reacting 'directly' in the van den Bergh test. Their absorption curves are similar, though not identical, and they differ from that of bilirubin.

3. The visible absorption curves of the azo compounds of these three pigments are indistinguishable.

4. The two direct-reacting pigments of serum occur in small amounts in the urine of patients with obstructive jaundice, and in large quantities in gallbladder bile obtained at necropsy, where they are more abundant than bilirubin.

5. The proportion between the two direct-reacting pigments of serum is variable.

6. There is evidence that other diazo-positive pigments occur in bile and urine.

We wish to express our thanks to the many clinical colleagues who have provided us with sera, and to Dr W. Hunt, St Mary's Hospital, Paddington, for obtaining specimens of autopsy bile. A grant from the Medical Research Council is also acknowledged by one of us (B.H.B.).

REFERENCES

- Andrewes, C. H. (1924). *Brit. J. exp. Path.* **5**, 213.
 Bergh, A. A. H. van den (1918). *Der Gallenfarbstoff im Blute*, Leiden. Quoted by Hunter, G. (1930). *Brit. J. exp. Path.* **11**, 415.
 Bergh, A. A. H. van den & Müller, P. (1916). *Biochem. Z.* **77**, 90.
 Claireaux, A. E., Cole, P. G. & Lathe, G. H. (1953). *Lancet*, **2**, 1226.
 Cole, P. G. & Lathe, G. H. (1953). *J. clin. Path.* **6**, 99.
 Cole, S. W. (1933). *Practical Physiological Chemistry*, 9th ed. p. 24. Cambridge: Heffer.
 Feruglio, F. S. & Salvini, M. (1947). *Boll. Soc. ital. Biol. sper.* **23**, 1183.
 Heilmeyer, L. (1943). *Spectrophotometry in Medicine*, p. 164. Hilger: London.
 Howard, G. A. & Martin, A. J. P. (1950). *Biochem. J.* **46**, 532.
 Siedel, W. (1951). In *Physiologische Chemie*, edited by Flaschenträger, B. Berlin: Springer.

Polyol Dehydrogenases

1. THE SPECIFICITY OF RAT-LIVER POLYOL DEHYDROGENASE

BY JEAN MCCORKINDALE AND N. L. EDSON

Department of Biochemistry, Medical School, University of Otago, New Zealand

(Received 22 February 1954)

The 'sorbitol' dehydrogenase of rat liver (Blakley, 1951) reacts with diphosphopyridine nucleotide (DPN) to catalyse the reversible oxidations:

Finding that glucose, gluconate, mannitol, dulcitol and D-iditol did not react with the enzyme, Blakley (1951) concluded that an oxidizable substrate must possess a primary alcohol group at C-1 adjacent to the site of oxidation (C-2) and a stereochemical pattern at C-2 and C-4 identical with that occurring in sorbitol and L-iditol.

Blakley's evidence eliminated the configuration at C-5 as a determinant of specificity but did not decide the importance of the configuration at C-3. In order to settle this point, and to gain more knowledge of the enzyme, we have investigated the oxidation of a wider range of polyhydric alcohols.

MATERIALS AND METHODS

Enzyme. The enzyme solution was the dialysed preparation B of Blakley (1951).

Coenzymes. Diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) were products of the

Sigma Chemical Co., St Louis, Missouri. The former was found to be 65% pure by the LePage (1947) assay and the latter 60% pure (assayed with glucose 6-phosphate dehydrogenase).

Acyclic polyols. Xylitol (m.p. 92–93.5°) and D-arabitol (m.p. 102°) were prepared by catalytic reduction of D-xylose and D-arabinose respectively, according to Carson, Waisbrot & Jones (1943). Allitol (m.p. 148–149°) was prepared by oxidation of divinyl glycol (Lespieau, 1946). Erythritol, ribitol and L-arabitol were obtained from Nutritional Biochemicals Inc., Cleveland, Ohio, U.S.A. Sorbitol monohydrate, D-mannitol and dulcitol were products (C.P. grade) of the Pfanstiehl Chemical Co., U.S.A. The following were gifts from Dr N. K. Richtmyer, National Institutes of Health, Bethesda, U.S.A.: D-gulitol, 6-deoxy-L-gulitol, D-talitol, volemitol, perseitol, β-sedoheptitol and L-gulo-D-gala-heptitol.

Cyclitols. Scyllitol (m.p. 347°) was prepared from scyllitol hexaacetate according to Posternak (1942). *meso*Inositol (C.P. grade) was obtained from the Pfanstiehl Chemical Co.; D- and L-inositol, L-viburnitol and scyllitol hexaacetate were gifts from Mrs E. Smith, Chemistry Department, University of Sydney.

Sugars. The sugars used were obtained from the following sources: D-xylose (C.P. grade), Pfanstiehl Chemical Co.; D-arabinose, Nutritional Biochemicals Inc.; D-mannoheptulose and D-glucoseheptulose, General Biochemicals Inc., Chagrin Falls, Ohio, U.S.A.; perseulose hemihydrate and

sedoheptulosan monohydrate (gifts), Dr N. K. Richtmyer; D-xylulose (gift) prepared from the crystalline monoacetone derivative according to Levene & Tipson (1934) by Mr R. G. Kulka; D-tagatose (gift) prepared according to Reichstein & Bosshard (1934) by Mr D. R. D. Shaw. Sedoheptulose: an equilibrium mixture containing approximately 20% of sedoheptulose and 80% of sedoheptulosan was prepared by heating (steam bath) a solution of sedoheptulosan in $n\text{-H}_2\text{SO}_4$ for 3 hr. and removing the acid by passage through an anion-exchange resin (Amberlite IR 4-B).

Analytical methods

Oxygen consumption at 38° was measured by the conventional Warburg manometric technique. In all experiments methylene blue was added in order to link the dehydrogenase system to molecular oxygen.

Reduction and re-oxidation of DPN. Oxidation and reduction of the coenzyme were followed by measuring optical density at 340 m μ . in a Beckman DU spectrophotometer. The reaction mixtures were contained in quartz cuvettes having a 1 cm. light path.

Reducing sugar was determined according to Somogyi (1945a) after deproteinization with $\text{Ba}(\text{OH})_2$ and ZnSO_4 (Somogyi, 1945b), and removal of methylene blue. Much of the methylene blue was adsorbed on the protein precipitate and the residue was removed by treatment of the protein-free filtrate with the minimum amount of Merck's activated charcoal.

Detection of ketoses. After removal of protein and methylene blue (as above) ketoses were identified by the resorcinol test using the reagents of Bacon & Bell (1948), the carbazole reaction (Dische & Berenfreund, 1951) and the isolation of phenylosazones. The ketoses were distinguished in the carbazole reaction by observation of the colour developed initially and by plotting absorption spectra in the wavelength range 440–630 m μ ., maximum absorption being at 560 m μ . for ketohexoses and at 538 m μ . for ketopentoses (see Dische & Berenfreund, 1951). In the resorcinol test the colours developed were green (ketopentose), cherry red (ketoheptose) and purplish red (ketoheptose), and the respective absorption curves in the visible region of the spectrum agreed with those which have been determined by Mr R. G. Kulka (unpublished observation) for pure sugars. The individual heptuloses were identified by paper chromatography with a solvent mixture composed of *n*-butanol, ethanol and water (Bevenue & Williams, 1951).

Optical rotations were measured in a standard Hilger polarimeter reading to $\pm 0.01^\circ$.

Preparation of phenylosazones

When the scale of an experiment was limited by the amount of polyol available, the osazones were prepared from the contents (deproteinized and cleared of methylene blue) of Warburg flasks. When there was no such restriction the enzyme solution (about 100 ml.) containing polyol (0.048M), DPN ($9.6 \times 10^{-5}\text{M}$), potassium phosphate buffer, pH 7.9 ($1 \times 10^{-3}\text{M}$) and methylene blue ($9.5 \times 10^{-4}\text{M}$) was divided into six equal parts and shaken in stoppered 50 ml. conical flasks for 12 hr. at 38°. The gaseous phase was air. Oxygen uptake was followed manometrically in a pilot Warburg flask containing a 3 ml. sample of the reaction mixture. The combined solutions were deproteinized either with HClO_4 according to Neuberg, Strauss & Lipkin (1944)

or with 0.1 vol. of 7% (w/v) metaphosphoric acid. Residual methylene blue was removed with Merck's activated charcoal, and after neutralization with K_2CO_3 the filtrate was concentrated at room temperature *in vacuo* over H_2SO_4 until its volume was reduced to 30–40 ml. The phenylosazones were prepared at pH 5 (Edson, 1940), recrystallized from 30% (v/v) aqueous ethanol and dried *in vacuo* at room temperature.

RESULTS

Substrate specificity and rates of polyol oxidation

The rate of oxidation of a substrate in the complete dehydrogenase system was followed manometrically by measurement of oxygen uptake under the conditions of Blakley (1951) with methylene blue as a hydrogen carrier. In these experiments (Table 1) the rate of oxygen uptake was almost linear in the first hour but declined slowly during the second hour. The enzyme preparation consumed a small amount of oxygen in the absence of substrate.

The substrate oxidized most rapidly is sorbitol (L-gulitol), but it is important to recall that L-iditol is oxidized at approximately the same rate (see Blakley, 1951). Allitol reacts at less than half this rate. The other hexitols (galactitol (dulcitol), D-gulitol, D-mannitol, D-talitol) and 6-deoxy-L-gulitol are not oxidized. Of the pentitols, xylitol is oxidized faster than ribitol; D- and L-arabitol are not attacked. In the heptitol series the compounds

Table 1. *Oxidation of polyols by rat-liver polyol dehydrogenase*

Each Warburg flask contained 1.5 ml. of enzyme solution, 214 μ moles of potassium phosphate buffer, pH 8.0, 2.72×10^{-1} μ mole of DPN, 51.2 μ moles of substrate and 3.2 μ moles of methylene blue (added from the side bulb after equilibration). Total volume, 3.2 ml. Gas, air. Temp., 38°. Time, 1 hr. The inseat contained 0.2 ml. of 2N-NaOH.

Substrate	O ₂ -uptake (μ moles)
Nil	0.60
Erythritol	0.60
D-Arabitol	0.55
L-Arabitol	0.50
Ribitol	6.83
Xylitol	11.79
Allitol	6.26
Galactitol (dulcitol)	0.50
D-Gulitol (L-glucitol)	0.60
L-Gulitol (D-glucitol, sorbitol)	13.84
6-Deoxy-L-gulitol (1-deoxysorbitol)	0.50
D-Mannitol	0.50
D-Talitol	0.50
D-manno-D-gala-Heptitol (perseitol)	0.50
D-manno-D-talo-Heptitol (volemitol)	0.45
L-gulo-D-gala-Heptitol	7.28
L-gulo-D-talo-Heptitol (β -sedoheptitol)	12.10
mesoInositol	0.50
d-Inositol	0.60
l-Inositol	0.60
Scyllitol	0.60
l-Viburnitol	0.55

containing the *L-gulo* configuration are oxidized but those which lack this configuration (perseitol and volemitol) are not attacked. The following are not oxidized: erythritol and the cyclitols (*meso*-inositol, *d*- and *l*-inositol, scyllitol and *l*-viburnitol).

The relative rates of oxidation of sorbitol, allitol, ribitol and xylitol found in the manometric experiments were confirmed by spectrophotometric measurement of reduced DPN (Fig. 1).

Coenzyme specificity

When TPN is substituted for DPN the enzyme system does not attack polyols.

Relationship between oxygen consumption and sugar formation

Table 2 shows that the dehydrogenation catalysed by the enzyme in the presence of methylene blue and oxygen corresponds almost quantitatively

Fig. 1. Reduction of DPN by polyols in the presence of rat-liver enzyme. The reaction mixture (3.0 ml.) contained initially 1.0 ml. of enzyme solution, 100 μ moles of potassium phosphate buffer, pH 7.9, 9×10^{-2} μ mole of DPN and 21.9 μ moles of substrate. Temp. 20°.

Table 2. Formation of reducing sugar during polyol oxidation

Warburg flasks contained the same quantities of enzyme and reagents as in Table 1. Gas, air. Temp. 38°. Time 3 hr.

Substrate	O ₂ -uptake* (μ moles)	Reducing sugar (μ moles)
Sorbitol	20.0	39.0
Allitol	6.8	12.6
Ribitol	8.5	15.5
Xylitol	15.7	29.7

* Oxygen uptake of the system without substrate has been subtracted.

to the equation: polyol + $\frac{1}{2}$ O₂ = reducing sugar (ketose) + H₂O. Reducing sugar was absent from controls in which substrates were omitted. Since accurate factors for the reducing powers of ketoses other than fructose were not available, the amount of each sugar formed was calculated on the assumption that its capacity for reducing the Somogyi reagent equals that of glucose.

Identification of the products of reaction

The isolation of sugar derivatives was restricted by the amount of substrate available and by the relatively poor yields of sugar due to progressive inactivation of the enzyme at 38°.

Allitol (60 mg.) formed 21 mg. of a reducing sugar which gave a red resorcinol reaction, a purple carbazole reaction and a yellow crystalline phenylosazone, m.p. (recryst.) 180–182.5° (decomp.). Levene & Jacobs (1910) reported the melting point of *D*-allosazone as 183–185° (decomp.) while Steiger & Reichstein (1935) gave 161–164° as the melting point of *L*-allosazone. The amount of osazone obtained was insufficient for measurement of optical rotation with the apparatus available, but the above evidence, considered in conjunction with the specificity of the enzyme, indicates that the product of allitol oxidation is *D*-allulose.

Ribitol (729 mg.) formed 165 mg. of reducing sugar giving a green resorcinol test and a purplish red carbazole reaction. The phenylosazone crystallized in yellow, flaky plates, m.p. (recryst.) 163–164°, $[\alpha]_D^{25} = -49.8^\circ$ in pyridine: absolute ethanol:2:3, v/v (c, 0.92). The osazone was identified as *D*-arabinosazone (cf. Reichstein, 1934) and the product of ribitol oxidation as *D*-ribulose.

Xylitol (729 mg.) formed 198 mg. of a reducing sugar which gave a green resorcinol reaction and a purplish red carbazole reaction. The phenylosazone crystallized as yellow rhombic needles, m.p. (recryst.) 158–160°, $[\alpha]_D^{25} = -38.7^\circ$ in absolute ethanol (c, 1.2). The osazone was identified as *D*-xylosazone and the product of xylitol oxidation as *D*-xylulose.

L-Gulo-D-gala-heptitol (18 mg.) formed a reducing sugar which gave a purplish red resorcinol test and a phenylosazone crystallizing in fine yellow needles, m.p. (recryst.) 197° (decomp.). The sugar migrated on a paper chromatogram at the same rate as perseulose.

β -*Sedoheptitol* (18 mg.) formed a reducing sugar which gave a purplish red resorcinol reaction. The phenylosazone crystallized in fine yellow needles, m.p. (recryst.) 194° (decomp.). The enzymically formed sedoheptulose gave one well-defined spot with the same R_F value as the chief spot in the reference mixture of sedoheptulose and sedoheptulosan (see also Noggle, 1953).

Reversibility of reactions

Blakley (1951) has shown that the oxidation of sorbitol and of *L*-iditol is reversible. The analogous oxidations of xylitol, *L-gulo-D-gala-heptitol* and β -sedoheptitol are also reversible (Fig. 2). *D*-Tagatose, *D*-mannoheptulose and *D*-glucoheptulose do not oxidize reduced DPN in the presence of the enzyme. There was no change in optical density at 340 m μ .

when these ketoses were added to an equilibrium mixture prepared from buffered enzyme, sorbitol and DPN.

The failure of the enzyme to reduce D-tagatose and D-mannoheptulose is consistent with its failure to oxidize the corresponding epimeric pairs of polyols, namely, dulcitol and D-talitol, volemitol and perseitol; and from the absence of reaction with D-glucoseheptulose it can be inferred that the enzyme will not oxidize *gluco-gulo-heptitol* or *D-gluco-D-ido-heptitol* (*D-ido-L-gulo-heptitol*).

Fig. 2. Reversibility of polyol oxidation. The reaction mixture (3.2 ml.) contained initially 1.0 ml. of enzyme solution, 100 μ moles of potassium phosphate buffer, pH 7.9, 9×10^{-2} μ mole of DPN, and 20 μ moles of polyol. Temp. 20°. Ketoses (20 μ moles) were added at points indicated by arrows. The experiment with xylitol was done with a dilute solution of very active sheep-liver enzyme (Todd, 1954); the other experiments with rat-liver enzyme.

DISCUSSION

Rat-liver polyol dehydrogenase catalyses a general reaction:

acyclic polyol + DPN⁺ $\rightleftharpoons$ ketose + DPNH + H⁺,
if the stereochemical pattern of the substrate conforms to the specific requirements of the apoenzyme. Comparison of the structures of the polyols listed in Table 3 will reveal that the vulnerable configurations are I and II:

* Site of oxidation.

The oxidation of fully hydroxylated acyclic polyols having five to seven carbon atoms will not occur unless the following conditions are satisfied.

(1) The secondary alcohol group involved in the oxidation must be adjacent to a primary alcohol group and its hydroxyl must possess an L configuration with respect to this primary alcohol group. The need for a primary alcohol group is indicated by the inability of the enzyme to oxidize glucose, gluconate (Blakley, 1951) and 6-deoxy-L-gulitol. (2) The hydroxyl of the secondary alcohol group next but one to the oxidizable group must also possess an L configuration. (3) The configuration of the intervening secondary hydroxyl may be either D or L. In this connexion it may be significant that most substrates possessing configuration I (sorbitol, L-iditol, xylitol, β -sedoheptitol) are oxidized more rapidly than those having configuration II (allitol, ribitol). The relative rates of oxidation, referred to sorbitol as 100, are: sorbitol, 100 > L-iditol, 96 (Blakley, 1951) > β -sedoheptitol, 87 > xylitol, 85 > L-gulo-D-gala heptitol, 52 > ribitol, 49 > allitol, 45. The specificity rules based on the evidence at hand do not exclude the possibility that certain ω -deoxy polyols (e.g. 6-deoxy-D-iditol, 6-deoxy-L-glucitol) will be attacked by the liver enzyme in a manner analogous to the L-fucitol oxidation of *Acetobacter suboxydans* (Richtmyer, Stewart & Hudson, 1950).

It is proposed to call the enzyme *L-iditol dehydrogenase* because L-iditol is an unambiguous substrate containing the specific configuration at both ends of the polyol chain. The enzyme occurs in all mammalian livers so far examined (Blakley, 1951; Todd, 1954), and may be widely distributed in micro-organisms and plants (McCorkindale, 1953; Edson, 1953).

The specificity can be explained in terms of the polyaffinity theory (Bergmann, 1935-6; Gottschalk, 1950) by assuming that oxidizable substrates are attached to the apoenzyme and definitely oriented on its surface by the formation of three or four hydrogen bonds. The stereochemical mechanism of the dehydrogenation has already been discussed in a preliminary way (Edson, 1953).

The specificity of L-iditol dehydrogenase is different from that exhibited by *Acetobacter* grown in cultures at pH 5-6.8. Bertrand (1904) discovered the selective oxidation of polyols by *A. xylinum* and described the phenomena in a well-known rule. Subsequent observations on *A. suboxydans*, too numerous and well-known to be cited in detail here, led to the confirmation of Bertrand's rule by Kluyver, Underkoffler, Reichstein, Hudson, Richtmyer and others, and to its extension (Hann, Tilden & Hudson, 1938; Richtmyer *et al.* 1950; Stewart, Richtmyer & Hudson, 1952; Pratt, Richtmyer & Hudson, 1952).

Table 3. Comparison of polyol oxidation by *Acetobacter suboxydans* and liver polyol dehydrogenase

Substrate	Ketose formed	
	Cultures of <i>A. suboxydans</i>	Liver polyol dehydrogenase
Erythritol	L-Erythrulose	Not oxidized
D-Arabitol	D-Xylulose	Not oxidized
L-Arabitol	Not oxidized	Not oxidized
Ribitol	L-Ribulose	D-Ribulose
Xylitol	Not oxidized	D-Xylulose
Allitol	L-Allulose	D-Allulose
Galactitol (dulcitol)	Not oxidized	Not oxidized
L-Gulitol (D-glucitol, sorbitol)	L-Sorbose	D-Fructose
D-Gulitol (L-glucitol)	Not oxidized	Not oxidized
6-Deoxy-L-gulitol (1-deoxy-D-glucitol)	6-Deoxy-L-sorbose	Not oxidized
D-Iditol	Not oxidized	Not oxidized
L-Iditol	Not oxidized	L-Sorbose
D-Mannitol	D-Fructose	Not oxidized
D-Talitol (D-altritol)	D-Tagatose	Not oxidized
L-gulo-D-gala-Heptitol (L-gala-D-gluc-heptitol)	Not oxidized	L-galaHeptulose (perseulose)
L-gulo-D-talo-Heptitol (D-altro-D-gluc-heptitol, β -sedoheptitol)	L-guloHeptulose	D-altroHeptulose (sedoheptulose)
D-manno-D-gala-Heptitol (L-gala-D-manno-heptitol, perseitol)	L-galaHeptulose	Not oxidized
D-manno-D-talo-Heptitol (D-altro-D-manno-heptitol, volemitol)	D-mannoHeptulose and sedoheptulose (approximately equal parts)	Not oxidized

The Bertrand-Hudson rule states that acyclic polyols possessing configuration III or IV are oxidized to ketoses by *A. suboxydans*.

* Site of oxidation.

The hydroxyl of the secondary alcohol group involved in the oxidation and the *cis*-vicinal secondary hydroxyl must have a D configuration with respect to the primary alcohol group (or the equivalent suggested by Richtmyer *et al.* (1950) in certain ω -deoxy polyols) adjacent to the site of oxidation.

Table 3 shows a comparison of the sugars formed during the oxidation of polyols by *A. suboxydans* and the L-Iditol dehydrogenase of rat liver. The contrast in specificity is readily apparent. The behaviour of *A. suboxydans* suggests that it contains a polyol dehydrogenase which also requires multipoint, but spatially different, attachment of the substrate. Since D-mannitol is a typical and unambiguous substrate for *A. suboxydans*, it is proposed to call the enzyme responsible for reactions described in the Bertrand-Hudson rule, D-mannitol dehydrogenase. It is a DPN-linked soluble dehydrogenase which can be extracted from *A. suboxydans* and other sources (Edson, 1953; Arcus &

Edson, 1954). Its properties will be described in a later communication. Dulcitol, which is not attacked by either D-mannitol or L-Iditol dehydrogenase, is well known to be metabolized by certain micro-organisms. This fact suggests the existence of a third type of DPN-linked enzyme, galactitol dehydrogenase, specific for the stereochemical pattern of dulcitol. A soluble enzyme of this kind oxidizing dulcitol to tagatose has been extracted from micro-organisms (Shaw, 1954).

Thus an examination of the specificity of rat-liver polyol dehydrogenase has led to the hypothesis that the liver enzyme is merely one member of a group of at least three DPN-linked polyol dehydrogenases, each of which is highly specific for a particular stereochemical pattern. The existence of these enzymes will account for many facts known about the metabolism of polyols in different organisms and a study of their distribution and properties may ultimately explain the peculiar occurrence of polyhydric alcohols in plants (Edson, 1953).

SUMMARY

1. The polyol dehydrogenase of rat liver catalyses the reaction:

acyclic polyol + DPN⁺ $\rightleftharpoons$ ketose + DPNH + H⁺, with a high degree of stereochemical specificity. It does not attack cyclitols.

2. The specificity of the enzyme is described by a rule which is contrasted with the Bertrand-Hudson rule for *Acetobacter suboxydans*. A suitable name for the liver enzyme is L-Iditol dehydrogenase.

3. Consideration of the metabolism of polyols in different organisms leads to the conclusion that there are at least three types of polyol dehydrogenases (L-iditol, D-mannitol and galactitol dehydrogenases) distinguishable by their stereochemical affinities.

We wish to thank the persons named above who have generously given us samples of rare materials, especially Dr N. K. Richtmyer whose courtesy made it possible for this study to be reasonably complete; and we are indebted to Prof. L. H. Briggs (Auckland University College) for use of high-pressure hydrogenation facilities in his laboratory.

REFERENCES

- Arcus, A. C. & Edson, N. L. (1954). *Proc. Univ. Otago med. Sch.* **32**, 6.
- Bacon, J. S. D. & Bell, D. J. (1948). *Biochem. J.* **42**, 398.
- Bergmann, M. (1935-6). *Harvey Lect.* **31**, 37.
- Bertrand, G. (1904). *Ann. Chim. (Phys.)* **3**, 181.
- Bevenue, A. & Williams, K. T. (1951). *Arch. Biochem. Biophys.* **34**, 225.
- Blakley, R. L. (1951). *Biochem. J.* **49**, 257.
- Carson, J. F., Waisbrot, S. W. & Jones, F. T. (1943). *J. Amer. chem. Soc.* **65**, 1777.
- Dische, Z. & Berenfreund, E. (1951). *J. biol. Chem.* **192**, 583.
- Edson, N. L. (1940). *N.Z. med. J.* **39**, 39.
- Edson, N. L. (1953). *Australian and New Zealand Association for the Advancement of Science. Report of 29th Meeting, Sydney, 1952*, **29**, 281.
- Gottschalk, A. (1950). *Advanc. Carbohydr. Chem.* **5**, 49.
- Hann, R. M., Tilden, E. B. & Hudson, C. S. (1938). *J. Amer. chem. Soc.* **60**, 1201.
- LePage, G. A. (1947). *J. biol. Chem.* **168**, 623.
- Lespieau, R. (1946). *Advanc. Carbohydr. Chem.* **2**, 107.
- Levene, P. A. & Jacobs, W. A. (1910). *Ber. dtsch. chem. Ges.* **43**, 3144.
- Levene, P. A. & Tipson, R. S. (1934). *J. biol. Chem.* **106**, 603.
- McCorkindale, J. (1953). *Proc. Univ. Otago med. Sch.* **31**, 7.
- Neuberg, C., Strauss, E. R. & Lipkin, L. E. (1944). *Arch. Biochem.* **4**, 101.
- Noggle, G. R. (1953). *Arch. Biochem. Biophys.* **43**, 238.
- Posternak, T. (1942). *Helv. chim. acta*, **25**, 746.
- Pratt, J. W., Richtmyer, N. K. & Hudson, C. S. (1952). *J. Amer. chem. Soc.* **74**, 2210.
- Reichstein, T. (1934). *Helv. chim. acta*, **17**, 996.
- Reichstein, T. & Bosshard, W. (1934). *Helv. chim. acta*, **17**, 753.
- Richtmyer, N. K., Stewart, L. C. & Hudson, C. S. (1950). *J. Amer. chem. Soc.* **72**, 4934.
- Shaw, D. R. D. (1954). *Proc. Univ. Otago med. Sch.* **32**, 5.
- Somogyi, M. (1945a). *J. biol. Chem.* **160**, 61.
- Somogyi, M. (1945b). *J. biol. Chem.* **160**, 69.
- Steiger, M. & Reichstein, T. (1935). *Helv. chim. acta*, **18**, 790.
- Stewart, L. C., Richtmyer, N. K. & Hudson, C. S. (1952). *J. Amer. chem. Soc.* **74**, 2206.
- Todd, C. M. (1954). *Proc. Univ. Otago med. Sch.* **32**, 9.

A Sodium-Yeast and some of its Properties

BY E. J. CONWAY AND P. T. MOORE

Department of Biochemistry, University College, Dublin

(Received 12 November 1953)

An observation made in this laboratory by Winder (1950) showed that if baker's yeast (*Saccharomyces cerevisiae*) be allowed to ferment in the presence of sodium citrate and glucose there is a large entrance of sodium ions into the cell. It appeared, therefore, that it would be possible to prepare a 'sodium-yeast', i.e. a yeast in which all or nearly all the potassium would be replaced by sodium, such a yeast being analogous to the 'ammonium-yeast' described by Conway, O'Brien & Boyle (1941) and Conway & Breen (1945). A comparison of the properties of such a yeast with those of a control 'potassium-yeast' and with those of the ammonium yeast might well throw light on the role of these ions in metabolic processes. It proved possible by the methods here described to prepare, as required, a yeast in which 98% of the cellular potassium was replaced by sodium and to measure its growth, fermentation and oxygen consumption rates.

METHODS

Preparation of a sodium-yeast. A fresh twice-washed sample of baker's yeast (usually 10 g. fresh wt.) was used; 1 vol. of the centrifuged material was suspended in 20 vol. of a medium containing 5% (w/v) glucose and 0.2M sodium citrate and fermentation was allowed to proceed for 2 hr. The yeast was then centrifuged, washed with 20 vol. of distilled water and resuspended in fresh fermentation medium. This procedure was repeated until the K content of the yeast was reduced as far as possible. As a control, a sample of the same fresh yeast was treated similarly, using a medium containing potassium citrate instead of the sodium salt. The yeast which has been treated in this way is referred to as 'K-yeast'. Whenever an analysis was to be carried out the yeast was twice washed with 20 vol. of distilled water to remove Na or K from the interspaces.

The fermentations were carried out under three different sets of conditions: (a) at room temperature with occasional stirring, (b) at 37° with occasional stirring, (c) at room temperature with continuous shaking. In the final stages of