ISOTONICITY OF FRUCTOSE, GALACTOSE AND MANNOSE SOLUTIONS

By E. Menczel, M. Rabinovitz and Y. Goldberg

From the Pharmacy Department, School of Pharmacy, Hebrew University, Jerusalem, and Haematologic Laboratory, Hadassah Hospital, Tel-Aviv

Received April 19, 1960

A haemolytic method has been used for the determination of the isotonic concentrations of dextrose, fructose, galactose and mannose. Fructose, galactose and mannose produce haemolytic effects deviating from those of dextrose. The isotonic concentration in mM per cent of fructose was found to be 20·3; of galactose, 38·9; of mannose, 33·3; whereas that of dextrose is 26 mM per cent.

The intravenous carbohydrate alimentation by fructose solutions has been recognised since 1954 in *New and Non-official Remedies*, now *New and Non-official Drugs*, for diabetic patients, since it is metabolised or converted into glycogen in the absence of insulin[1,2]. Galactose and mannose have also been given parenterally in several studies of diabetes and sugar metabolism[3,4]. The molecular weights of these monosaccharides are the same as that of dextrose (anhydrous) and it might be reasonably assumed that their similar properties imply similar isotonic concentrations (about 5 per cent). However, as these sugars might be partially permeable to erythrocytes, only by haemolytic tests can their isotonicity be assessed. A quantitative haemolytic method for determining the degree of disintegration of erythrocytes produced by hypotonic solutions was described by Hunter[5]. Husa and others[6–14] used this method for the determination of isotonic coefficients *i* of various salts and organic medicinal substances including some sugars.

Cadwallader and Husa[11] emphasised the difference between the physico-chemical Van't Hoff's factor (isotonic coefficient *i*) and the haemolytic one (haemolytic *i*) of those compounds which are permeable or which affect the erythrocytes in other ways. Grosicki and Husa[7] suggested the use of the *i* value of sodium chloride as a standard for the evaluation of the haemolytic data of other compounds, as sodium chloride is practically impermeable to erythrocytes; its physico-chemical *i* value is also its haemolytic *i* value, and by reference to this the haemolytic *i* value of any compound at equivalent molar concentrations could be computed. Thus the haemolytic *i* value could be substituted for the physico-chemical *i* value in the equation for determining isotonicity using the freezing point method[15]. The isotonic concentration will coincide with the iso-osmotic concentration only where the erythrocytes are not affected by increased permeability, agglutination or even slight haemolysis.

Experimental

Materials

Sodium Chloride B.P.; Dextrose B.P.; D-(−)-fructose, analytical reagent grade, optical rotation of 9·579 per cent solution = − 16·7°; specific rotation = − 87·2°; D-(+)-galactose, analytical reagent grade, optical rotation of 10·04 per cent solution = + 16°, specific rotation = + 79·6°;

D-(+)-mannose, analytical reagent grade, optical rotation of 9·77 per cent solution $= +2{\cdot}6°$, specific rotation $= +14°$; water for injection U.S.P. and B.P., boiled before use.

Method

Solutions were made of 0·7 per cent sodium chloride and 10 per cent of each sugar respectively, in water for injection. The solutions were serially diluted. Blood was added in 0·02 ml. portions to 4 ml. of each solution in a test tube. After admixing, the test tubes were set aside at room temperature for 2 hours, and centrifuged at 3,000 r.p.m. for one minute. The light transmission of the supernatant fluid containing the liberated oxyhaemoglobin was determined by a Klett-Summerson photoelectric colorimeter using No. 54 green filter; the sensitivity being increased by a Kipp galvanometer[16]. Two readings were made for each concentration and were then averaged. The amount of haemolysis at the different concentrations of salt and sugars was calculated as a percentage of haemolysis obtained by laking the erythrocytes in a 0·1 per cent sodium carbonate solution which might reasonably be considered as complete haemolysis.

The resistance of the erythrocytes to haemolysis can also be evaluated by determining the maximum concentration of sodium chloride causing 100 per cent haemolysis of the blood of different donors; the lower this concentration, the greater is the erythrocyte's resistance to haemolysis. The haemolytic tests are therefore reproducible only with the same blood but the variations from one healthy donor's blood to another are slight. The effects of these variations could be minimised by averaging the results of the haemolytic tests for each of the compounds of the different donors' blood at equivalent conditions. At concentrations where haemolysis is increased by subsequent dilution, the per cent haemolysis is directly proportional to the hypotonicity, and therefore can be used for computing the haemolytic *i* value and isotonicity.

The *i* value calculation is essentially similar to that suggested by Grosicki and Husa[7] but concentrations are expressed in millimols per cent, in accordance with the following equation:

$$i \text{ (haemolytic) value of compound} = [i \text{ (haemolytic) value of NaCl}] \times \frac{(\text{mM}a + \text{mM}b + \text{mM}c + \text{mM}d + \text{mM}e)}{(\text{mM}f + \text{mM}g + \text{mM}h + \text{mM}i + \text{mM}j)} \quad \ldots \quad (1)^*$$

$$\text{mM per cent of an isotonic solution} = \frac{\text{R}}{i \text{ (haemolytic)}} \times 100 \quad \ldots \quad \ldots \quad (2)^\dagger$$

* *a*, *b*, *c*, *d*, and *e* represent concentrations of sodium chloride in millimols per cent, at 25, 35, 50, 60, and 75 per cent haemolysis whereas *f*, *g*, *h*, *i* and *j* represent the equivalent concentrations of the test compound in millimols per cent, respectively.

† R is the constant osmotic ratio T_f/K_f derived from the freezing point equation of isotonicity[15] which could be rewritten as following: $m = T_f/K_f \times 1/i$; R being a ratio is entirely independent of the freezing point method (and its units) and is equivalent to 0·28 (m = molal concentration, T_f = freezing point depression of blood ($-0{\cdot}52°$), K_f = molal freezing point depression constant for water ($-1{\cdot}855°$), i = Van't Hoff's factor).

The multiple of mM concentration and the haemolytic *i* value gives the haemolytic milliosmol units, which should be differentiated from the milliosmol units defined as the multiple of Van't Hoff's factor and millimolar concentration. For isotonicity, the haemolytic milliosmol units should be adopted, and from equation (2) a 0·28 haemolytic-osmolar concentration is equivalent to 28 haemolytic milliosmols per cent, which represents the isotonic concentration with blood serum.

This method for the evaluation of isotonicity is applicable to any of the pharmaceutical injectable compounds including those producing exo-osmotic or endo-osmotic effects on erythrocytes provided that the compounds are non-haemolytic in iso-osmotic concentrations with blood serum.

Experimental and Results

Preliminary Tests

The equivalent per cent haemolysis at each concentration of sodium chloride of the preliminary tests is recorded in Table I. The results confirm those of Grosicki and Husa[7] that the haemolytic tests are sensitive in the range of 0·30 to 0·50 per cent sodium chloride. By plotting the per cent haemolysis against salt concentration a sigmoid curve is obtained. The sensitive range with the monosaccharides (see Table II) is about 1 to 3 per cent and plots produce similar sigmoid curves.

TABLE I

Haemolysis per cent of sodium chloride solutions at various concentrations

Concentration units	Sodium chloride concentrations								
g. per cent	0·15	0·20	0·25	0·30	0·35	0·40	0·45	0·50	0·60
mM per cent	2·56	3·42	4·27	5·13	5·98	6·84	7·69	8·55	10·26
mOsmols per cent	4·76	6·36	7·94	9·54	10·02	12·74	14·20	15·90	19·08
Haemolysis per cent	100	100	100	96	93	76	10	2	0

Haemolysis Tests

Three healthy donors were chosen for the subsequent haemolysis tests which were made at close arithmetic dilutions within the sensitive range of concentrations as following:

Sodium chloride: 0·36, 0·4, 0·44, 0·48, 0·52 and 0·9 g. per cent respectively.

Monosaccharides: 0·2, 0·5, 0·8, 1·2, 1·5, 1·8, 2·1, 2·4, 2·7, 3·0, 3·3, 3·6, 3·9, 4·2, and 4·5 g. per cent respectively.

Haematologic Data of the Blood Donors

Donor Z (female), age 20, erythrocytes = 4,500,000, haemoglobin 90 per cent. The highest concentration of sodium chloride causing about complete haemolysis was 0·4 per cent.

Donor M (male), age 23, erythrocytes = 4,400,000, haemoglobin = 89 per cent. The highest concentration of sodium chloride causing complete haemolysis was 0·36 per cent.

Donor Y (male), age 24, erythrocytes = 4,400,000, haemoglobin = 88 per cent. The highest concentration producing about complete haemolysis was 0·1 per cent.

TABLE II

HAEMOLYSIS* PER CENT PRODUCED BY DEXTROSE, FRUCTOSE, GALACTOSE AND MANNOSE SOLUTIONS

Concentrations per cent			Dextrose	Fructose	Galactose	Mannose
g.	mM	mOsmol.	Per cent haemolysis	Per cent haemolysis	Per cent haemolysis	Per cent haemolysis
1	5·55	5·55	100	92	98	100
2	11·10	11·10	93	10	97	93
3	16·65	16·65	60	2	50	40
4	22·02	22·20	2	0	12	2

* Blood was from the donor of blood for the results in Table I.

Calculation of the Haemolytic i *Value and Isotonicity*

The average readings of light transmission of each blood sample at the concentrations of sodium chloride and monosaccharides used were converted into per cent haemolysis with reference to the total haemolysis obtained by laking each blood sample in 0·1 per cent sodium carbonate. Plotting the per cent haemolysis against the strength of sodium chloride and monosaccharides, respectively, the concentrations producing 25, 35, 50, 60, 75 per cent haemolysis were derived.

The data obtained from the three blood specimens was averaged for each degree of haemolysis produced by the sodium chloride and the dextrose, fructose, galactose and mannose respectively using equation (1). The haemolytic *i* values of the monosaccharides were computed reckoning the *i* value of sodium chloride as 1·86[7]. These calculated haemolytic *i* values at the respective per cent haemolysis are tabulated in Table III, the last column representing the average value which might reasonably be considered as the haemolytic *i* value.

TABLE III

HAEMOLYTIC *i* VALUES OF MONOSACCHARIDES

Monosaccharide	At 25 per cent haemolysis	At 35 per cent haemolysis	At 50 per cent haemolysis	At 60 per cent haemolysis	At 75 per cent haemolysis	Haemolytic *i* value
Dextrose ..	1·014	1·075	1·073	1·099	1·122	1·076
Fructose* ..	1·346	1·346	1·375	1·432	—	1·376
Galactose ..	0·724	0·722	0·718	0·728	0·715	0·721
Mannose ..	0·852	0·836	0·834	0·842	0·845	0·842

* The blood specimen *y* did not yield to 75 per cent haemolysis within the range of concentrations of fructose studied and therefore the haemolytic *i* value at this degree of haemolysis was not ascertained. Based on the other two blood specimens, the haemolytic *i* value for this sugar was 1·408.

Our results for dextrose differ slightly from those obtained by Grosicki and Husa[7], who found the *i* value to be 1·17. The haemolytic effects of fructose deviated considerably from those of the other monosaccharides and indicate significant exosmosis; in one sample, fructose agglutinated the red blood corpuscles, which might be interpreted as a further effect on

the permeability of the cells. On the other hand, galactose and mannose solutions produce the opposite effect to that of fructose, and even dextrose, on the permeability of erythrocytes indicating a certain degree of endosmosis[7].

From the haemolytic *i* values isotonicity was calculated using equation (2). The results are in Table IV.

TABLE IV

ISOTONIC CONCENTRATIONS OF DEXTROSE, FRUCTOSE, GALACTOSE AND MANNOSE

	Haemolytic *i* value	Isotonic concentrations per cent		
		g.	mM or milliosmols	haemolytic milliosmols
Dextrose	1·076	4·68	26	28
Fructose	1·376	3·65	20·3	28
Galactose	0·721	7·00	38·9	28
Mannose	0·842	6·00	33·3	28

Acknowledgements. We wish gratefully to acknowledge the assistance given by the late Professor F. Rappaport and the colleagues of the Central Pharmacy for Government Hospitals. The preliminary tests were made by Y. Yossifov (graduate thesis, M. Pharm., Hebrew University, Jerusalem).

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