The Metabolism of the Oral Flora

2. THE OXIDATION OF SOME SUGARS BY MIXED HUMAN SALIVA

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The breakdown of carbohydrate by the oral micro-organisms has been the subject of numerous investigations. It is believed by many that the intra-oral production of acids from carbohydrate, arising from incomplete oxidation, is a factor in the production of dental caries. With a view to finding the agent causing acid production, specific organisms have been isolated from the mouth and their biochemical behaviour has been studied; this work has been outlined in a previous paper (Hartles & McDonald, 1950). More recently, the anaerobe *Clostridium welchii*, which can break down glucose into lactic acid more rapidly than does *Lactobacillus acidophilus* (Hartles & McLean, 1952), has been found in carious teeth (Hartles & McDonald, 1951; Evans & Prophet, 1951). Undue emphasis must not be placed on the results obtained by studying cultures of organisms isolated from the mouth, for the presence of a particular acidogenic organism may be relatively unimportant if enzymes which can complete the oxidation of the carbohydrate are contributed to the system by other organisms. For example, lactic acid may be the end point of glucose metabolism for an *in vitro* culture of *Lb. acidophilus*, but it certainly is not so in the mouth, for frequently other organisms exist there which can metabolize lactic acid (Douglas, 1950; Neuwirth & Summerson, 1951). Fosdick & Hansen (1936) were perhaps the first to realize fully the importance of this symbiotic relationship. Thus, whilst recognizing the contribution which has been made by the investigation of specific bacteria, we consider it desirable to study the enzymic properties of saliva as a whole. We have already studied the oxygen uptake of saliva samples with and without the addition of glucose (Hartles & McDonald, 1950); a similar study by Neuwirth & Summerson (1951) reached substantially the same conclusions. It seemed desirable to extend this work using other sugars, primarily to find out if there were any differences in the rates of oxygen uptake by a series of salivas when each sugar was added separately. The sugars chosen were glucose, fructose, maltose, and sucrose, since they are the most relevant to man. We found that in the saliva of all the subjects studied, fructose was metabolized less rapidly than the other three sugars.

EXPERIMENTAL

Material. Samples of saliva were obtained from ten volunteers with varying caries experience.

Collection of saliva. The method of collection of early-morning saliva was that described by Hartles & McDonald (1950).

Manometry. The saliva samples (0.5 ml. or 1.0 ml., depending on the enzymic activity) were buffered with 0.2M phosphate buffer, pH 7.4. O_2 consumption was measured in Warburg manometers at 38°. The final concn. of sugar, when used, was 0.005M. Increasing the concn. to 0.01M had no noticeable effect on respiration. The difference in O_2 uptake between saliva incubated alone and with added sugar was a measure of oxidation of the sugar.

RESULTS

The oxidation rates of the four sugars are shown in Table 1. The subjects have been arranged in two groups of five in order of increasing DMF number (i.e. the number of teeth decayed, missing or filled); those in group I have an average DMF of 8 and those in group II of 19.

In group I fructose and maltose showed a consistently significant difference in metabolic rate for each subject; the difference between fructose and sucrose was significant for two subjects, J.M. and J.R.D. ($P < 0.05$), while that between glucose and fructose was not significant at the 5% level. The analysis of variance shows that combining the data for all five subjects accentuates the difference between sugars; fructose is metabolized at a significantly lower rate than any of the other three sugars ($P < 0.001$), whilst maltose is metabolized at a significantly higher rate than either glucose ($P < 0.001$) or sucrose ($P < 0.01$). There is no significant difference in the rates of oxidation of glucose and sucrose.

Results for group II show that each subject (except M.R.W. with sucrose) metabolizes glucose, maltose, and sucrose, at a significantly higher rate than fructose; also, that the variation in the values for each sugar is greater than that displayed by the subjects of group I. This is established by an analysis of variance of the combined data for group II in which the residual variance used to assess the

Table 1. *Mean oxygen consumption of salivary samples due to the addition of different sugars*(All values expressed as $\mu\text{l. O}_2/\text{ml. saliva/hr.}$ Final concn. of added sugar, 0.005M. Temp. 38°.)

Subject	Teeth decayed, missing, or filled (DMF)	Glucose	Fructose	Maltose	Sucrose	*s.e.	No. of days
Group I							
S.F.S.	4	91.7	87.1	98.5	91.1	4.0	12
J.M.	6	68.5	62.9	77.5	77.8	4.0	10
J.G.	8	63.7	59.6	74.7	63.0	3.1	12
J.R.D.	10	180.4	170.2	186.7	186.3	5.8	12
A.G.L.	12	143.0	132.4	149.8	140.5	5.7	11
Mean	8	109.4	102.4	117.4	111.7	—	—
Weighted mean		110.2	103.4	118.1	112.5	—	—
Group II							
R.L.H.	17	397.1	334.8	390.2	411.5	14.7	13
M.R.W.	17	136.4	113.5	128.9	121.4	5.1	14
L.G.	18	108.0	77.5	103.1	110.8	5.1	10
I.A.R.	20	60.6	46.8	68.0	65.0	3.9	11
P.D.B.	23	134.6	115.5	138.5	135.6	5.2	12
Mean	19	167.3	137.6	165.7	168.9	—	—
Weighted mean		173.9	143.3	172.0	175.0	—	—

* s.e. is the standard error of the difference between any two means: thus, roughly speaking, a difference of more than twice this value between any two means in a given row is statistically significant.

significance of the variance between sugars is much greater than that in the analysis for group I. Thence it can be seen from the overall analysis for group II that, again, fructose is the sugar least rapidly metabolized ($P < 0.001$), but that there is no significant difference in the rates of metabolism of the other three sugars.

An analysis of variance of the whole data for the ten subjects shows that the difference in metabolic rate between fructose and the other sugars is highly significant ($P < 0.001$). When deliberate selection is exercised and the subjects are arranged in two groups according to their DMF numbers, certain tendencies are observed. The differences between sugars with subjects of lower DMF (group I) are sharpened, because the general pattern of metabolism amongst these subjects is the same, that is, that fructose is metabolized less and maltose more rapidly than the others, whilst there is no significant difference between rates of glucose and sucrose metabolism. On the other hand, small differences between sugars with subjects of higher DMF (group II) are blurred because these subjects tend to behave differently toward different sugars and thus the only significant difference in this group is that between fructose and the three other sugars.

DISCUSSION

Dixon & Needham (1946) reported that yeast hexokinase had a lower affinity for fructose than for glucose. Meyerhof & Wilson (1949) obtained similar results for brain hexokinase. Slein, Cori & Cori

(1950) found that yeast and brain hexokinases were similar in many respects, and they produced evidence to show that a single enzyme was responsible for the action on both glucose and fructose. Our results suggest that a hexokinase is concerned in the oxidation of glucose and fructose by the oral flora.

We found that sucrose was, in all except one of the subjects, metabolized at least as rapidly as glucose by the saliva. There is some disagreement in the literature concerning the existence of invertase in saliva (Bourquelot, 1910; Lisbonne, 1910; Tauber & Kleiner, 1933; Wohrinz, 1938; Volker, 1950). The fact remains, however, that unfiltered whole saliva can metabolize the disaccharides sucrose and maltose no less readily than glucose. Doudoroff, Kaplan & Hassid (1943) demonstrated that *Pseudomonas saccharophila* contained an enzyme which phosphorylated sucrose directly. There are, then, two possible pathways for sucrose breakdown. If sucrose is acted upon by invertase to produce glucose and fructose in equimolecular proportions, and if glucose and fructose are competing for hexokinase, it is not unreasonable to expect sucrose to be oxidized at a rate significantly less than glucose. For our group of salivas this was not so, and our results suggest that preliminary hydrolysis of sucrose may not be the first step in its metabolism. Further work is necessary to decide this point, and also whether the non-glycolytic enzymes concerned are a secretion of the salivary glands or are contributed by the oral flora. The only salivary carbohydrase which has been shown to be a product of the salivary glands is α -amylase.

Roberts & Whelan (1951) have shown that this enzyme, when purified, has no action on maltose.

We are not at this stage particularly concerned with differences between 'caries active' and 'caries inactive' groups of subjects. We also realize that the present DMF number is not necessarily an indication of the present caries activity. Nevertheless, certain interesting observations can be made. In group I (low DMF) fructose is metabolized at a significantly lower rate than any of the other sugars. There are also significant differences in the rates of oxidation of maltose and sucrose, and of maltose and glucose. There is no such difference in the case of glucose and sucrose. In group II (high DMF) the only conclusion which can be drawn is that fructose is metabolized at a lesser rate than the other three sugars. Our results therefore suggest that the pattern of utilization of different sugars by group I subjects differs from that of group II subjects. It is, however, unwise to deduce more from these observations on so few subjects.

SUMMARY

1. The salivary flora metabolize fructose at a significantly lower rate than glucose, maltose, or sucrose.

2. Subjects with a comparatively low DMF number (number of teeth decayed, missing or filled) also show differences in oxidation rate between glucose and maltose, and between sucrose and maltose, but not between sucrose and glucose.

3. Subjects with a high DMF number did not show the differences enumerated in paragraph 2.

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The Breakdown of Urea in Cats not Secreting Gastric Juice

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Although Luck (1924*a, b*) had shown urease to be present in the stomachs of a wide variety of animals, urea was until recently regarded as solely an end-product of mammalian nitrogen metabolism. Experiments with [¹⁴C]urea (Leifer, Roth & Hempelmann, 1948; Langham, 1949; Jones, 1950; Hastings, Langham, Carter & Roth, 1950; Skipper *et al.* 1951; Kornberg, Davies & Wood, 1953*a*) and [¹⁵N]urea (Davies & Kornberg, 1950; Von Korff, Ferguson & Glick, 1951; Kornberg & Davies,

1952*a, b*) showed, however, that cats, dogs, mice, and guinea pigs, could hydrolyse urea *in vivo* and that urea was therefore not metabolically inert.

The experiments reported in this and a subsequent paper (Kornberg, Davies & Wood, 1954) were undertaken to study the rate of, and the conditions affecting, the breakdown of [¹⁴C]urea in cats, whose stomachs were allowed either to rest or to secrete gastric juice. Previous investigations of the behaviour in the cat of [¹⁴C]carbon dioxide liberated into the blood (Kornberg, Davies & Wood, 1952*a*) had shown that the amount of [¹⁴C]urea hydrolysed

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