Technical Papers

Oxidation of Disaccharide Alcohols by *Acetobacter suboxydans*[1]

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In connection with work on the constitution of planteose (*1*), we have been interested in various possibilities for the synthesis of oligosaccharides in which the reducing group is that of a ketose. Although the biological oxidation (*2*) of sugar alcohols by *Acetobacter* species has been used to prepare monosaccharide ketoses, we have been unable to find any record that this selective oxidation has ever been used to convert disaccharide sugar alcohols into the corresponding disaccharide ketoses.

We wish to report now some preliminary experiments on the behavior of disaccharide alcohols in *A. suboxydans* cultures. Typical 10-ml cultures contained 50 mg of yeast extract and 25 mg of sorbitol or mannitol (to ensure good growth of the organism) with or without 100 mg of the disaccharide alcohol. Samples were taken at intervals and analyzed for total reducing sugar and for the presence of reducing sugars (alkaline copper, heated, followed by phosphomolybdic acid) and ketoses (phloroglucinol-hydrochloric acid) on paper chromatograms.

Melibiitol (*3*), obtained by sodium borohydride reduction (*4*) of melibiose, was completely unaffected by the organism. Even after 46 days there was no evidence for the formation of any reducing sugar other than sorbose (from the added sorbitol). Since glycosides in general are not oxidized, the only position in melibiitol which would be configurationally suitable for oxidation would be position 5 of the sorbitol moiety. However, substitution of the galactosyl group on position 6 blocked oxidation.

Maltitol (*3*), similarly obtained from maltose and purified through the crystalline acetate, behaves in the same fashion. Here, substitution on position 4 of the sorbitol unit blocked oxidation at the otherwise available position 5.

Epimelibiitol (1-galactosyl mannitol) (*1*) was obtained in crude form by the acid-catalyzed condensation of galactose and mannitol. The preparation contained much mannitol, some mannitol anhydrides, and a mixture of the α and β galactosyl mannitols. On subjecting this mixture to the action of *A. suboxydans*, coincident with the formation of fructose there appeared a reducing disaccharide which had the same R_f value as planteobiose (*1*) and gave the characteristic color test for a ketose when sprayed with the phloroglucinol reagent. In this case, substitution of a glycosyl group at one end of the mannitol chain still leaves the other end unsubstituted and of the correct configuration for oxidation.

It is proposed, then, that this oxidation can be used for the preparation of keto disaccharides or higher oligosaccharides, provided that the corresponding sugar alcohols are available. We are now attempting to prepare workable quantities of planteobiose from purified epimelibiitol, and maltulose from turanose (through epimaltitol [*5*]).

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Rh and ABO Blood Group Distributions in Japanese and Ethiopians

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Soon after the outbreak of hostilities in Korea a large-scale blood bank was activated in Tokyo. It supplied whole blood to medical units supporting United Nations forces in Korea and Japan. Most of its donor procurement and all its blood-processing operations centered in Tokyo.

Among the diverse racial and national groups volunteering as donors were numerous Japanese of the metropolitan area. Since these donors represented a realistic cross section of the indigenous population, it seemed appropriate to report some observations pertinent to them.

This paper records the Rh and ABO blood group distributions in 4541 native Japanese. It compares present findings with those described previously in Japan and in countries where persons of Japanese ancestry constitute a population minority. Data concerning frequencies in Ethiopian troops are also presented.

To ensure the accuracy of Rh[1] determinations, each blood was typed both by slide and by tube-centrifugation techniques. Fifty per cent concentrations of cells in their own serum were deposited on slides, mixed

[1] Refers to the Rh antigen most significant clinically, Rh_o (D). Its presence in erythrocytes of any phenotype combination is demonstrated by agglutination with anti-Rh_o serum. Lack of agglutination denotes its absence. Accordingly, persons with reactive cells are regarded as clinically Rh-positive; those with nonreactive cells are considered clinically Rh-negative.

TABLE 1

RH AND ABO FREQUENCIES IN JAPANESE AND ETHIOPIANS

Place	No. persons studied	Rh* (%)		Group (%)			
		Positive	Negative	O	A	B	AB
Japanese							
New York (1)	150	98.0	2.0	26.0	40.0	23.3	10.7
Denver† (2)	280	98.9	1.1	29.4	37.2	22.2	11.2
Canada (3)	606	98.9	1.1	—	—	—	—
Japan (Kumamoto Prefecture) (4)	459	98.5	1.5	—	—	—	—
Japan (Kumamoto Prefecture) (5)	1,011	98.7	1.3	—	—	—	—
Japan (nationwide) (6)	302,928	—	—	30.5	38.2	21.9	9.4
Japan‡	4,541	99.56	0.44	33.1	36.5	21.6	8.8
Ethiopians							
Ethiopia (6)	400	—	—	42.8	26.5	25.2	5.5
Korea‡	878	95.8	4.2	41.2	28.5	24.0	6.3

* Refers to reactions with anti-Rh_0 (anti-D) serum only.
† Group distributions based on 180 persons.
‡ Present study: Japanese in metropolitan Tokyo; Ethiopian troops with UN forces in Korea.

with 1 drop of anti-Rh_0 (anti-D) serum[2] and examined for agglutination within 5 min. All slide results were verified by tube tests in which cells from clotted blood were suspended to 2% concentration in one drop of anti-Rh_0 serum, centrifuged briefly, then read.

Blood groupings were carried out by tube-centrifugation procedures. Known anti-A and anti-B sera were used to classify the donor's cells. Conversely, known group A and B cells served to identify isoagglutinins in the donor's serum. Thus, the ABO group of each blood was established by its agglutinin as well as its agglutinogen content.

Table 1 compares data from representative surveys of Japanese and Ethiopians. It shows that of 4541 Japanese donors examined in the present investigation only 0.44% (20) were Rh-negative. This frequency is notably lower than the others listed. It is probable that the use of more effective diagnostic sera and techniques has minimized the occurrence of false negative reactions. Similarly, some Rh_0 antisera contained a mixture of D and D^u (Rh_0 intermediate) agglutinins in relatively high titer. The presence of this second antibody may have identified cells with D^u variant as Rh-positive, thus reducing the Rh-negative findings further.

In comparison with the others, the present survey indicates a moderately higher percentage of group O and slightly lower percentages of groups A, B, and AB Japanese. Since the earlier Ethiopian study did not include Rh data, only ABO group frequencies can be compared. These do not differ materially.

Table 2 shows that whereas the sexes in each group and the Rh-negatives in both sexes are almost equally distributed, the Rh-negative frequencies within each group vary substantially. To determine the statistical significance of these differences, the χ^2 test was applied only to the data for groups A and O, since groups B and AB do not have sufficient Rh-negatives for statistical test. Analysis reveals that the deviation in group A is not significant. However, the deviation in group O yields a χ^2 of 6.2 for 1 degree of freedom, giving a probability of less than 0.02. It therefore seems unlikely that the observed difference is due to chance. Conceivably, a sample containing considerably

[2] Procured from American commercial sources, all antisera conforming to National Institutes of Health standards.

TABLE 2

RH-NEGATIVE DISTRIBUTION IN JAPANESE BY SEX AND BLOOD GROUP

Total persons studied	4541		
Rh-negative			20 (0.44)*
Male		3075	
Rh-negative			14 (0.46)
Female		1466	
Rh-negative			6 (0.41)
Group O	1501		
Rh-negative			13 (0.87)
Male		998 (32.5)†	
Rh-negative			9 (0.90)
Female		503 (34.3)‡	
Rh-negative			4 (0.80)
Group A	1659		
Rh-negative			6 (0.36)
Male		1145 (37.2)	
Rh-negative			4 (0.35)
Female		514 (35.1)	
Rh-negative			2 (0.39)
Group B	979		
Rh-negative			1 (0.10)
Male		663 (21.6)	
Rh-negative			1 (0.15)
Female		316 (21.6)	
Rh-negative			0 (0.00)
Group AB	402		
Rh-negative			0 (0.00)
Male		269 (8.7)	
Rh-negative			0 (0.00)
Female		133 (9.0)	
Rh-negative			0 (0.00)

* Figures in parentheses indicate percentages.
† Percentage of 3075 males; calculated similarly for groups A, B, and AB.
‡ Percentage of 1466 females; calculated similarly for groups A, B, and AB.

more Rh-negative individuals might alter present implications.

Although not recorded in the table, intergroup Rh-negative frequencies in Ethiopian troops showed no significant differences.

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Rooting Lemon Cuttings with Fruits Attached

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Detached lemon fruits are utilized in many research problems, of both a general and a specialized nature. A major drawback to their use has been the relatively short period during which they would remain turgid and more or less normal. The authors were concerned with prolonging the useful life of lemon fruits for studies of a physiological, biochemical, and entomological nature. A simple solution to the problem seemed to be the production of roots on stems attached to the fruits. Such a technique should not only result in maintaining healthy turgid fruits for long periods under the usual conditions of high humidity but should permit studies involving low relative humidity.

This paper presents the results obtained in an experiment to determine the rooting response of lemon cuttings with fruits attached.

Two hundred and forty-nine medium-sized lemons ranging in color from yellow to light-green were clipped from several Eureka lemon trees on Feb. 7. Stems on the fruits varied from 1 to 2 in. in length, and approximately half of them had one or two leaves attached. The fruits were segregated into three color groups of yellow, silver, and light-green. The silver category is a packing-house designation for yellow fruit which still retains a slight amount of green color, usually at the ends. Each color group was subdivided into cuttings with and without leaves. These groups were further divided into groups to be treated with a rooting preparation (0.2% naphthalenacetic acid on talc, ANA) or left untreated. The cuttings were placed in a rooting bed with sand as a rooting medium and were usually sprinkled two or three times daily during the rooting period.

FIG. 1. Rooted lemon cutting without leaf but with light-green lemon attached.

A count of rooted cuttings and roots was made on Mar. 6, 4 weeks after the start of the experiment. The results are presented in Table 1 and Fig. 1. Cuttings with light-green lemons attached rooted most readily, whereas those with yellow lemons rooted least readily. The presence of leaf tissue appeared to be unnecessary in the cuttings with light-green and silver lemons but necessary for root formation in the cuttings with yellow lemons. Naphthalenacetic acid increased the percentage of rooted cuttings in all comparisons.

TABLE 1

ROOTING RESPONSE OF LEMON CUTTINGS

	Light-Green				Silver				Yellow			
	Leaves		No leaves		Leaves		No leaves		Leaves		No leaves	
	No ANA	ANA	No ANA	ANA	No ANA	ANA	No ANA	ANA	No ANA	ANA	No ANA	ANA
No. cuttings	38	37	31	26	21	22	13	12	13	15	10	11
Percentage rooted	31	59	52	81	10	68	15	67	8	67	10	18
Roots per rooted cutting	1.8	3.8	3.3	3.0	1.5	4.4	1.5	2.4	1.0	3.9	5.0	7.5

Leafy lemon cuttings have been reported to root better than leafless ones (*1*), even when treated with a growth regulator such as indolacetic acid (*2*). Cooper (*3*) suggested that the role of indolacetic acid was to mobilize, at the base of the cutting, rhizocaline, a root-forming factor produced in the leaves. On the