methochloride. The Decalso is eluted with KCl solution, which is then made alkaline and extracted with *iso*butanol. The fluorescence produced is compared with that of standards similarly treated.

This investigation forms part of an investigation of nicotinamide deficiency carried out on behalf of the Air Ministry. We wish to thank Air Marshal Sir H. E. Whittingham, K.B.E., K.H.P., Director-General of the Medical Services of the Royal Air Force, for facilities provided. Our thanks are also due to L.A.C.W. A. E. Wrigglesworth for technical assistance. One of the authors (R. A. C.) is a member of the Civilian Technical Corps (Air Ministry), attached to the Lister Institute for an investigation of nicotinamide deficiency. The authors wish to thank Roche Products, Ltd., for a generous supply of nicotinamide.

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Enzyme Formation and Polysaccharide Synthesis by Bacteria

2. POLYSACCHARIDE FORMATION BY *RHIZOBIUM RADICICOLUM* STRAINS

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An investigation into polysaccharide production by various strains of *Rhizobium* growing on the basal culture medium of Cooper, Daker & Stacey (1938) is described. The strains used were provided by Dr Hugh Nicol, and it was found that his two clover strains termed 'Clover Bartel A' and 'Clover 2027' were remarkably prolific in polysaccharide production. In general the purified polysaccharides gave laevo-rotatory, viscous solutions in water, although some differences in properties and yields of the polysaccharide were apparent from strain to strain. About 200 g. of the polysaccharide were obtained from the Bartel A strain.

In order to prevent contamination of the polysaccharide with the agar medium some modifications to the normal cultural conditions were made. For example, the usual agar culture medium, distributed in large Petri dishes, was covered with a layer of sterile cellophan according to the method of Birch-Hirschfeld (1933–4) and the surface inoculated with the bacterial suspension. The polysaccharide, uncontaminated by agar, was produced on the cellophan surface as a white mucilaginous growth which could easily be washed off and purified. In another method, suggested by Prof. W. N. Haworth, F.R.S., thick porous plates were embedded in agar medium so that nutrient material could freely diffuse through and be utilized by the organisms inoculated on to the surface of the plates. Polysaccharide production was good. In a third method the agar in the basal medium was replaced by acid-free kieselguhr, to provide a pasty solid medium which was distributed in Roux bottles and conical flasks. The organism grew well on the medium, covering the surface with a white mucilaginous material from which the polysaccharide could be obtained by extraction with water. The product made in this way generally had a much higher ash content than that made by other methods.

By an experimental procedure involving an examination of the hydrolysis products of its methylated derivative, which will be described elsewhere, part of the structure of the Clover Bartel A polysaccharide has been determined. The hydrolysis products were 2:3:6-trimethylglucopyranose (1 part), 2:3-dimethylpyranose (1 part) and 2:3-dimethylglucuronic acid (1 part). The type of structure assigned to the repeating unit is that of a polymerized aldobionic acid in which the carboxyl groups of the glucuronic acid residues are free. It has been shown previously (Cooper *et al.* 1938) that *Rhizobia* polysaccharides belong to the same class of compound as the specific polysaccharides from types II and III *Pneumococcus* (see Heidelberger & Goebel, 1927; Hotchkiss & Goebel, 1937). The structure of the latter has been worked out in some

detail by Reeves & Goebel (1941), and it may be formulated as follows:

$$\underline{4}\,\text{glucose}\underline{1{:}3}\,\text{glucuronic acid}\underline{1{:}4}\,\text{glucose}\underline{1{:}3}\,\text{glucuronic acid}\underline{1},$$

in which the numerals indicate the points of attachment.

In view of the precipitin reaction given in high dilution by the *Rhizobium* polysaccharide with anti-*Pneumococcus* horse serum type III and of the known close relationship between structure and serological specificity (Heidelberger, Kabat & Shrivastava, 1937; Heidelberger & Hobby, 1942), it may well be that the aldobionic acid units in the *Rhizobium* polysaccharide are of the cellobiuronic acid type. The function of the polysaccharide may be that of a defence mechanism against soil Protozoa and fungi.

EXPERIMENTAL

Large-scale production

For large-scale production the following medium described by Cooper *et al.* (1938) was used: K_2HPO_4 1·0 g., asparagine 1·0 g., caffeine 0·2 g., NaCl 0·2 g., $MgSO_4.7H_2O$ 0·2 g., $FeCl_3$ 0·01 g., $MnCl_2$ 0·01 g., $CaCO_3$ 6·0 g., agar 1·8 g., sucrose 80·0 g. These were dissolved in 1 l. of lucerne root extract (made by boiling 10 g. of powdered lucerne root in 1 l. of tap water for 1 hr.). After one steaming the hot medium was transferred in 200 and 100 ml. amounts into Roux bottles, which were then subjected to two further steamings on successive days. Test-tubes containing 10 ml. amounts were also prepared. These were inoculated with the organism and, after an incubation period of 3 days at room temperature, 15 ml. of sterile water were added. 2 ml. of this bacterial suspension were transferred under sterile conditions to the Roux bottles. The bottles were incubated at room temperature for 10 days and the heavy yield of the gum was removed by cautiously skimming it from the solid agar surface after addition of a few ml. of distilled water. The resulting viscous solution was filtered through cotton-wool and run into twice its volume of ethanol, which was stirred continuously. The polysaccharide collected round the rod as a fibrous mass. The solvent was pressed out and the product dried under absolute ethanol and ether and in a vacuum. Thus obtained the polysaccharide had the appearance of asbestos fibre. For purification purposes the 'kieselguhr filtration method' as described by Cooper *et al.* (1938) was applied. This process removed the bulk of the bacterial debris.

Investigation on various strains of Radicicola *species*

The organisms received from Dr H. Nicol were grown on the medium by the method of Cooper *et al.* (1938). Some of the strains—for instance those of the soya bean—failed completely to grow on this medium while other strains gave the yields shown in Table 1. Thus Clover Bartel A and Clover 2027 gave the optimum yields and of these Clover Bartel A was chosen for large-scale production since it had less ash than Clover 2027. Clover Bartel A gave a precipitin reaction in dilutions up to 1 in 100,000 with types III and VI anti-*Pneumococcus* horse sera (Dr M. Heidelberger, private communication).

Table 1. *Polysaccharide production by* Rhizobium

Strain of *Rhizobium radicicolum*	Average yield of crude polysaccharide (g./l. of medium)	Ash (%)	$[\alpha]_D$ in water (corrected for ash)
West Australian lupin	4·0	23·4	−22°
Lucerne A and H	2·5	57·2	−14°
Pea 313	5·6 (mainly protein)	—	—
Pea B33	6·2	73·0	−16°
Clover Bartel A	7·5	29·5	−14°
Clover Coryne	5·6 (mainly protein)	—	—
Clover 202	2·1	28·0	−17°
Clover 205	0·3 (mainly protein)	—	—
Clover 2027	7·5	38·9	−14°

Investigations on polysaccharides obtained from various Clover strains

Product from strain Clover Bartel A. The crude polysaccharide (68·7% ash) gave very opalescent solutions in water, and polarimetric readings could not be taken. The solution was therefore acidified with a few drops of conc. HCl, and the polysaccharide reprecipitated by running the solution with continuous stirring into ethanol (2 vol.). This process was repeated, and finally the polysaccharide was precipitated from a neutral aqueous solution. This partially purified material contained 29·5% ash.

On hydrolysis with 0·5 N-H_2SO_4 the crude material gave the following readings: $[\alpha]_D^{20°}$, −10·4° (initial); −9·5° (15 min.); −6·6° (45 min.); −2·8° (75 min.); +1·9° (135 min.); +5·6° (240 min.); +9·5° (360 min.); +10·4° (420 min.); +12·3° (540 min.); +12·3° (600 min.). The hydrolysate gave, with naphthoresorcinol, a positive test for uronic acid residues.

Product from strain Clover 202. The polysaccharide, after reprecipitation twice from dilute HCl and once from a neutral solution by addition of ethanol, contained 28% ash. On hydrolysis with 0·5 N-H_2SO_4 the following readings were obtained: $[\alpha]_D^{20°}$, −11·1° (30 min.); −3·1° (60 min.); +1·0° (90 min.); +13·1° (180 min.); +17·2° (240 min.); +18·2° (300 min.); +21·2° (540 min.). The hydrolysate gave a positive naphthoresorcinol test for uronic acid residues.

Product from strain West Australian Lupin. This polysaccharide was hydrolyzed by 0·5 N-H_2SO_4, during which the rotation changed from $[\alpha]_D^{20°}$, +21·9° to $[\alpha]_D^{20°}$, +26·3° (equilibrium value after 450 min.).

Product from strain Clover Coryne. The initial precipitate disappeared completely when reprecipitation from an acid solution was attempted, showing that the material obtained consisted mainly of bacterial protein.

Product from strain Clover 2027. After repeated purification as described above, the polysaccharide contained 38·9% ash. On hydrolysis with 0·5 N-H_2SO_4 the following readings were obtained: $[\alpha]_D^{20°}$, +19·8° (3 hr.); +24·8° (4½ hr.). The naphthoresorcinol test for uronic acid residues was positive.

Experiments on the prevention of agar contamination of the polysaccharides

Cellophan paper method. Agar medium (75 ml.) was poured into each of six large Petri dishes and, after they had cooled, sterile cellophan paper was transferred on to the agar surface by means of sterile forceps, so that the whole surface was covered with cellophan. The dishes were then inoculated with a heavy dose of the usual bacterial suspension of *Rhizobium radicicolum* Bartel A and kept at room temperature for 8 days. The growth which had formed on the cellophan was then scraped off with a glass rod, the cellophan rinsed with distilled water and, after filtration through cotton-wool, the polysaccharide was precipitated in the usual way. Yield, 3·6 g./l. of medium; ash, 15·1%; N, 3·7%.

Porous plate method. A large crystallization dish was filled with 75 ml. of agar medium and a sterile porous plate was embedded in the semi-solid agar by means of sterile forceps. After inoculation with a bacterial suspension, it was kept for 8 days at room temperature. The gummy growth on the porous plate was scraped off, the plate rinsed with distilled water and the polysaccharide obtained as in the usual way. Yield, 0·93 g./l. of medium; ash, 16·8%; N, 5·75%.

Kieselguhr method. Nutrient medium (25 ml.), containing the same contents as the usual lucerne root-agar medium with the exception of agar, was poured into each of eight conical flasks. Acid-free kieselguhr (25 g.) was stirred in until a thick paste was obtained. The flasks were sterilized on 3 successive days and then inoculated as above and kept at room temperature for 10 days. Distilled water (40 ml.) was added, the flasks warmed for 3 hr. at 40° and the mixture filtered and carefully washed out with warm distilled water. The combined filtrates were concentrated *in vacuo* at 40–50° and the resultant syrup run into ethanol. Yield, 1·9 g./l. of medium; ash, 34·5%; N, 3·8%.

Large-scale production of polysaccharide by the Clover Bartel A strain

As had been mentioned above, strain Clover Bartel A was chosen for large-scale production. It remained active as gum-producer over a very long period and was subcultured every fortnight on slopes of the lucerne-agar metabolism medium. The gum which was obtained on precipitation from ethanol was purified by redissolving it in water and filtering the very dilute solution through kieselguhr. Final ash content, 38·4%.

Purified polysaccharide (60 g.) was obtained, and 200 g. of unpurified material with ash, 68·7%. It was decided not to purify the latter, since it was found that the impurities were separated during a process of methylation and dialysis, which will be described elsewhere.

SUMMARY

1. Various strains of *Rhizobium radicicolum* species were examined with regard to their ability to produce polysaccharides. Strain Clover Bartel A was found to be the most efficient for large-scale production.

2. Experiments on methods for elimination of agar impurities showed that the use of cellophan was the most satisfactory with regard to yield and purity of the polysaccharide formed.

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The '*Q*' Notation for Expressing Metabolic Activities in Tissue Slices. A Critical Study

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Ever since Warburg introduced the Q_{O_2} in experiments with tissue slices it has been widely used as a convenient term for comparing experiments performed under different conditions. By expressing the quantity of any substance formed or consumed during a reaction in terms of

$$Q = \frac{\mu\text{l. substance (formed or consumed)}}{\text{mg. tissue (dry wt.)} \times \text{hours of incubation}},$$

it was thought possible to obtain a comparable index of the activity of the tissue even when the conditions of the experiments differed with respect to the amount of tissue used and the period of incubation. While '*Q*' was originally designed to express gaseous changes it has become a general habit of workers to use the term also for changes of non-gaseous substances by assuming the compound formed or consumed to be a gas, of which