321. *Studies in Hydrogen-bond Formation. Part VII.* Reactions between Carbohydrates and Proteins in Water and their Relation to Transfer of Carbohydrates across Red-cell Membranes.*

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The refractometric method [1] of detecting intermolecular complexes has been used with dilute aqueous solutions of glucose, sucrose, or *meso*-inositol and proteins or models of proteins. These and other results suggest that: (*a*) normal carbohydrates do not form hydrogen-bond complexes with proteins in dilute aqueous or aqueous alkaline solutions because of the protective effect of the water; and (*b*) *meso*-inositol and simpler solutes, *e.g.*, alcohols, do form complexes, probably by hydrogen-bonding. Differences in transport rates of various solutes including carbohydrates across red-cell membranes can thus be explained. Apparently the more readily the solute forms hydrogen bonds with a protein, the slower is its diffusion rate in the membrane.

Carbohydrates and proteins may react in aqueous media in many biological systems. We have tried to assess the probability of such interactions' being due to hydrogen bonding, which at first appears the most likely mechanism.

Glucose and cellobiose in water form simple complexes [2a] of the same ratio, usually 1 : 1, with several types of nitrogen-containing group in second solutes, but are inactive towards phenols or alcohols. Fructose and sucrose appeared not to form any complex with second solutes in water. The explanation given was that the cyclic forms of these carbohydrates are so strongly hydrogen-bonded to water that no second solute can react with them in aqueous solution. The open-chain form of glucose and cellobiose, however, is apparently less strongly bound to water and can react with certain other solutes through its aldehyde group; the hydroxy-groups remain inactive, probably through weak chelation (cf. the chelation of polyhydric alcohols [2a]). Fructose or sucrose cannot react even as the open-chain molecule, because the end-group in that form, *viz.* the keto-group, is itself unreactive in water.

Several authors have examined the reactions of carbohydrates with proteins. Some complexes appear to be formed in water, but only in fairly concentrated solutions.[3–5] There is evidence, for example, that under alkaline conditions some carbohydrates, but not sucrose, interact with amino-groups in proteins,[3,4] but the products are unstable under biological conditions.[4] It seems likely that only when the solutions are concentrated can the carbohydrate–protein attraction break down the barrier of solvated water and lead to formation of complexes.

We examined refractometrically three proteins (casein, edestin, and gelatin) and several models for the polar groups in proteins [*N*-methylacetamide, glycine, dimethylformamide, diacetamidomethane (cf. ref. 2*b*), ethylamine, and propionic acid]. Since glucose and cellobiose behave as aldehydes in water,[2a] a few tests were made with simple aldehydes. *meso*-Inositol was included for comparison with the normal carbohydrates, because of its different biological properties.

* Part VI, *J. Soc. Dyers and Colourists*, in the press; Part V, Arshid, Giles, and Jain, *J.*, 1956, 1272.

[1] Arshid, Giles, McLure, Ogilvie, and Rose, *J.*, 1955, 67.

[2] (*a*) Arshid, Giles, and Jain, *J.*, 1956, 559 (in Table 1 of this paper the mol. ratio of the D-cellobiose–aniline complex *should read* 2 : 1. On p. 569, first line, *for* free acid *read* no free acid); (*b*) Arshid, Giles, Jain, and Hassan, *J.*, 1956, 72.

[3] Przylecki, *Acta Biol. Exptl.* (*Warsaw*), 1938, **12**, 82; *Chem. Abs.*, 1939, **33**, 6351.

[4] Przylecki and Cichocka, *Biochem. Z.*, 1938, **299**, 92.

[5] Haurowitz, "Chemistry and Biology of Proteins," Academic Press Inc., New York, 1950.

Experimental

Full details of the method and the general significance of the results have been given.[1] Distilled water was used as solvent. Casein (" light white soluble ") and edestin were obtained from British Drug Houses Ltd.; gelatin was of pure photographic quality; other reagents including sodium chloride were " AnalaR " or were purified by normal methods. A Pulfrich

Results of complex-detection tests.

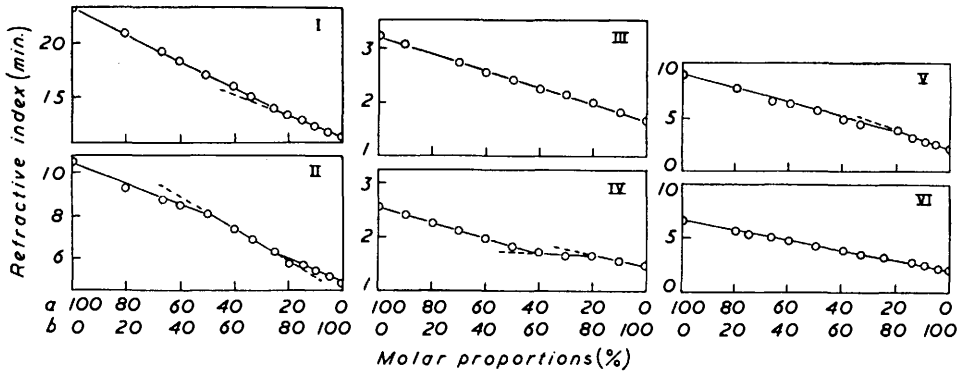
Solute *a*	Concn. (M)	Solute *b*	Concn.*	Solvent †	Mol. ratio of complex, *a* : *b* ‡
Acetaldehyde	0·5	*N*-Methylacetamide	0·5M	W	0
	0·5	Dimethylformamide	0·5M	W	(1 : 1)
	0·5	Diacetamidomethane	0·5M	W	0
	0·5	Propionic acid	0·5M	W	0
*iso*Butyraldehyde	0·5	*N*-Methylacetamide	0·5M	W	0
Terephthaldehyde	0·1	*N*-Methylacetamide	0·1M	WEl	0
	0·1	Dimethylformamide	0·1M	WEl	0
D-Glucose	0·01	Casein	1·25 g./l.	B9	0
	0·0085	Edestin	1·07 g./l.	S	0
	0·1	Gelatin	10·6 g./l.	W	0
	0·1	Glycine	0·1M	W	0
	0·5	*N*-Methylacetamide	0·5M	W	0
	0·5	Ethylamine	0·5M	W	1 : 1
Sucrose	0·1	*N*-Methylacetamide	0·1M	W	0
	0·01	Casein	1·25 g./l.	B9	0
meso-Inositol	0·1	*N*-Methylacetamide	0·1M	W	1 : 3
	0·1	*p*-Cresol	0·1M	W	(1 : 1); 1 : 3
	0·1	Phenol	0·1M	W	1 : 1; 1 : 3
	0·01	Casein	1·25 g./l.	B9	0
	0·0085	Edestin	1·07 g./l.	S	See Fig.
	0·1	Gelatin	10·6 g./l.	W	See Fig.

* Weights of proteins refer to the normal air-dry condition.

† Solvents: B9 = pH 9·0 Buffer solution; S = 1·0M aqueous sodium hydroxide solution; W = water; WEl = 50% (v/v) aqueous ethanol.

‡ Data in parentheses denote uncertain indications; 0 denotes no evidence of complex formation.

Relation between refractive index (as instrument reading) and ratio of components.



Solvent: water
I: *a*, *meso*-Inositol; *b*, *N*-methylacetamide.
II: *a*, *meso*-Inositol; *b*, phenol.

Solvent: 1·0M-NaOH soln.
III: *a*, D-Glucose; *b*, edestin.
IV: *a*, *meso*-Inositol; *b*, edestin.

Solvent: water
V: *a*, *meso*-Inositol; *b*, gelatin.
VI: *a*, Glucose; *b*, gelatin.

refractometer (Bellingham and Stanley), on which an experienced operator's standard deviation was about 3·5 sec., was used with sodium light, all measurements being made at room temperature; the mixed solutions containing proteins were kept overnight before being tested.

Casein was dissolved in pH 9 buffer, edestin in 1·0M sodium hydroxide solution, and gelatin in water, the second solutes in each case being dissolved in the same respective liquid. The

refractive indices are proportional to concentration and additive, and the theory of the method [1] shows that the two solutions need not be of the same molarity, although if they are not, any complex-ratio shown in the graphs must be adjusted.

Results and Discussion

The results (Table and Figure) give no evidence that hydrogen-bond interaction in dilute aqueous solution can take place between normal carbohydrates and proteins, but they suggest that it may occur between inositol and proteins.

The aldehydes, including glucose, react in 1 : 1-ratio with several simple model compounds, *e.g.*, dimethylformamide, triethylamine, pyridine, etc.,[2a] but, surprisingly, none gives any evidence of reaction with the $-CO\cdot NH-$ group in *N*-methylacetamide or with the peptide link in casein, or reacts with aliphatic carboxylic acids. Aldehydes and glucose react with amino-groups (cf. ref. 2*a*), but we obtained no evidence that they do so with the side-chain amino-groups in proteins in alkaline or neutral solution, probably because under such conditions these groups are either in the zwitterion form or bound in covalent salt links. Sucrose was known not to react with several other solutes,[2a] and now appears unreactive towards *N*-methylacetamide or casein. Apparently, adsorption from dilute aqueous solutions of carbohydrate to protein or *vice-versa* can take place only by non-polar van der Waals forces.

Inositol (hexahydroxy*cyclo*hexane), unlike other carbohydrates, does form complexes with the phenolic and amide groups and with edestin and gelatin in water. The difference must reflect the relative affinity of the two types of compound for water (cf. water solubility: *meso*-inositol, 4·5; D-glucose, 83 g., per 100 c.c. of water at 15° and 17·5° respectively). Inositol is therefore less firmly bound to the solvent and its solvated water can more readily be replaced by another solute molecule.

We cannot suggest possible modes of attachment of inositol to the proteins; about one molecule appears to be combined for every 5 amino-acid residues in gelatin and for every 1·5 or 4 in edestin, for which there is evidence of two different complexes (Figure), the average molecular weight per residue in gelatin [6] being taken as about 75 and in edestin [5] about 105 (that for casein [5,6a] is about 105).

Stein and Danielli [7] state that sugars penetrate cell membranes in particular instances much more rapidly than simpler hydrogen-bonding molecules, *e.g.*, water, methanol, urea, and formamide; Bowyer and Widdas state [8] that *meso*-inositol differs from normal sugars, *viz.*, fructose, glucose, sorbose, and sucrose, in being unable to penetrate red blood cells. Various authors [8] have observed also that the rate of penetration of glucose into cells decreases at higher concentrations. These facts can be related to the hydrogen-bonding properties of the solutes concerned by a consideration of the present and earlier results.[2a] If the solute in an aqueous medium forms hydrogen bonds with a component of the cell-wall membrane it may be adsorbed thereto and its transport across the membrane will then be retarded or prevented. This would explain why *meso*-inositol, which forms bonds in dilute aqueous solution, is not transported, and the normal carbohydrates, which are too highly solvated to form bonds, are transported. The decreased penetration rate of glucose with rise in its concentration in solution can be explained by an increase in its ability to form hydrogen bonds with the solid subtrate when the protective effect of the solvent becomes less pronounced.

Bowyer and Widdas consider that the penetration of sugars into human red cells is not a simple diffusion and probably first requires the formation of a complex between the sugar and some (unidentified) membrane component; we suggest that this complex appears to be formed by non-polar forces. Bowyer and Widdas suggest that the hexose-transfer

[6] (*a*) Traill, *J. Soc. Dyers and Colourists*, 1951, **67**, 257; (*b*) Eastoe, *Biochem. J.*, 1955, **61**, 589.
[7] Stein and Danielli, *Discuss. Faraday Soc.*, 1956, **21**, 238.
[8] Cf. Bowyer and Widdas, *Discuss. Faraday Soc.*, 1956, **21**, 251.

system is of wide biological significance, occurring as it does not only in human red cells, but in other species and tissues.

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