fraction rather than extrapolating the results to represent total serum drug concentrations. With drugs such as phenytoin, whose measurement in saliva has been validated, salivary rather than serum bioavailability studies may be clinically more relevant, as comparisons of free pharmacologically active drug fractions would appear to give a better measurement of a drug's concentration at its receptor site and better quantitation of clinical response.

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REFERENCES

Koch-Weser, J. (1974) New Eng. J. Med. 291: 233–237

Lund, L. (1974) Eur. J. Clin. Pharmacol. 7: 119–124

Neuvonen, P. J. (1979) Clin. Pharmacokinet. 4: 91–103

Paxton, J. W., Rowell, F. J., Ratcliffe, J. G. (1977a) Clin. Chim. Acta 9: 81–92

Paxton, J. W., Rowell, F. J., Ratcliffe, J. G., Lambie, D. G., Nanda, R., Melville, I. D., Johnson, R. H. (1977b) Eur. J. Pharmacol. 11: 71–74

Paxton, J. W., Whiting, B., Stepehn, K. W. (1977c) Br. J. Clin. Pharmacol. 4: 185–191

Tyrer, J. H., Eadie, K. N., Sutherland, J. M., Hooper, W. D. (1970) Br. Med. J. 3: 271–273

Freeze-drying of haemoglobin in the presence of carbohydrates

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Compounds that make it possible to freeze-dry haemoglobin without oxidation have been studied in our laboratory for several years (Labrude et al 1976; Labrude & Vigneron 1980). Of such compounds, glucose is known to be very effective (Amberson et al 1942; Farr et al 1947; Smith & Pennell 1952; Fateeva & Gruzova 1979). In 1976 we demonstrated the effectiveness of several other carbohydrates. More recently Pristoupil et al (1978) and De Venuto et al (1979) have confirmed these results.

To our knowledge however, no systematic study has been reported of the protective actions of hexoses, of substituted derivatives of glucose or levulose, or of other carbohydrates and related C4, C5, C7, etc. compounds. Therefore we have tried to discern possible relationships between the properties of these compounds and their protective activities.

All the compounds were of analytical quality and were from Sigma, Calbiochem, Boehringer, Merck, Fluka, and Prolabo. Only water-soluble compounds were studied, since the absence of this characteristic is incompatible with effective protection. The compounds studied, in order of increasing complexity, are as follows:

Trioses: DL-glyceraldehyde, dioxyacetone, glycerol, propane-1,3-diol.

Tetroses: D-erythrose, erythritol.

Pentoses: D-ribose, D-arabinose, D-xylose, D-lyxose and substituted derivatives of ribose (Table 1).

α-D-glucose (Sigma G 5000 with 5% β anomer) β-D-glucose (Sigma G 5250), L-glucose (Sigma G 5500, mixed anomers);

D-series of aldohexoses: allose, altrose, glucose, mannose, idose, galactose, talose. Substituted derivatives of glucose (Table 2).

Ketohexoses: fructose, L-sorbose, D-tagatose, sodium fructose-1-phosphate, trisodium fructose-1,6-disphosphate . $7H_2O$, and barium fructose-6-phosphate.

Heptoses: D-mannoheptulose, D-sedoheptulose hydrate, α-D-heptagluco-γ-lactone, calcium heptagluconate.

Disaccharides: D-sucrose, D-trehalose hydrate, D-cellobiose, lactulose, palatinose, maltose hydrate, gentobiose.

The haemoglobin solution, at a concentration of 45 ± 5 g litre^{-1}, was prepared as described before (Labrude & Vigneron 1980). The carbohydrates were dissolved in it, without adjustment of the pH, to a concentration of 0·2 M. All other operations were as described by Labrude & Vigneron (1980). Of the parameters studied (colour, dissolution, saturation, p 50, etc.), we report only the methaemoglobin concentration, measured according to Evelyn & Malloy's method (1938). Each carbohydrate was studied twice. A compound was considered to be effective if it led to a methaemoglobin concentration less than or equal to 10% (Tables 1, 2); in the absence of protector the mean value was 49% (n = 30).

The trioses and tetroses were ineffective. The four aldopentoses were very effective, as were almost all the ribose derivatives studied. The α, β, L, or D character of the glucose did not affect its protective activity. The six aldehexoses were as effective as glucose. In the substituted aldohexoses series the most unfavourable modifications for the protective activity were those on carbon atoms 1 and 2. Changes at position 6 seemed to be less important, although most of the borderline cases (leading to 10% methaemoglobin) occurred there and the double modification on positions 1 and 6 seems best avoided. Of the six ketohexoses, only the 6-phosphate and 1,6-diphosphate derivatives of fructose were

* Correspondence.

Table 1. Lyophilization of haemoglobin with pentoses.

	Structural modification	Compound studied ribose-arabinose-xylose-lyxose	methaemoglobin (%) 3 ± 1
	Reduction	Ribitol (= adonitol)	5·9
		Arabitol	1
		Xylitol	4·6
1	Oxidation	D-Ribonic acid γ-lactone	10
		D-Arabonic acid, potassium salt	10
1	Etherization	Methyl β-D-arabopyranoside	13
1 & 2	Ketopentose	D-Ribulose	4·7
2	Reduction	2-Deoxy-D-ribose	17
5	Esterification	D-Ribose-5-phosphate (Na salt)	5·5
		Pentaerythritol	60

ineffective. The four heptoses were good protectors, and all seven disaccharides were very effective.

For a hypothesis of some specificity in the protective action there should be structural elements common to all the effective compounds. Although the short-chain sugars were not good protectors, the length of the chain seemed irrelevant, at least for C5, C6, C7, and C12. Likewise, the presence of an aldehyde function at position 1 did not seem to be important, since the ketoses were effective. Lastly, cyclization is not essential, since ribitol, sorbitol, and sodium gluconate, for example, gave good results. Conversely, the effect of structural differences as small as those that separate sorbitol from mannitol, or methyl α-D-glycopyranoside from methyl α-D-mannoside, favours specificity of action. The protection afforded by the pentoses and hexoses showed that modification at positions 1, 2, 6, and 1, 6 gave rise to the greatest amount of oxidation of the haemoglobin. The effectiveness of sucrose weakens the hypothesis that a reducing capability plays a role in maintaining the iron in the II state.

Thus, it is very difficult to discern a relation between the structure and the activity. The diversity of the natures and structures of the effective compounds, plus the necessity for a high molar ratio of protector to haemoglobin (at least 100), suggests that other explanations should be sought. No definitive answer on the lyoprotective effect of such compounds has been forthcoming but their stereospecific interactions with water are believed to be related to the protective effect (see Suggett 1975; MacKenzie 1977: Franks 1979). This mechanism would perhaps explain the differences of efficacy observed for small steric variations between some of the molecules. So far, Pristoupil et al (1978) have recently invoked a non-specific mechanic stabilization of hydrational shell of haemoglobinic molecules by the low molecular carbohydrates.

The present results and the absence of interaction of glucose with the iron atom or the haem seen by E.S.R. (Labrude et al 1979) are not in disagreement with the precedent stereospecific hypothesis.

Table 2. Study of hexoses derivatives.

	Structural modification	Compound studied Hexoses	methaemoglobin (%) $1{\cdot}2 \pm 0{\cdot}5$
1	Reduction	D-Sorbitol	1·5
		D-Mannitol	29
		D-Galactitol (dulcitol)	50
1	Oxidation	Gluconic acid, Na salt	5
		D-Gluconic acid lactone	20
		D-Galactonic acid-γ-lactone	0
		L-Gulonic acid-γ-lactone	49
1	Etherization	Methyl α-D-glucopyranoside	3
		Methyl α-D-mannoside	37
1	Esterification	D-Glucose 1-phosphate (K salt)	40
1	Amination and reduction	*N*-Methyl-D-glucamine (Meglumine)	8
2	Reduction	2-Deoxy-D-glucose	9·7
		2-Deoxy-D-galactose	6·5
2	Amination	D-Glucosamine HCl	17–40
		N-Acetyl-D-glucosamine	40
		D-Galactosamine, HCl	2–10
1 & 2	Oxidation Reduction	D-Glucosaminic acid	40
3	Amination	3-Amino-3-deoxy D-glucose, HCl	4·1
5	Thiol	5-Thio D-glucose	2·6
6	Oxidation	Glucuronic acid (Na salt)	1·6
6	Reduction	L-Rhamnose	1·2
		L-Fucose	2·1
		6-Deoxy-D-glucose	10
6	Esterification	D-Glucose-6-phosphate (Na salt)	7
		D-Galactose-6-phosphate (Na salt)	10
6	Amination	6-Deoxy-6-amino-D-glucose, HCl	10
		6-Deoxy-6-amino-D-allose, HCl	10
1 & 6	Oxidation	Saccharic acid, Na salt	45
		Mucic acid, Na salt	50
1 & 6	Esterification	Glucose 1,6-diphosphate (cyclohexylammonium salt)	10
2 & 6	Reduction Esterification	2-Deoxy-D-glucose-6-phosphate (Na salt)	2·9
2 & 6	Reduction cyclization	D-Digitoxose	4·8
		Inositol	54

REFERENCES

Amberson, W. R., Jacobs, J. E., Hisey, A. (1942) Charles C. Thomas Springfield. 156

De Venuto, F., Zegna, A. I., Busse, K. R. (1979) Surg. Gynecol. Obstet. 148:69–75

Evelyn, K. A., Malloy, H. I. (1938) J. Biol. Chem. 126: 655–662

Farr, L. E., Hiller, A., Van Slyke, D. D. (1947) J. Exp. Med. 86: 465–475

Fateeva, I. M., Gruzova, L. M. (1979) Ukr. Biokhim. Zh. SSSR. 51: 179–184

Franks, F. (1979) in: Blanshard, J. M. V. (ed) Polysaccharides in foods. Butterworth, London

Labrude, P., Loppinet, V., Vigneron, C. (1976) Ann. Pharm. Fr. 34: 143–149

Labrude, P., Chaillot, B., Vigneron, C., Simatos, D. (1979) Communication 15th Congr. intern. Refrigeration, Venice

Labrude, P., Vigneron, C. (1980) J. Pharm. Pharmacol. 32: 305–307

MacKenzie, A. P. (1977) Phil. Trans. Roy. Soc. B 278: 167–190

Pristoupil, T. I., Kramlova, M., Ulrych, S. (1978) Cesk, Farm. 27: 311–313

Smith, W. E., Pennell, R. B. (1952) Blood 7: 368–372

Suggett, F. (1975) in: Franks, F. (ed.) Water a comprehensive treatise. Plenum Press, N.Y.