Table 1. IRON CHELATING ABILITY OF 1, 2 AND 3 CARBON ATOM COMPOUNDS WITH HYDROXYL, ALDEHYDE AND KETONE FUNCTIONAL GROUPS

| Compound | Iron chelating ability* | Compound | Iron chelating ability |
|---|---|---|---|
| Methanol | < 1 | Propane dialdehyde | † |
| Ethanol | < 1 | Acetone | < 1 |
| Ethylene glycol | < 1 | Glycollic aldehyde | † |
| *n*-Propanol | < 1 | 3-Hydroxy propanal | † |
| Iso-propanol | < 1 | Lactaldehyde | † |
| Propylene glycol | < 1 | Glyceric aldehyde | † |
| Propane-1,3-diol | 17·5 | 2-Hydroxy propan-1,3-dial | † |
| Glycerol | 12 | 1-Hydroxy acetone | < 1 |
| Formaldehyde | < 1 | Dihydroxy acetone | 100 |
| Acetaldehyde | < 1 | Methyl glyoxal | < 1 |
| Glyoxal | † | 2-Keto propan-1,3-dial | † |
| Propionaldehyde | < 1 | 3-Hydroxy-2 keto propanal | † |

* Iron chelating ability equals per cent ferric ion held in solution at *p*H 8·0 when equal volumes of 0·1 M solution of the substance under test and $10^{-3}$ M ferric chloride are mixed and the *p*H raised to 8·0.

† Compound not available or too unstable for testing by this procedure.

Table 2. IRON CHELATING ABILITY OF SOME KETO HEXOSES, ALDO HEXOSES AND DISACCHARIDES

| Type | Compound | Iron chelating ability* |
|---|---|---|
| Keto hexose | Fructose | 100 |
| | Sorbose | 100 |
| | Tagatose | 100 |
| Aldo hexose | Glucose | < 1 |
| | Galactose | < 1 |
| | Mannose | < 1 |
| Disaccharide | Sucrose | < 1 |
| | Lactose | < 1 |
| | Maltose | < 1 |

* Measured as for Table 1.

acetone which shows strong chelating ability, all share the common structural features of hydroxyl groups on carbon atoms one and three, and are the only compounds among the twenty-four possibilities which do so. The dihydroxy acetone structure is found in the open chain fructose molecule, but is not present in any aldohexoses or disaccharides. On the basis of this evidence it seems reasonable to propose that the ability of fructose to chelate iron is related to the dihydroxy acetone structure found in the open chain fructose molecule. The superiority of dihydroxy acetone over 1-3,dihydroxy propane and glycerol is presumably caused by the electron withdrawing effect of the carbonyl group, which enables the hydroxyl groups to dissociate exposing oxygen atoms which can chelate iron and form a stable six-membered ring structure.

If this hypothesis is correct, only sugars possessing the dihydroxy acetone structure will chelate iron as powerfully as fructose does. On this basis we predicted that the other keto hexoses sorbose and tagatose would demonstrate iron chelating ability and that no aldo hexoses or disaccharides would chelate iron to the same extent. Table 2 shows the results of tests carried out on the keto hexoses sorbose and tagatose and on a number of aldo hexoses and disaccharides. Of all the sugars tested, only sorbose and tagatose chelate iron as powerfully as does fructose.

We conclude that the interesting biological phenomenon of iron chelation by sugars and polyols is exhibited most strongly by those compounds which contain the dihydroxy acetone structure in their molecule; that this property is one which distinguishes keto from aldo sugars; and that the structure of the iron–fructose chelate proposed by Saltman's group[1,4] should be modified to one in which the iron atom is incorporated into a six-membered ring, bound through the hydroxyl oxygen atoms to carbon atoms one and three of the fructose molecule.

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## Cholinesterase Inhibitory Property of Dimethyl Sulphoxide

DIMETHYL sulphoxide (DMSO) has long been known for its solvent properties and its ability to prevent freezing damage to living cells[1]. More recently, it has been found to cross intact cellular membranes and the skin very readily and to effect rapid penetration through these tissues of some substances dissolved in it[2,3]. Despite these remarkable abilities, very few undesirable effects in either experimental animals[4] or in man have been reported. As a result, DMSO is coming into rather widespread use as therapy for a number of human diseases[5].

During the course of toxicity investigations on the isolated innervated guinea-pig atrium, it was observed that a 0·4 M bath concentration of DMSO in the Krebs Henseleit bicarbonate buffer[6] depressed the vagal threshold to electrical stimulation by about 50 per cent, an effect also produced by $3{\cdot}3 \times 10^{-4}$ M neostigmine. It was further observed that a concentration of 0·4 M DMSO in the bath of the isolated guinea-pig diaphragm preparation caused spontaneous fasciculations. These fasciculations were also readily demonstrated with $6{\cdot}7 \times 10^{-4}$ M neostigmine in the bath. Skeletal muscle fasciculations have been reported to occur in neostigmine poisoning in humans[7].

DMSO at concentrations of 0·01, 0·1, 0·5 and 1·0 mole/l. was incubated for 30 min at 23° C with bovine erythrocyte cholinesterase (Sigma, type I) in buffer of the following composition: 0·1 mole sodium monohydrogen phosphate/l., adjusted to *p*H 7·4, 0·1 mole sodium chloride/l., 0·008 mole magnesium chloride/l. and 0·1 per cent gelatine. Neostigmine methyl sulphate, $8{\cdot}5 \times 10^{-7}$ mole/l., was similarly incubated with the enzyme.

After the 30 min period, acetylcholine chloride (Sigma) was added to a final concentration of 0·004 mole/l. and the incubation was continued for a second 30 min period.

Unhydrolysed acetylcholine was assayed by the method of Hestrin[8]: To 1 ml. of the incubation mixtures and to a standard solution of 0·004 molar acetylcholine was added 1 ml. of 2·0 mole hydroxylamine and 1 ml. of 3·5 N sodium hydroxide. After 1·5 min the solution was brought to a *p*H of $1{\cdot}2 \pm 0{\cdot}2$ with 1 ml. 4 N hydrochloric acid. One ml. of 0·37 molar ferric chloride was then added and the resulting colour immediately read at 540 mμ in a spectrophotometer.

A 90 per cent inhibition was found for neostigmine and a 3, 16, 44 and 85 per cent inhibition, respectively, for the 0·01, 0·1, 0·5 and 1·0 molar DMSO.

Thus it seems that DMSO, in relatively large concentration, is able to inhibit cholinesterase *in vitro*—this inhibitory property might account for the lowering of the vagal threshold induced by DMSO in the isolated innervated guinea-pig atrium, and for the fasciculations observed in the isolated guinea-pig diaphragm.

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