SEPARATION OF TWO RECEPTOR SITES IN A SINGLE LABELLAR SUGAR RECEPTOR OF THE FLESH-FLY BY TREATMENT WITH p-CHLOROMERCURIBENZOATE

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Abstract—A 3 min treatment of a single sugar receptor with 0.5 mM p-chloromercuribenzoate (PCMB) did not affect its response to D-fructose, but depressed completely its response to D-glucose. This is the first direct evidence of the presence of two different sites in the sugar receptor of the fly.

No specific protection by D-glucose on PCMB treatment suggested that PCMB did not react at a glucose-binding site but did react at a specific site indispensable to simulation by D-glucose.

Various sugars were examined and classified into two groups according to the effects of PCMB treatment on the sugar receptor. They correspond to those effective in the furanose and pyranose forms, respectively. The pyranose group was further divided into two subclasses according to the presence or absence of three successive equatorial hydroxyl groups regardless of their positions. The results are discussed in relation to the structures that are common to furanose stimulating sugars.

INTRODUCTION

There are two approaches to clarify the mechanism of the initial process of chemoreception. One approach to this problem is to study the relative stimulating effectiveness of different stimulants and to evaluate the relation between the molecular structure and its stimulating effectiveness (e.g. Moncrieff, 1944; Shallenberger and Acree, 1971).

The other approach is to study directly the properties of the receptor substances. This promising type of research has just begun. One of the earliest attempts was that of Dastoli and Price (1966). They isolated a 'sweet-sensitive protein' from bovine tongues. More recently Parisi et al. (1973) have isolated the special proteolipid from the electric organs of Electrophorus and have shown a biophysical response to acetylcholine when the proteolipid is embedded in artificial bilayered membranes.

Von Frisch (1935) and Dethier (1955), using Apis mellifera and Phormia regina, respectively, attempted unsuccessfully to discover configurations that were common to the stimulating carbohydrates and were absent in the tasteless ones. But one
of the most important results was the extreme specificity of the insect sugar receptor compared with that of mammals. Only certain carbohydrates were effective. Dethier observed that mixtures of the very weakly stimulating sugar, mannose, with fructose markedly reduced stimulation by fructose. Similar mixtures of glucose and mannose were as effective as if the glucose was present alone. Consequently Evans (1961) insisted on the presence of at least two different sites, i.e. the 'glucose site' and the 'fructose site' on the sugar receptor of the blowfly. The results of his own experiments suggested that the stimulating effectiveness of fructose and glucose was depressed by rearing the larvae in the presence of the sugar in question. But his result was almost denied by Dethier and Goldrich (1971) behaviorally.

On the other hand, Evans (1963) studied the stimulating effectiveness of a series of derivatives of d-glucose on the sugar receptor of the blowfly to determine the structural requirements for stimulation considering not only their molecular configurations but also their conformations. He tentatively concluded that only the equatorial hydroxyl groups of C-3 and C-4 of the molecule combined with the receptor site. Afterwards there were some developments on the extension of his research. Namely, equatorial hydroxyl residues on the C-2, C-3, and C-4 in the pyranose ring are the most important for the effectiveness, while ring oxygen and residues on C-1 and C-5 are less important (Evans, 1963; Omand and Dethier, 1969; Jakinovich et al., 1971; Hanamori et al., 1972). Hanamori et al. (1974) arranged these results quite successfully and classified the conformation of monosaccharides in pyranose form according to their stimulating effectiveness (Pflumm, 1971). Above all, they proved that d-fructose was effective only in the β-D-fructofuranose form. With all these results, however, there are some considerable exceptions in their arrangements. For example, they are unable to account for the inactivity of methyl β-L-glucopyranoside (Jakinovich et al., 1971). Furthermore, there is no information about the conformation of stimulating monosaccharides in furanose form except for d-fructose.

In order to discover a general rule for the stimulating effectiveness of sugars on the receptor of the fly, we must surmount some serious difficulties. (1) In aqueous solutions, there can be simultaneously several conformations of the same sugar such as the pyranose form, furanose form, α- and β- anomers, and so on. Their composition in the equilibrium aqueous solution cannot easily be determined. Nuclear magnetic resonance measurement is the best method for determination of the composition of a sugar solution, but there are few available data (cf. Pigman and Isbell, 1968). (2) There often appears to be no direct and simple correspondence between molecular structure and stimulating effectiveness. For example, maltose shows a concentration–response curve similar to that of sucrose in spite of a considerable difference in the structure between them (Morita and Shiraishi, 1968; Omand and Dethier, 1969). There could be many factors for stimulating effectiveness. Effectiveness may result from the summation of those factors. After all we can get no more than indirect and complex information about the mechanism of the initial process of chemoreception through the study of the relative stimulating effectiveness of different stimulants.
With respect to the direct approach to the properties of the sugar receptor substance of the fly, Hansen (1969) proposed that an α-glucosidase was the receptor protein for disaccharides. Though further studies revealed many parallelisms between the properties of the α-glucosidase and those of the sugar receptor of the fly, no direct proof supporting his working hypothesis has yet been given (AmaKawa et al., 1972; Morita, 1972; Kawabata et al., 1973; Kijima et al., 1973; Koizumi, Kijima, Kawabata, and Morita, 1973; Koizumi, Kijima, and Morita, 1974).

In our previous work (Shimada et al., 1972) on the structure and the substance of the receptor, we adopted a technique of chemical modification. Analysing the effects of the sulphhydryl reagent, p-chloromercuribenzoate (PCMB), on the response of the sugar receptor to sucrose, we concluded that PCMB reacted with sulphhydryl groups in proteins of the sugar receptor, and we suggested that it reacted at a different site from the sugar-binding site and blocked a change in the receptor membrane permeability.

We report here the results of a further study on the effects of PCMB on the response of the sugar receptor to fructose, glucose, and other sugars.

MATERIALS AND METHODS

The fleshfly, Boettcherisca peregrina, 4 to 6 days after emergence, was used in the experiments. The chemosensory hairs used were of the largest type. A side-wall recording was employed (Morita, 1959; Morita and Yamasita, 1959). The methods were described in detail in our previous paper (Shimada et al., 1972). PCMB, d-fucose, and l-glucose were purchased from Sigma Chemical Co. (St. Louis, Mo.). d-Fructose, d-glucose, d-galactose, sucrose, and maltose were of a special grade from Wako Pure Chemicals, Japan. d-Galactose was used after recrystallization four times. d-Arabinose, l-arabinose, and d-xylose were obtained from Merck, Germany. l-Sorbose was of a special grade from Nakarai Chemicals, Japan.

RESULTS

We examined here the effects of PCMB treatment on the response of the sugar receptor to various sugars such as glucose, fructose, several other monosaccharides, and two disaccharides. The results are shown in Tables 1 and 2. They are also summarized in the form of a histogram in Fig. 4.

Marked difference between the effect of PCMB on the response to glucose and that to fructose.

Fig. 1 shows the time course of response of the labellar chemoreceptors of the fleshfly to several stimulants after treatment with 0.5 mM PCMB in 1/15 M phosphate buffer (pH 7.5) for 3 min. The response of the sugar receptor to 0.2 M d-glucose was completely depressed while that to 0.1 M d-fructose was unaffected after PCMB treatment. Furthermore, the response to 1 M glucose was also depressed almost completely, while that to 1 M d-fructose was nearly normal. In
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Figure 1. Time course of response to fructose, glucose, NaCl, and H₂O after PCMB treatment obtained from a single chemosensory hair. P, treatment with 0.5 mM PCMB; R, treatment with Waterhouse's saline. Length of lines above P and R indicates the time of treatment.

In other words, with a direct attack of PCMB on the receptor cell, the effectiveness of D-glucose was blocked, but that of D-fructose was unchanged.

The duration of the PCMB treatment was critical. Treatment with 0.5 mM PCMB for more than 5 min often reduced subsequent response to 0.1 M D-fructose and the depression of the response to 0.2 M D-glucose after treatment for less than 3 min often recovered spontaneously. Excitability of the salt receptor was depressed, but the water receptor was scarcely affected by PCMB treatment. Therefore 0.2 M D-glucose caused only a firing of the water receptor cell after PCMB treatment.

Dependency on PCMB concentration

In terms of the relative response, i.e. the ratio of the magnitude of the response to each sugar after PCMB treatment to that before the treatment, Fig. 2 shows the dependency of the response on the concentration of PCMB over a range of concentration from 0.004 to 2.0 mM. White precipitate appeared above 2.0 mM. At low concentrations the depression was often recovered as time passed. Each point in Fig. 2 represents the mean value of initial three successive measurements whose intervals were about 3 min. Duration of the treatment was always kept to 3 min. The response to 0.2 M D-glucose decreased; in other words, the effect of PCMB treatment on the response increased as its concentration increased. On the other hand, the response to 0.1 M D-fructose was unchanged.

The concentration of PCMB in Fig. 1, 0.5 mM, was the lowest that could cause the most marked difference between the response to glucose and that to fructose after the treatment. The condition of treatment with 0.5 mM PCMB for 3 min was kept constant in all the following experiments reported here.
These results shown in Figs. 1 and 2 are the first direct demonstration of the existence of two different sites, that is, a 'glucose site' and a 'fructose site' on the sugar receptor of the fly, that has been suggested since Drether (1955). Thus, PCMB might react with sulphhydryl groups in proteins of the 'glucose site'.

Fig. 2. Depression of responses to fructose and to glucose by treatment with PCMB of various concentrations. Ordinate is relative value of response to that before the treatment. Duration of the treatment with PCMB was always kept at 3 min.

Two groups of monosaccharides with respect to PCMB depression

We next examined the effects of PCMB treatment of the receptor on the stimulating effectiveness of various sugars to determine which site, 'glucose site' or 'fructose site', they reacted with. When the treatment decreased the response to a monosaccharide, such as the response to glucose, it was stated to react with the 'glucose site'. On the other hand, it was stated to react with the 'fructose site' when the treatment made no effect on the response to the sugar. The results are shown in Table 1 and also in Fig. 4. Here, relative response means the ratio of the magnitude of the response to each sugar after PCMB treatment to that before the treatment as shown in Fig. 2. The responses to several sugars were sometimes observed to decrease gradually after PCMB treatment, so the value of the relative response of each test was the average of initial two measurements whose intervals were about 9 min, during which most responses were constant in amplitude.

The sugars tested can be classified into two groups. One group of sugars such as D-fructose, D-fucose, and D-galactose are more than 0·6 in relative response. The other group of sugars such as D-arabinose, L-fucose, L-arabinose, L-sorbose, D-xylose, L-glucose, and D-glucose show the values of the relative response to be smaller than 0·4. The former sugars may chiefly react with the 'fructose site', and the latter may respond to the 'glucose site' for the most part.
### Table 1—Relative response after PCMB treatment

<table>
<thead>
<tr>
<th>Monosaccharides</th>
<th>Molar sugar concentration</th>
<th>Control* response</th>
<th>Relative response M±S.D.‡</th>
<th>No. of tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Fructose</td>
<td>0.1</td>
<td>7.6</td>
<td>1.006 ± 0.252</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>7.2</td>
<td>0.940 ± 0.129</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1.197 ± 0.267</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>0.180 ± 0.150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Fucose</td>
<td>0.2</td>
<td>6.4</td>
<td>0.605 ± 0.232</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.941 ± 0.218</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>0.109 ± 0.151</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Galactose</td>
<td>0.2</td>
<td>9.8</td>
<td>0.383 ± 0.203</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.964 ± 0.174</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>0.104 ± 0.165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Fucose</td>
<td>0.1</td>
<td>8.7</td>
<td>0.225 ± 0.197</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.915 ± 0.210</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>0.104 ± 0.170</td>
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<td></td>
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<tr>
<td>L-Arabinose</td>
<td>0.3</td>
<td>4.5</td>
<td>0.050 ± 0.100</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1.167 ± 0.136</td>
<td></td>
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<td></td>
<td>G</td>
<td>0 ± 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Sorbose</td>
<td>0.2</td>
<td>4.7</td>
<td>0 ± 0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1.031 ± 0.240</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>0 ± 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Xylose</td>
<td>0.3</td>
<td>3.0</td>
<td>0 ± 0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.771 ± 0.071</td>
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<tr>
<td></td>
<td>G</td>
<td>0 ± 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Glucose</td>
<td>0.2</td>
<td>6.0</td>
<td>0 ± 0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.907 ± 0.103</td>
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<tr>
<td></td>
<td>G</td>
<td>0 ± 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Glucose</td>
<td>0.2</td>
<td>8.6</td>
<td>0.084 ± 0.140</td>
<td>98</td>
</tr>
</tbody>
</table>

* The mean value of the magnitude of the response to each sugar before PCMB treatment (impulses/0.2 sec).
† The mean value of the relative responses of each test (see text).
‡ Standard deviation.
F and G indicate 0.1 M d-fructose and 0.2 M d-glucose respectively.

**Effect of PCMB on the response to disaccharides**

Fig. 3 shows the effect of PCMB on the response to sucrose compared with those to d-fructose and to d-glucose. The response of the sugar receptor to 0.1 M sucrose was markedly decreased in the same way as that to 0.2 M d-glucose after PCMB treatment, but that to 0.1 M d-fructose was almost invariable. This indicates that sucrose as well as d-glucose may react with the 'glucose site', where the glucose moiety of sucrose played a decisive role, while its fructose moiety is the secondary factor for its stimulating effectiveness.

Maltose was also examined. The result is shown in Table 2 together with that of sucrose. It appears that maltose like sucrose reacts with the 'glucose site'.
The different effects of PCMB on the response of the sugar receptor to mono- and disaccharides are summarized in the form of a histogram in Fig. 4.

The locus of PCMB reaction

We examined the effects of glucose of a high concentration on PCMB treatment (receptor protection) in order to locate the site of the PCMB action. The results are shown in Fig. 5. Treated with 0.5 mM PCMB mixed in 1 M D-glucose solution, the response to 0.2 M D-glucose was deeply depressed and recovered only by treatment with 10 mM β-mercaptoethanol. We next considered the slight effect of 1 M D-glucose mixed into PCMB solution and examined the effect of D-glucose of a higher concentration, 3 M, on PCMB treatment together with that of 3 M D-fructose. Fig. 6 shows a considerable protection effect by 3 M D-glucose, but shows also the same protection effect by 3 M D-fructose, which was not less
Fig. 4. Comparison of relative response to several mono- and disaccharides after PCMB treatment. Results here are summarized from data in Tables 1 and 2. F and G indicate the response to 0.1 M D-fructose and 0.2 M D-glucose, respectively, as the controls. The range of standard deviation is shown by bars associated with each rectangle. The boundary of two groups is shown by the horizontal dotted line.

P: 0.5 mM PCMB
M: 10 mM β-mercaptoethanol
P+G: 0.5 mM PCMB + 1 M glucose
△: 0.1 M fructose
○: 0.2 M glucose

Fig. 5. The effect of 1 M glucose on PCMB treatment. P + G, treatment with 0.5 mM PCMB mixed in 1 M D-glucose solution; M, treatment with 10 mM β-mercaptoethanol in 1/15 M phosphate buffer (pH 7.5). Other details are the same as in Fig. 1.
in effectiveness than that by 3 M d-glucose. If the treatment with only 3 M d-glucose or 3 M d-fructose activates the response to glucose, the treatment with PCMB mixed in 3 M d-glucose or 3 M d-fructose solution should also cause a superficial protection effect. As is shown in Fig. 7, however, the treatment caused neither a noticeable activation nor depression of the response to glucose. A high concentration of sugars, in other words, caused non-specific receptor protection. The existence of non-specific protection was further proved by the study of the

![Graph](image)

**Fig. 6.** The effect of 3 M glucose and 3 M fructose on PCMB treatment. P + G, treatment with 0.5 mM PCMB mixed in 3 M d-glucose solution; P + F, treatment with 0.5 mM PCMB mixed in 3 M d-fructose solution. Other details are the same as in Fig. 5.

![Graph](image)

**Fig. 7.** The effect of treatment with either 3 M glucose or 3 M fructose only on the sugar receptor. G, treatment with 3 M d-glucose; F, treatment with 3 M d-fructose. Other details are the same as in Fig. 5.
effect of PCMB mixed with 50 per cent glycerol on the response to D-glucose. The results are shown in Fig. 8. Here also a remarkable protection effect by 50% glycerol was observed. A non-specific protection may be caused by the general interference with the PCMB reaction by some viscous and concentrated carbohydrate solutions.

These results thus show no specific receptor protection, and suggest that PCMB reacts at a different site from the glucose-binding site.

In our previous paper (SHIMADA et al., 1972) we showed the effect of PCMB treatment on the response-concentration relationship in the stimulation by sucrose. There, we suggested that PCMB reacted at a different site from the sucrose-binding site.

The above-mentioned results, therefore, do not contradict with our previous work. PCMB may not react at a glucose-binding site, but reacts at a different site that is indispensable to the establishment of stimulation by D-glucose.

DISCUSSION

There have been several suggestions with regard to the different sites of the sugar receptor of the fly such as the 'glucose site' and 'fructose site' (DETHIER, 1955; EVANS, 1963; MORITA, and SHIRAISHI, 1968; OMAND and DETHIER, 1969; JAKINOVICH et al., 1971; PFLUMM, 1971; MORITA, 1972; HANAMORI et al., 1974). Our results in Figs. 1 and 2 present direct evidence of the existence of two different sites in the sugar receptor from a special point of view, namely, chemical modification.

We could then classify the sugars examined into two groups. Members of one group were D-fructose, D-fucose, and D-galactose which chiefly reacted with the 'fructose site'. Those of the other group were D-glucose, D-arabinose, L-fucose, L-arabinose, L-sorbose, D-xylose, and L-glucose which responded to 'glucose site' for
the most part. OMAND and DETHIER (1969) insisted the existence of the different stimulus characteristics of ketose and aldose sugars. These differences, however, may be superficial and non-essential. For example, D-fucose and D-galactose (aldose) are classified into the group of D-fructose (ketose), whereas L-sorbose (ketose) is in the group of D-glucose (aldose) from our results.

On the effectiveness of sucrose we can conclude that sucrose responds to the 'glucose site' and is predominantly effective by its glucopyranosyl moiety. This conclusion does not agree with that of EVANS (1961), but agrees with those of JAKINOVICH et al. (1971) and HANAMORI et al. (1972).

With all our results we cannot easily determine in what conformation sugars react with the receptor. HANAMORI et al. (1974) analysed the response-concentration curves for both fresh (0.5 min after dissolution) and equilibrium solutions of D-fructose and the changes in the magnitude of response accompanied by the mutarotation starting from β-D-fructopyranose. They concluded that only β-D-fructofuranose was effective. From this result and ours we may conclude that D-fucose and D-galactose as well as D-fructose react with the 'fructose site' in the form of furanose. The result may be extended to a general interpretation that the effective structure to the 'fructose site' is the furanose form. But HANAMORI et al. (1973) again concluded that β-D-galactopyranose was effective in galactose solution. If examined in detail, however, their data do not reject the possibility that the furanose form is effective. Their experimental data agree rather well with the assumption that both β-D-galactopyranose and D-galactofuranose are effective. As shown in Table 1, the relative response to D-galactose is partially depressed to about 0.6 after PCMB treatment, whereas the responses to D-fructose and D-glucose are 0.94 and 0.11, respectively, for the same preparation. This means the existence of a portion of D-galactose which reacts with the 'glucose site'. The values of the relative response, however, may depend not only on the relative amounts of the pyranose and furanose forms in the sugar solution but also on the effectiveness of each form. Therefore, it may happen that the minor component of the two forms in the sugar solution contributes mainly to the response of the sugar receptor. At any rate it is impossible to determine the ratio of D-galactose in the furanose form in the solution from the data in Table 2, but it may be concluded that the sugar receptor responds predominantly to D-galactose in the furanose form and partially to the one in the pyranose form. D-fucose may react with the receptor in the same way as D-galactose. However, the sugar receptor may respond preferentially to D-arabinose in the pyranose form by similar reasoning in spite of the existence of D-arabinose which is effective in the furanose form to some extent.

Based on the above discussion, we now adopt more precise terms, namely, pyranose site and furanose site instead of 'glucose site' and 'fructose site'. They represent the different sites of the sugar receptor of the fly which respond especially to sugars in the pyranose form and those in the furanose form, respectively.

The pyranose form is subdivided into two groups by their conformations. They are the chair form and the boat form. But we consider the chair form exclusively because the boat form is generally unstable in solution (REEVES, 1950).
The chair form is further subdivided into two groups, C1 and 1C conformation based on the asymmetry according to the ring oxygen. Their discrimination, however, may be regarded as of minor importance because they are easily superimposable owing to the fact that the ring oxygen is not essential (Evans, 1963; Jakinovich et al., 1971). Pflumm (1971) insisted on the existence of C1 and 1C sites that respond to the monosaccharide with C1 conformation and to the one with 1C conformation, respectively. There seemed no experimental necessity for introducing those two sites though they may be useful for increasing the degree of freedom in order to account for the data of the various stimulating effectiveness of many monosaccharides. We recognize no essential difference between them and try to treat them all as a pyranose form.

We can, therefore, consider rather many possibilities where the molecules of sugars are superimposed freely over α-D-glucopyranose without fixing the ring oxygen. According to the results obtained so far, we will first mark three successive equatorial hydroxyl groups on the positions C-2, C-3, and C-4 of α-D-glucopyranose as indispensable to stimulation (Jakinovich et al., 1971; Hanamori et al., 1974).

As an example, we will consider β-L-glucopyranose. Fig. 9(1) shows β-L-glucopyranose of 1C conformation which is more stable than that of C1 conformation (H₂OH on C-6 was omitted; similarly in the following (2), (3), and (4)). α-D-Glucopyranose of C1 conformation is shown in Fig. 9(4).

There are two ways to superimpose β-L-glucopyranose of 1C conformation over α-D-glucopyranose of C1 conformation under the condition of exact overlapping of three successive equatorial hydroxyl groups with each other. The

![Diagram of sugar molecules](image-url)

**Fig. 9.** Superimposition of β-L-glucopyranose of 1C conformation over α-D-glucopyranose of C1 conformation. The numbered circles represent carbon atoms 1 to 6. The middle dark circle and the small ones indicate the ring oxygen atom and hydrogen atoms respectively. The shadowed circles with dotted lines represent oxygen atoms of the three successive hydroxyl groups that are essential for stimulation. (1) β-L-Glucopyranose of 1C conformation; (2) inversion of (1) fixing ring oxygen and C-3; (3) 60° rotation of (1) about the axis; (4), α-D-glucopyranose of C1 conformation.
orientation in Fig. 9(2) is obtained by inverting \(\beta\)-L-glucopyranose in Fig. 9(1), fixing its ring oxygen and C-3. On the other hand, the orientation in Fig. 9(3) is obtained by a rotation of 60° about the axis passing perpendicularly through the centre of the ring. In both, three successive equatorial hydroxyl groups of \(\beta\)-L-glucopyranose can be superimposed over those at C-2, C-3, and C-4 of \(\alpha\)-D-glucopyranose. But in (2), the equatorial hydroxyl methyl group of \(\beta\)-L-glucopyranose is put on the position C1 of \(\alpha\)-D-glucopyranose. Here we next note that the equatorial substitute of a certain size at the position C-1 hinders stimulation such as that by methyl \(\beta\)-D-glucoside and \(p\)-nitrophenyl \(\beta\)-D-glucoside. Thirdly, the C-1 hydroxyl group both in the \(\alpha\) and \(\beta\)-positions was not necessary for stimulation (HANAMORI et al., 1972). It may be concluded that the orientation in Fig. 9(3) of \(\beta\)-L-glucopyranose is much more effective for stimulation than that in Fig. 9(2). The former orientation may well explain the relatively high stimulating effectiveness of L-glucose which consists of \(\alpha\)- and \(\beta\)-anomers in aqueous solution. The inactivity of methyl \(\beta\)-L-glucopyranoside suggests also that the orientation in Fig. 9(2) is not effective. Otherwise, methyl \(\beta\)-L-glucopyranoside would be as effective as L-glucose since the equatorial substituents at C-5 of \(\alpha\)-D-glucopyranose are less important for stimulation (JAKINOVICH et al., 1971). The orientation in (3) of methyl \(\beta\)-L-glucopyranoside results in O-methylation of one of the three successive equatorial hydroxyl groups and causes a due decrease in its effectiveness somewhat more than that of 2-O-methyl D-glucopyranose.

In short, three successive equatorial hydroxyl groups in the chair form of the pyranose ring are first of all essential for stimulation regardless of their positions. This also means that the equatorial hydroxyl group at C-1 may play an important role as one of the three successive equatorial hydroxyl groups such as in L-glucose, since sugar solutions usually contain both \(\alpha\) - and \(\beta\)-anomers of the same monosaccharides.

With this general rule for the stimulating effectiveness of sugars, monosaccharides are classified into two groups according to the presence or absence of three successive equatorial hydroxyl groups. The classification is shown in Table 3 with respect to our results in Table 1 and those of others (JAKINOVICH et al., 1971; HANAMORI et al., 1974). Here we have parenthesized some sugars such as D-fructose, D-fucose, and D-galactose since they may be effective in the furanose form for the most part as is shown in our results. There are some exceptions to our general rule, but these are easily explained with further consideration.

Inactivity of 4-O-methyl-D-glucose with three successive equatorial hydroxyl groups at C-1, C-2, and C-3 is explained by the presence of the equatorial methoxy group on the corresponding C-1 position of \(\alpha\)-D-glucopyranose of C1 conformation when it is superimposed over \(\alpha\)-D-glucopyranose. Inactivity of methyl \(\alpha\)-L-glucoside is explained in almost the same way as that of methyl \(\beta\)-L-glucoside.

The ineffectiveness of \(\beta\)-altrose of 1C conformation seems to need a somewhat different explanation than that proposed so far. Some useful information is given from the study of the specificity of the sugar receptor to some isomers of myo-inositol that have three successive equatorial hydroxyl groups except allo-inositol.
TABLE 3—CLASSIFICATION OF PYRANOSES

<table>
<thead>
<tr>
<th>Stimulating effectiveness</th>
<th>With three successive hydroxyl groups</th>
<th>Without three successive hydroxyl groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective</td>
<td>C1: D-Glucose, poligalitol, D-xylose, α-D-glucose, methyl α-D-glucoside, methyl β-D-glucoside, α-L-arabinose, (β-D-galactose), (β-D-fucose)</td>
<td>C1: 2-Deoxy-D-glucose, 2-O-methyl-D-glucose</td>
</tr>
<tr>
<td></td>
<td>1C: L-Glucose, L-sorbose, L-xylose, α-D-arabinose, β-L-fucose</td>
<td></td>
</tr>
<tr>
<td>Ineffective or little effective</td>
<td>C1: 4-O-methyl D-glucose</td>
<td>C1: D-Allose, D-mannose, D-ribose, D-lyxose, 3-deoxy-D-glucose, 3-O-methyl-D-glucose, D-glucose, D-tagatose, D-altrose, D-glucose</td>
</tr>
<tr>
<td></td>
<td>1C: α-D-Aльтrose, methyl α-L-glucoside, methyl β-L-glucoside, (α-D-fructose)</td>
<td>1C: D-Lyxose, D-glucose, L-rhamnose</td>
</tr>
</tbody>
</table>

(JAKINOVICH AND AGRANOFF, 1972). Ring carbon atoms are numbered corresponding to those of α-D-glucopyranose of C1 conformation under the condition of exact overlapping of three successive equatorial hydroxyl groups. First, the axial substituent at C-5 may cause drastic steric hindrance for stimulation since epiniositol with two axial substituents at C-1 and C-5 shows no stimulating effectiveness while myo-inositol with an axial substituent at C-1 is very effective. Second, a steric hindrance for stimulation also may happen by an axial substituent at C-6, the position corresponding to ring oxygen of α-D-glucopyranose as D-chiro-inositol with two axial substituents at C-1 and C-6 is less effective compared with myo-inositol with an axial one at C-1. D-Aльтrose is ineffective because of its axial hydroxyl group at C-5 and the hydroxyl methyl group at the position corresponding to C-6 of D-chiro-inositol. α-D-Fructose may be ineffective in the pyranose form because of its axial hydroxyl methyl group at the same position as one of the three successive equatorial hydroxyl groups. The slight effectiveness of 2-deoxy-D-glucose and 2-O-methyl-D-glucose may suggest a less stringent requirement for the first equatorial hydroxyl group of the three successive ones. D-Idose may be the only one exception for this general rule since it has been shown to be ineffective in spite of its three successive equatorial hydroxyl groups (DETHIER, 1955; PFLUMM,
It is thought to be necessary to reinvestigate this problem especially by electrophysiological methods. But it might be possible to explain its ineffectiveness based on an assumption that the pyranose form is not predominant in an aqueous solution. Its furanose form is discussed later.

There has been little information about the structure of the sugars, except D-fructose, which react with the furanose site of the sugar receptor. In Table 4 we list a few sugars, which are thought to react with the furanose site with reference to our results and those of others (Evans, 1963; Jakinovich et al., 1971; Hanamori et al., 1974). The presence of the furanose form in sugar solutions was proved on

### Table 4—Classification of Furanoses

<table>
<thead>
<tr>
<th>Stimulating effectiveness</th>
<th>Effective</th>
<th>Ineffective or little effective</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D-Fructose, D-fucose, D-galactose</td>
<td>D-Ribose, D-mannose, (D-idose), methyl α-D-fructoside, methyl β-D-fructoside, (α-D-fructose-6-phosphate)</td>
</tr>
</tbody>
</table>

Fig. 10. Basic structure of monosaccharides in pyranose and furanose forms essential for stimulation. The shadowed circles represent the position of common residues essential for stimulation. Ring atoms are numbered arbitrarily regardless of their being carbon or oxygen. R₁ and R₂ represent the residues which are different with different sugars. Furanose is shown tentatively in an envelope conformation though there is no reason for excluding a twist conformation. Others are the same as in Fig. 9.
the limited monosaccharides such as D-fructose, D-galactose, D-ribose, and D-mannose. D-Fucose may be in the furanose form to some extent since L-fucose in the furanose form coexists with that in the pyranose form (cf. Pfumm, 1971). According to Hanamori et al. (1973), only β-D-fructofuranose is effective in the equilibrium solution of D-fructose. Inactivity of methyl β-D-fructofuranoside means an important role for the hydroxyl group in the β-position, and the ineffectiveness of α-D-fructofuranose-6-phosphate may suggest some stringent requirement for substituents at C-6 as compared with the effectiveness of glucose-6-phosphate (Dethier, 1955). Based on the assumption that the ring oxygen is not essential for stimulation as it is at the pyranose site, we find a common structure among β-D-fructofuranose, D-fucofuranose, and D-galactofuranose on applying a suitable rotation. The results are tentatively shown in Fig. 10 together with those of the pyranose form. The essential and common structure may be two hydroxyl groups at C-1, C-2, and the R₃ residue shown by shadowed circles in Fig. 10(2). Their orientation shown in Fig. 10 may also be important.

D-Ribose has a different hydroxyl group at C-2 whose orientation is in the opposite direction to that in Fig. 10. The hydroxyl group at C-3 of D-mannose is in the opposite direction to that in Fig. 10 and causes a steric hindrance against the hydroxyl group at R₂. This renders a twisted conformation of the R₃ residue to D-mannose. These two sugars may be classified in the group of ineffective sugars in the furanose form. D-Idose in the furanose form has an hydroxyl group at C-1 in the opposite direction though there has been no proof of its existence as yet. This may be the explanation for its ineffectiveness.

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REFERENCES


