- Lynen, F. & Koenigsberger, R. (1951). *Leibigs Ann.* **573**, 60.
- Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
- Passonneau, J. V. & Lowry, O. H. (1962). *Biochem. biophys. Res. Commun.* **7**, 10.
- Peters, R. A., Sinclair, H. M. & Thompson, R. H. S. (1946). *Biochem. J.* **40**, 516.
- Petruschka, E. & Giuditta, A. (1959). *J. biophys. biochem. Cytol.* **6**, 129.
- Potter, V. R. & Reif, A. E. (1952). *J. biol. Chem.* **194**, 287.
- Price, V. E. & Levintow, L. (1952). *Biochemical Preparations*, vol. 2, p. 22. Ed. by Ball, E. G. London: Chapman and Hall.
- Racker, E. (1956). *Ann. N.Y. Acad. Sci.* **63**, 1017.
- Rendina, G. & Hellerman, L. (1961). *Fed. Proc.* **20**, 83.
- Schwartz, A. & Lee, K. S. (1960). *Biochim. biophys. Acta*, **44**, 590.
- Segal, S., Blair, A. E. & Wyngaarden, J. B. (1956). *J. Lab. clin. Med.* **48**, 137.
- Siekevitz, P., Löw, H., Ernster, L. & Lindberg, O. (1958). *Biochim. biophys. Acta*, **29**, 378.
- Sisakian, N. M. & Pinus, E. A. (1958). *Biokhimiya*, **23**, 904.
- Somogyi, M. (1945). *J. biol. Chem.* **160**, 69.
- Somogyi, M. (1952). *J. biol. Chem.* **195**, 19.
- Terner, C. (1954). *Biochem. J.* **56**, 471.
- Tiedemann, H. & Born, J. (1958). *Biochim. biophys. Acta*, **27**, 656.
- Tiedemann, H. & Born, J. (1960). *Hoppe-Seyl. Z.* **321**, 205.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1945). (editors). *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*, 3rd ed. Minneapolis: Burgess Publishing Co.
- Von Korff, R. W. (1959). *Biochim. biophys. Acta*, **31**, 467.
- Von Korff, R. W. & Twedt, R. M. (1957). *Biochim. biophys. Acta*, **23**, 143.
- Webb, J. L. & Elliott, K. A. C. (1951). *J. Pharmacol.* **103**, 24.
- Weil-Malherbe (1937). *Biochem. Z.* **31**, 2202.
- Whittaker, V. P. (1959). *Biochem. J.* **72**, 694.

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The Intracellular α -Galactosidase of a Rumen Strain of *Streptococcus bovis*

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The α -galactosidases have not been studied as extensively as β -galactosidases. Thus a review of these enzymes by Wallenfels & Malhotra (1961) devotes 50 pages to an account of β -galactosidases but only 8 pages to the corresponding α -enzymes. In addition almost all of the α -galactosidases that have been examined in any detail have come from yeasts, moulds or higher plants. Bacterial enzymes of this class appear to be rare. Carrere, Lambin & Courtois (1960), for example, investigated the intestinal flora of many animal species, including ruminants, and obtained only one bacterial strain, from guinea-pig intestine, which produced an α -galactosidase.

Many pasture plants contain α -linked galactose present in oligosaccharides such as raffinose and the galactosylgalactosyl glycerides of the lipid fraction (Weenink, 1961). Cattle and sheep feeding on pasture metabolize these compounds in their rumens, the microflora of which should, therefore, contain organisms producing α -galactosidases. A preliminary study (Bailey, 1962) suggested that this was the case and cell extracts from mixed rumen bacteria were found to exhibit α -galactosidase activity. The enzyme was also found in the cells of one rumen bacterial species, *Streptococcus*

bovis. Extracts prepared from the cells of this organism grown in a glucose medium contained scarcely any other simple oligosaccharidases. A detailed study of the *S. bovis* α -galactosidase has now been made and the results obtained form the subject of the present paper.

EXPERIMENTAL

Buffers. The following buffers were used: sodium citrate (0.1M, pH 6.0), sodium acetate (0.1M, pH 6.0), sodium phosphate (0.1M, pH 6.0) and citric acid (0.1M)-disodium hydrogen phosphate (0.02M) (McIlvaine, 1921; pH 6.0 except in studies on the effect of pH on enzyme activity).

Organism. *S. bovis*, strain I (Bailey & Oxford, 1958), was revived from a freeze-dried culture prepared from cells that had been grown in the glucose medium described below. The organism was grown in a medium containing glucose (1%, w/v), sodium acetate (anhydrous) (1.2%, w/v), Bacto-Yeast Extract (0.5%) and Bacto-Tryptose (1.4%, w/v). Cultures were incubated at 37° under aerobic conditions with the atmosphere in the flasks enriched with CO₂ gas.

Cell extracts. Medium was inoculated and incubated for 18–20 hr., by which time good growth had occurred and the cultures contained 2–3 g. wet wt. of cells/l. Cells were harvested by centrifuging at 25 000g for 30 min. and washed by suspending in either acetate or citrate buffer and

re-centrifuging. The washed cells from 1 l. of culture were suspended in the same buffer (10 ml.), disintegrated at 2° in a Nossal (1953) shaker, diluted with additional buffer to 25 ml., centrifuged twice at 25 000g for 30 min. at 2° and stored at 2° under toluene. The extracts were used without further purification.

Carbohydrates. Isomaltose, laminaribiose, manninotriose, and verbascotetraose were isolated from partial acid hydrolysates of dextran, laminarin, stachyose and verbascose respectively. Melibiitol and manninotriitol were prepared by borohydride (tetrahydroborate) reduction of the corresponding sugars. Galactosylglycerol and galactosylgalactosylglycerol were isolated, on a charcoal column, from an alkaline-methanol hydrolysate of red-clover lipids (Wintermans, 1960). Sophorose was isolated from *Sophora japonica* pods. Dextran and leucrose were prepared from an *S. bovis*-sucrose culture (Bailey & Oxford, 1958). 4-O- α -D-Galactopyranosyl-D-galactose was supplied by Professor R. L. Whistler, Purdue University, U.S.A. All other carbohydrates were from commercial sources (L. Light and Co. Ltd., Colnbrook, Bucks.; Mann Research Laboratories Inc., New York, N.Y., U.S.A.; or Bios Laboratories, New York, N.Y., U.S.A.). All compounds were shown, by paper-chromatographic analysis, to contain no contaminating sugars, or were freed from such contaminants by preparative paper chromatography.

Analysis of digests. Digests were analysed qualitatively for sugars by paper partition chromatography, with the solvents and sprays listed by Bailey & Robertson (1962). In addition ketose-containing sugars were located with a spray reagent consisting of naphtharesorcinol (0.2%, w/v) in ethanol containing 10% (v/v) of syrupy orthophosphoric acid.

Reducing sugars in digests were measured by the micro-cuprimetric method of Nelson (1944), except for those containing melibiose as substrate when glucose oxidase (Huggett & Nixon, 1957) was used. With most of the substrates studied the α -galactosidase liberated either galactose from a non-reducing oligosaccharide or glucose from a compound that did not react with glucose oxidase. In these cases a direct measure of the hydrolysis rates could be obtained by using the results from the cuprimetric or glucose-oxidase procedures. With two sugars, manninotriose and verbascotetraose, the cuprimetric reducing value of the enzymic hydrolysate at any particular stage was due to liberated galactose and lower homologue, and residual substrate. The rates of hydrolysis of these substrates were calculated on the assumption that the contribution of the original reducing glucose unit to the reducing value of the solution did not change and that the measured increase in reducing value was due, therefore, to the liberated galactose. The cuprimetric reduction values of melibiose, manninotriose and verbascotetraose were measured by the Nelson (1944) method and, on a molar basis, found to be 97, 94 and 90% of that of glucose, indicating that the assumption made above was substantially correct.

RESULTS

Carbohydrase activity of Streptococcus bovis cell extracts

Extracts (25 ml.) from 2–3 g. wet wt. of cells were prepared from several cultures. All of the

extracts readily hydrolysed melibiose. Extract I, prepared in citrate buffer, was used in most of the quantitative studies. It contained, after dialysis, 0.64 mg. of N/ml. and hydrolysed 3.7 μ moles of melibiose/hr./mg. of N at pH 6.0 and 37°. A second extract (II) contained 0.22 mg. of N/ml. and hydrolysed 1.33 μ moles of melibiose/hr./mg. of N under the same conditions. Extract IIA was prepared from the same batch of cells as extract II except that acetate buffer was used.

Action on glucose and galactose. Solutions containing extract I (0.1 ml.), glucose or galactose (0.2 mg.) and citrate-phosphate buffer (0.4 ml.) were incubated for 6 hr. at 37°. Reducing sugars were measured in portions (0.1 ml.) of the digests at the beginning and end of the incubation period. The results showed that there was no loss of either sugar during incubation.

Hydrolysis of carbohydrates. Solutions containing extract I (0.05 ml.), citrate buffer (0.05 ml.) and carbohydrate (2 mg.) were incubated under toluene for 24 hr. at 37° and analysed for liberated sugars, both before and after incubation, by paper chromatography. Appropriate controls showed that the cell extract itself produced no reducing sugars and that none of the substrates, when incubated alone, was hydrolysed under the conditions used.

The following compounds were hydrolysed: melibiose, raffinose, stachyose, verbascose, manninotriose, verbascotetraose, methyl α -D-galactopyranoside, 6-O- α -D-galactopyranosyl- β -D-galactopyranosyl-1-glycerol, isomaltose and amylose. The intensity of the spots corresponding to liberated sugars indicated considerable variation in the rates of hydrolysis of the various sugars. The following compounds were not hydrolysed: 4-O- α -D-galactopyranosyl-D-galactose, methyl β -L-arabinopyranoside, methyl β -D-galactopyranoside, β -D-galactopyranosylglycerol, lactose, methyl α -D-glucopyranoside, α -trehalose, arbutin, salicin, sophorose, laminaribiose, turanose, cellobiose, maltose, leucrose, gentiobiose, sucrose and dextran. Similar results were obtained when extract II was used except that isomaltose was not hydrolysed. In the solutions containing raffinose, stachyose and verbascose, galactose was the only monosaccharide detected on the papers; glucose and fructose were clearly absent. Sucrose was not hydrolysed in solutions containing the citrate buffer but when this buffer was replaced with citrate-phosphate buffer definite hydrolysis occurred; fructose but not glucose was detected on the chromatograms.

Hydrolysis of verbascose and verbascotetraose. A solution containing extract I (0.3 ml.), citrate buffer (0.45 ml.) and verbascose (2 mg.) was incubated at 37° for 12 hr. At intervals portions (0.05 ml.) were removed, desalted with a little Bio-Deminrolit (The Permutit Co. Ltd., London,

Table 1. *Hydrolysis of verbascose by Streptococcus bovis α -galactosidase*

A solution containing extract I (0.3 ml.), citrate buffer (0.45 ml.) and verbascose (2 mg.) was incubated at 37° and spotted on papers at intervals. Papers were sprayed with naphtharesorcinol and acetone-silver nitrate spray reagents respectively. +, Compound present; -, compound absent.

Sugar	Incubation time (hr.)							
	0	1	2	4	6	8	10	12
Verbascode	+	+	+	+	+	+	+	+
Stachyose	-	+	+	+	+	+	+	+
Raffinose	-	-	-	-	+	+	+	+
Sucrose	-	-	-	-	-	-	+	+
Galactose	-	+	+	+	+	+	+	+

Fig. 1. Hydrolysis of verbascotetraose by *Streptococcus bovis* α -galactosidase. A solution containing extract I (0.4 ml.), citrate buffer (0.6 ml.) and verbascotetraose (2 mg.) was incubated at 37° and analysed at intervals for increases in total reducing sugars and free glucose. $\blacktriangle$, Total reducing sugars; $\bullet$, free glucose.

W. 4) and spotted on paper sheets. The sugars that were detected on the developed papers are listed in Table 1. The galactose spots showed a steady increase in intensity as the incubation proceeded. There was no sign at any stage of the incubation of any reducing di- or tri-saccharide spots.

A second solution containing extract I (0.4 ml.), citrate buffer (0.6 ml.) and verbascotetraose (2 mg.) was also incubated at 37° for 12 hr. At intervals duplicate portions (0.05 ml.) were removed, with a micropipette, for the measurement of total reducing sugars and free glucose by the cuprimetric and glucose-oxidase procedures respectively. The results

obtained are shown in Fig. 1 as a plot of increase in total reducing sugars and of free glucose with time. At the end of incubation the concentrations of sugars corresponded to 22% liberation of glucose and approximately 45% total hydrolysis.

Effect of various conditions on α -galactosidase activity

Preliminary experiments showed that optimum activity occurred at about pH 6.0 and 37°. In most of the quantitative studies melibiose was used as substrate; the rate of hydrolysis of this compound was linear provided that not more than 30–40% of the substrate had been hydrolysed. Solutions were standardized to contain extract (I, 0.1 ml.; II, 0.2 ml.), buffer (0.4 ml.) and melibiose (0.8 mg.). During incubation portions (0.05–0.1 ml.) were removed, with a micropipette, for the analysis of liberated sugars. With these standard solutions about one-third of the melibiose was hydrolysed under conditions of optimum activity in 3 and 4 hr. by cell extracts I and II respectively.

Effect of buffer ions on enzyme activity. Standard solutions were prepared containing extract I with added citrate, phosphate or acetate buffer respectively. A similar series was prepared with extract IIA (prepared in acetate buffer). Measurement of liberated glucose after incubation for 3 hr. showed the same amount of hydrolysis of the melibiose in the three members of each series.

Effect of pH on enzyme activity. Standard enzyme solutions in the pH range 3.0–7.5 were prepared containing extract I and McIlvaine's (1921) citrate-phosphate buffer. Liberated glucose was measured after incubation at 37° for 1.5 and 3 hr. and the pH values of duplicate solutions were also measured after incubation. The results obtained are given in Fig. 2 as a plot of μ moles of melibiose hydrolysed against pH.

Effect of temperature on enzyme activity. The degree of hydrolysis of melibiose by extract I was measured in standard solutions containing citrate-phosphate buffer, pH 6.0, and incubated for 3 hr.

at various temperatures. The results showed maximum hydrolysis over the range 37–42°. Above 42° enzyme activity declined rapidly and was almost nil at 46°.

Rate of hydrolysis of various carbohydrates

Standard solutions containing cell extract (I or II), various added sugars (equivalent to 0.8 mg. of melibiose) and citrate buffer were incubated for

Fig. 2. Effect of pH on *Streptococcus bovis* α -galactosidase activity. Solutions containing cell extract I (0.1 ml.), citrate-phosphate buffer (0.4 ml.) and melibiose (0.8 mg.) were incubated at 37° and the liberated glucose was measured after incubation for 1.5 and 3 hr. The hydrolysis of (anhydrous) melibiose after 1.5 hr. (Δ) and 3 hr. ($\bullet$) was then calculated.

from 3 to 12 hr. depending on the rate of hydrolysis of the particular compound. Liberated reducing sugars were analysed at intervals. With compounds containing one potentially hydrolysable glycosidic link the rates of hydrolysis were calculated from results obtained during the incubation period when not more than 30 % of the link was hydrolysed. With higher saccharides the rates were calculated from results obtained during the incubation period when, on the basis of endwise multi-chain hydrolysis, not more than 30 % of the link at the non-reducing end of the sugar had been hydrolysed. The rates of hydrolysis by extract I of a number of sugars containing α -linked galactose are given in Table 2, expressed as μ moles of substrate hydrolysed/hr./ml. of extract during the hydrolysis period defined above.

The rates of hydrolysis of sucrose and of isomaltose by cell extracts I and II were also measured in standard solutions and compared with the rates of hydrolysis of melibiose (Table 3). The results are expressed as μ moles of disaccharide hydrolysed/hr./ml. of extract. Where no hydrolysis was detected chromatograms sprayed with the

Table 2. Hydrolysis of sugars containing α -linked galactose by *Streptococcus bovis* α -galactosidase

Solutions containing extract I (0.1 ml.), sugar (0.8 mg. of disaccharide or equivalent) and citrate buffer (0.4 ml.) were incubated at 37° and analysed for liberated galactose at intervals. Hydrolysis rates were calculated under the conditions detailed in the text.

Sugar	Substrate hydrolysed (μ moles/hr./ml. of cell extract)
Melibiose	2.4
Manninotriose	0.9
Verbascotetraose	0.9
Melibi-itol	0.1
Manninotri-itol	0.3
Raffinose	1.0
Stachyose	1.4
Verbascose	1.0
6-O- α -D-Galactopyranosyl- β -D-galactopyranosyl-1-glycerol	1.0
Methyl α -D-galactopyranoside	0.1

Table 3. Hydrolysis of disaccharides by *Streptococcus bovis* cell extract

Solutions containing cell extract (0.1 ml.), sugar (0.8 mg.) and buffer (0.4 ml.) were incubated for 3 hr. at 37° and the liberated sugars measured.

Cell extract	Disaccharide	Buffer	Disaccharide hydrolysed (μ moles/hr./ml. of cell extract)
I	Melibiose	Citrate	2.38
I	Isomaltose	Citrate	0.55
I	Sucrose	Citrate	0
I	Sucrose	Citrate-phosphate	1.69
II	Melibiose	Citrate	0.29
II	Isomaltose	Citrate	0
II	Sucrose	Citrate	0

sensitive acetone-silver nitrate spray reagent clearly confirmed that no hydrolysis had occurred. Additional quantitative studies also confirmed that β -D-galactopyranosylglycerol and methyl β -L-arabinopyranoside were not hydrolysed by extract I.

Transferase action of the α -galactosidase

Solutions containing extract I (0.1 ml.), citrate buffer (0.4 ml.) and melibiose (0.8–20 mg.; 0.16–4.0 %, w/v) were incubated for 24 hr. at 37°. Paper chromatograms of the incubated solutions showed a definite trisaccharide spot when the solutions contained 1 % or more of melibiose and no trisaccharide spot when the melibiose concentration was 0.5 % or less. In all of the solvents used the trisaccharide moved as a single spot at the same rate as authentic manninotriose. When a similar solution was prepared containing 15 % (w/v) of melibiose, chromatograms of the incubated solution showed a similar single trisaccharide spot which was chromatographically identical with that of manninotriose. Intense glucose and galactose spots were present on chromatograms of all of the solutions. When similar solutions containing melibiose (5 mg.) and sucrose or raffinose (10 mg.) were incubated the chromatograms showed the presence of a single higher saccharide spot which was chromatographically identical with that of raffinose or stachyose. Transferase activity towards glucose and galactose was investigated in solutions containing raffinose (5 mg.) as substrate plus glucose or galactose (20 mg.). No sign of any reducing disaccharides could be detected on chromatograms of the incubated solutions. In the solution containing glucose the much weaker intensity of the spot indicating liberated galactose suggested that enzyme activity had been suppressed. All of the solvents used to develop the papers in this work normally give good resolution of di- and tri-saccharide mixtures. Whenever transferase action was demonstrated only a single spot corresponding to synthesized saccharide was detected.

DISCUSSION

The strain of *S. bovis* used in the present study had been isolated from the rumen contents of a cow, with a sucrose medium (Bailey & Oxford, 1958). Before freeze-drying, from glucose medium, it had passed through several sucrose cultures but had never been grown in media containing added raffinose or melibiose. It had possibly, at one stage, been cultured in a Difco-tomato juice-agar medium which may have contained traces of these sugars. Apart from this latter possibility the culture conditions under which the α -galactosidase occurs suggest that it is a constitutive enzyme.

Bailey & Bourne (1961) examined the carbohydrazidase activities of extracts prepared from cells of the same strain of *S. bovis* after growth in a sucrose medium. In this case the extracts contained strong α -galactosidase, isomaltase and invertase activities; action on amylose was not tested. A re-examination of the chromatograms suggests that the extracts contained true invertase in addition to any sucrose phosphorylase. In contrast the present extracts, from cells grown on glucose, contain little or no isomaltase and are entirely without action on sucrose if phosphate is absent. The conditions of hydrolysis of sucrose by the extracts suggest the presence of a sucrose phosphorylase. The presence of an amylase is to be expected as *S. bovis* is a well-known rumen starch fermenter. The cell extracts were tested against a wide range of sugars, and, in the absence of phosphate, apart from amylose, only compounds containing α -linked galactose were consistently hydrolysed.

α -Galactosidases of general specificity should hydrolyse a wide range of α -D-galactosides and also β -L-arabinopyranosides and α -D-fucopyranosides. Enzymes of this type from higher plants, yeasts and moulds do hydrolyse many aryl and alkyl α -galactosides as well as oligosaccharides and occasionally β -L-arabinopyranosides; for an account of α -galactosidases from these sources see Wallenfels & Malhotra (1961). In contrast the *S. bovis* enzyme only hydrolyses oligosaccharides containing α -(1 $\rightarrow$ 6)-linked galactose. It does not attack methyl β -L-arabinopyranoside or 4-O- α -D-galactopyranosyl-D-galactose, and only slowly hydrolyses methyl α -D-galactopyranoside; α -(1 $\rightarrow$ 1)-, α -(1 $\rightarrow$ 2)- and α -(1 $\rightarrow$ 3)-linked digalactosides are rare and were not available for testing. A different enzyme is probably responsible for the hydrolysis of isomaltose as not all cell extracts hydrolyse this sugar. On the evidence available it seems that the *S. bovis* α -galactosidase is an oligosaccharidase and possibly an α -(1 $\rightarrow$ 6)-galactosidase.

Of the substrates studied melibiose is the most rapidly hydrolysed and all of the tri- and higher saccharides are attacked at a slower rate. The nature of the substituent on the reducing carbon of an oligosaccharide has a marked effect on hydrolysis. Thus the presence of a fructosyl unit (raffinose, stachyose, verbascose) or glycerol unit (galactosylgalactosylglycerol) halves the rate of hydrolysis, as compared with melibiose, whereas a glucitol unit (melibi-itol and mannintri-itol) almost completely suppresses hydrolysis. A study of the hydrolysis of verbascose and verbasco-tetraose suggests that, under the experimental conditions used, hydrolysis is by a partly multi-chain process and involves the cleavage of successive galactosyl units from the non-reducing end of the

molecule. Random cleavage would have liberated reducing di- and tri-saccharides in the verbascone solution and glucose at a much earlier stage in the verbasconetetraose solution (see Fig. 1).

The *S. bovis* enzyme differs in one important aspect from all of the plant, yeast and mould α -galactosidases. All of these latter enzymes have broad pH optima over the range 3.5–5 or 6. *S. bovis* α -galactosidase, however, has optimum activity over a narrow pH range (5.6–6.3) and is inactive at pH 3.0–4.0. The enzyme possesses normal transferase activity when the concentration of acceptor is above 1%; melibiose, sucrose and raffinose act as acceptors. The compounds synthesized by transferase action were not isolated and examined chemically. The evidence from paper chromatograms, however, supports the conclusion that their formation involves galactosyl transfer to C-6 of the galactosyl unit of melibiose and raffinose or of the glucosyl unit of sucrose.

The properties of *S. bovis* α -galactosidase with regard to pH and temperature optima reflect the conditions of the organism's natural habitat in the rumen. Likewise substrate specificity is in agreement with the known occurrence of raffinose and galactosylgalactosylglycerol in many pasture plants. Apart from any interest in the enzyme itself it should be of value in structural studies on some of the more complex naturally occurring galactosyl-sucroses, particularly as, provided that phosphate is absent, the extract does not hydrolyse sucrose.

SUMMARY

1. Cell extracts prepared from a rumen strain of *Streptococcus bovis* grown on glucose contain α -galactosidase, amylase, sucrose phosphorylase and

occasionally isomaltase, but no other carbohydrates.

2. In the absence of phosphate sucrose is not hydrolysed and sugars of the raffinose series are hydrolysed to galactose and sucrose.

3. The α -galactosidase readily hydrolyses melibiose and other oligosaccharides containing α -(1 $\rightarrow$ 6)-linked galactose, but only hydrolyses methyl α -D-galactoside, melibi-itol and mannantri-itol slowly; methyl β -L-arabinopyranoside and 4-O- α -D-galactosyl-D-galactose are not hydrolysed.

4. The enzyme has optimum activity in the pH range 5.6–6.3 and temperature range 37–42°.

5. It may act as a transferase giving, with melibiose, sucrose or raffinose as acceptors, a single tri- or tetra-saccharide.

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REFERENCES

- Bailey, R. W. (1962). *Nature, Lond.*, **195**, 79.
 Bailey, R. W. & Bourne, E. J. (1961). *Nature, Lond.*, **191**, 277.
 Bailey, R. W. & Oxford, A. E. (1958). *J. gen. Microbiol.* **19**, 130.
 Bailey, R. W. & Robertson, A. M. (1962). *Biochem. J.* **82**, 272.
 Carrere, C., Lambin, S. & Courtois, J. E. (1960). *C.R. Soc. biol., Paris*, **154**, 1747.
 Huggett, A. St G. & Nixon, D. A. (1957). *Lancet*, **273**, 368.
 McIlvaine, T. C. (1921). *J. biol. Chem.* **49**, 183.
 Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
 Nossal, P. M. (1953). *Aust. J. exp. Biol.* **31**, 583.
 Wallenfels, K. & Malhotra, O. P. (1961). *Advanc. Carbohydr. Chem.* **16**, 239.
 Weenink, R. O. (1961). *J. Sci. Fd Agric.* **12**, 34.
 Wintermans, J. G. F. M. (1960). *Biochim. biophys. Acta*, **44**, 49.

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The Crystal Structure of Phenoxymethylpenicillin

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Phenoxymethylpenicillin [penicillin V, (I)] was first prepared and its formula established by Behrens, Corse & Edwards (1948). Its potential use in the oral administration of penicillin was not

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realized until Brandl & Margreiter (1954) showed that it was much more stable to acids than other then known penicillins. The free acid itself is unusual among penicillins in being easily crystallizable in a solvent-free state.

Brandl & Margreiter (1954) suggested that these unexpected properties might be caused by some conformational change within the molecule which