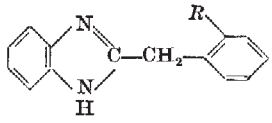
Table 4. PROTECTION GIVEN BY 1-ETHYL-2-[α-(HYDROXYMETHYL)-BENZYL]-BENZIMIDAZOLE TO CELLS INFECTED WITH POLIOVIRUS

Virus		Mean delay (days) before onset of cytopathic effects		
Type	Conc.	Control	1-Ethyl compound 0·5 M.T.C. (100 μM)	HBB (100 μM)
1	10^{-1}	0·75	1·0	1·0
	10^{-2}	1·0	1·25	1·5
	10^{-3}	1·5	2·0	2·0
3	10^{-1}	0·75	1·25	1·0
	10^{-2}	1·5	1·75	1·75
	10^{-3}	1·75	2·0	2·0
2	10^{-1}	0·75	1·75	1·25
	10^{-2}	1·25	3·25	2·75
	10^{-3}	1·75	4·75	3·75

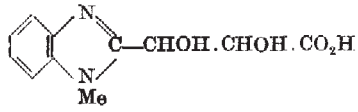
Initial virus concentrations (before dilution) ~ $10^{7\cdot5}$ TCD_{50} units per ml.
Otherwise footnotes to Table 1 apply.
Each assay (Tables 1 to 4) has been carried out on two separate occasions.

Thus it is not necessary for a hydroxy group to be attached directly to the α-carbon atom in order to preserve reasonable activity against type 2 poliovirus. The activity of the cyclic ether (II) indicates that an alkoxy group might be as effective (Table 2). 2-(α-Methoxybenzyl)-benzimidazole (I; $R = R' = H$, $R'' = OMe$) (ref. 2) (m.p. 160°–161°) was examined in order to discover the effect of replacing the α-hydroxy by an α-methoxy group. It is less toxic to the cells than HBB and shows similar protective action at half maximum tolerated concentration. Table 2 shows that the selective protective action against the type 2 virus is slightly less than that of HBB, while it is slightly more effective than HBB with respect

to the types 1 and 3 viruses. Thus the α-hydroxy group is not a necessary feature for high activity against the type 2 virus. However, it is just conceivable that some dealkylation might occur in the tissue-culture system. Investigations of other α-substituted derivatives of 2-benzylbenzimidazole and of their D- and L-isomers are in progress.



(III)



(IV)

2-(*o*-Carboxybenzyl)-benzimidazole (III; $R = CO_2H$) was tested because of the activity of the *o*-hydroxy compound[5]. However, at 30 μM (half maximum tolerated concentration) it shows no protective action against the type 1 or type 2 virus and only a small effect, of doubtful significance, against the type 3 virus. Another carboxylic acid (IV) was tested as the hydrochloride at 60 μM (half maximum tolerated concentration) and was found to have no activity with respect to the types 1 and 3 viruses but to offer slight protection against the type 2 virus (Table 3). Mandelic acid at 90 μM (half maximum tolerated concentration) provides no protection against any of the 3 virus types.

2-(*o*-Carboxybenzyl)-benzimidazole (III; $R = CO_2H$) was obtained by heating under reflux for 7 h, *o*-phenylenediamine (2·7 g, 0·025 mole) and homophthalic acid (4·5 g, 0·025 mole) in 2M hydrochloric acid (37 ml.). Cooling, filtering and drying the crystals gave a greenish product (5·9 g, yield 94 per cent). The solid, suspended in water, was dissolved in a slight excess of M sodium hydroxide, filtered, and treated with carbon dioxide until no more precipitation occurred. On crystallization from methanol, it gave the *o*-carboxy derivative (III; $R = CO_2H$) as white needles, m.p. 320° (dec.) (found: C, 71·2; H, 4·5; N, 10·7; $C_{15}H_{12}N_2O_2$ requires C, 71·5; H, 4·8; N, 11·1 per cent).

The glyceric acid derivative (IV) was prepared at the same time as the *bis*-derivative[1] by heating under reflux for 7 h, *N*-methyl-*o*-phenylenediamine[1] (2·44 g, 0·02 mole), tartaric acid (1·5 g, 0·01 mole) and 4M hydrochloric acid (20 ml.). The dihydrochloride of 1,2-*bis*-[2′-(1′-methylbenzimidazolyl)]-ethylene glycol[1] (1·65 g) separated on cooling. On standing the filtrate at 0°, a further crop of crystals (1·4 g) separated. Crystallization from ethanol-ether after charcoal treatment gave β-[2-(1-methylbenzimidazolyl)]-glyceric acid hydrochloride as white prisms, soluble in saturated sodium bicarbonate, m.p. 212°–213·5° (yield 51 per cent) (found: C, 47·1; H, 5·0; N, 10·1; $C_{11}H_{13}ClN_2O_4$ requires C, 47·5; H, 4·8; N, 10·2 per cent).

Table 5 summarizes the approximate relative activities of compounds, all at half maximum tolerated concentration, in protecting rabbit-embryo kidney (ERK) cells against the cytopathic effects of poliovirus type 1 (*L Sc*, 2 *ab*), type 2 (*P* 712, *Ch*, 2 *ab*) and type 3 (*Leon* 12 *ab*) (the virus strains used in this present work). The information is based on our most up-to-date work. Activities are given on an arbitrary scale from α (very high protection) to ε (slight protection), as described previously[1]. Until we have completed our investigation of percentage inhibitions of virus growth, Table 5 provides a rough guide to the relative effectiveness of the most interesting compounds we have examined[1,3,4].

Table 5. Approximate Relative Effectiveness in Protecting ERK Cells Infected with Poliovirus

Compound	M.T.C. (μM)	Protective influence* Type 1	Type 2	Type 3
DL-HBB	210	ε	γ	ε
D-HBB	210	δ	β	δ
1-Methyl-HBB†	210	δ	β	δ
1-Ethyl-HBB†	180	γ(−)	β	γ
1-Propyl-HBB†	80	α	α	β
1-Butyl-HBB†	60	β	α	γ
1-Pentyl-HBB	100	γ	β	δ
1-Isopropyl-HBB	100	ε	γ	ε
1-Benzyl-HBB	100	α	α	β
1-β-Methoxyethyl-HBB	300	β	β	γ
1,3-Diethyl-2-(α-hydroxybenzyl)-benzimidazolium iodide	70	ε	δ	ε
2-[α-(Hydroxymethyl)-benzyl]-benzimidazole	200	0	γ	0
1-Ethyl-2-[α-(hydroxymethyl)-benzyl]-benzimidazole	200	ε	γ	ε
2-(α-Methoxybenzyl)-benzimidazole	300	ε	γ	ε
Cyclic ether (II)	600	β	β	γ

* Ranges are given from α (very high) to ε (low); 0 means no activity.
† Same data apply to the DL- and D-isomers.

Experiments on the highly active compounds show that they do not inactivate the virus particles in the absence of cells and that they possess little or no retarding effects on the passage of virus into the cells. Presumably the compounds exert their actions within the cells. The DL- and D-1-propyl-, DL- and D-1-butyl- and DL-1-benzyl-2-(α-hydroxybenzyl)-benzimidazoles are the most effective compounds discovered so far in our investigations[1,3,4].

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AMINO-ACID COMPOSITION OF HUMAN DERMAL COLLAGEN

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THE amino-acid composition of collagens from various mammalian and other[1] species reveals a uniform pattern characterized by high concentrations of glycine, proline and hydroxyproline, low concentrations of aromatic and sulphur-containing amino-acids, and significant amounts of hydroxylysine. The amino-acid composition of human collagens from bone, tendon[2], dura mater and uterus[3] has been reported. This investigation reports the isolation and amino-acid composition of the citrate-soluble, insoluble and urea-extractable collagens from human skin.

Specimens of normal adult skin were obtained from the abdominal wall of fresh autopsy material. The epidermis was removed from the stretched skin by scraping with a scalpel and the skin was lyophilized. The dry weights of the samples ranged from 1·5 to 3 g. They were passed through a micro Wiley mill, resulting in a fine powder which greatly facilitated the subsequent isolation of the collagens. The lipids were removed by extracting with

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