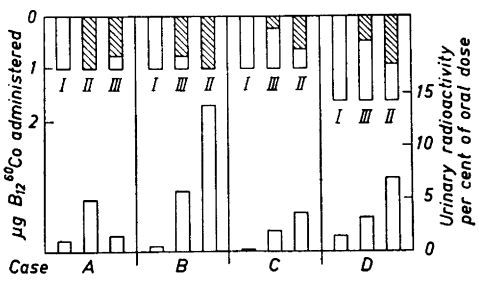

Fig. 1. Intrinsic factor assay of chymotrypsin digested gastric juice. Upper columns: Material administered. White columns: Free B_{12}. Shaded columns: Bound B_{12}. I = Basal test without intrinsic factor, II = Sample digested in presence of B_{12}, III = Sample digested in absence of B_{12}. Lower columns: Patient's response in the Schilling test [6]. *N.B.* In cases A and B sample II was not saturated with B_{12}.

some B_{12}-binding and intrinsic factor activity is retained after 20 h of digestion with high concentrations of chymotrypsin (Fig. 1).

As regards the action of chymotrypsin the present study confirms similar observations made during the preparation of commercial intrinsic factor preparations [8].

Preliminary ultracentrifugal studies [9] indicate that the B_{12}-binder — vitamin B_{12} complex remains a relatively large molecule after trypsin-chymotrypsin digestion (mol.wt. 52 000 both before and after digestion for cobalamin complex from pepsin-inactivated gastric juice). It therefore appears that the B_{12}-binder (and apparently also intrinsic factor) contains only a few bonds sensitive to the action of these enzymes.

The author is greatly indebted to Professor H. Theorell for valuable advice, to Dr. K. Yamashina for the chymotrypsin, to Dr. J. Gad Andresen for the mucinase, to Dr. H. Wijmenga for the intrinsic factor preparations, and to Dr. B. Josephson for arrangements in connection with the collection of gastric juice. Dr. J. Schuberth was kind to assist in the UV studies, and the Schilling-tests were carried out by Dr. G. Amnell. The investigation was supported by generous grants from *N. V. Organon* and *Sigrid Jusélius Stiftelse.*

1. Gräsbeck, R. *Acta Med. Scand.* Suppl. **314** (1956).
2. Schilling, R. F. and Schloesser, L. L. in *Vitamin B_{12} und Intrinsic Factor*, Enke, Stuttgart 1957, p. 194.
3. Gregory, M. E. and Holdsworth, E. S. *Biochem. J.* **59** (1955) 335.
4. Haas, W. J., Sizer, I. W. and Loofbourow, J. R. *Biochim. et Biophys. Acta* **6** (1951) 589.
5. Ada, G. L. and French, E. L. *Australian J. Sci.* **13** (1950) 82.
6. Schilling, R. F. *J. Lab. Clin. Med.* **42** (1953) 860.
7. Neurath, H. and Schwert, G. W. *Chem. Revs.* **46** (1950) 69.
8. Wijmenga, H. G. in *Vitamin B_{12} und Intrinsic Factor*, Enke, Stuttgart 1957, p. 156.
9. Gräsbeck, R. and Ehrenberg, A. *Unpublished data.*

Received December 22, 1957.

Barium Hydroxide as a Selective Precipitating Agent for Hemicelluloses

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A widely applied method of fractionating and purifying hemicelluloses is by precipitating them as their insoluble copper complexes. The separation of acidic hemicelluloses from neutral ones has been achieved by fractional precipitation using long-chain quaternary ammonium salts (*e. g.* "cetavlon") [1].

Another method has now been found which on its own, or in conjunction with known methods, may enable a more efficient fractionation to be achieved. We have observed that barium ions readily form insoluble complexes with mannans and glucomannans, probably by reaction with the vicinal *cis*-hydroxyl groups on carbon atoms 2 and 3 of the mannose units. Those complexes are precipitated from aqueous solutions on the addition of small quantities of barium hydroxide.

Ivory nut mannan A and spruce glucomannans were both precipitated comple-

Table 1.

Sample	Galactose %	Glucose %	Mannose %	Arabinose %	Xylose %
Hemicellulose mixture from spruce	7.1	10,4	19.8	6.6	56.1
Fraction I: $Ba(OH)_2$-precipitate	12.9	17.1	51.8	3.8	14.4
Fraction II: material left in solution	1.8	3.6	1.4	9.8	83.4

tely from aqueous solutions at barium hydroxide concentrations below 0.03 M. The barium complexes of the mannans and glucomannans are easily soluble in dilute acids and even in water, but are insoluble in organic solvents and in copper ethylene diamine.

Although the use of barium hydroxide did not lead to the precipitation of a larch arabo-galactan it caused a spruce galactan to be precipitated. This may be attributed to the fact that galactose, when present either as an end-group, or as a 1,6-linked residue in a polysaccharide, contains the requisite vicinal *cis*-hydroxyl groups on carbon atoms 3 and 4. As the hemicellulose galactans are highly branched molecules they will possess a high percentage of end-groups.

A birch xylan (2.5 % glucose + galactose, 85.6 % xylose, 11.9 % uronic acids) was also precipitated from aqueous solution when the barium hydroxide concentration was made up to 0.15 M. This may be due to the formation of an insoluble barium salt by the acidic xylan.

When barium hydroxide was added to a hemicellulose mixture (extracted from spruce holocellulose with 14 % potassium hydroxide) the glucomannans were precipitated almost completely. They were, however, contaminated by other polysaccharides containing galactose, xylose and arabinose residues. The precipitation of a galactan can be attributed to the presence of vicinal *cis*-hydroxyl groups as mentioned above. If precipitation is due only to them, then it would be difficult to explain why a xylan or an arabo-xylan should be precipitated by barium hydroxide as readily as were the glucomannans. When fraction I (*cf.* Table 1) was dissolved in water and "cetavlon" was added, xylose and arabinose together with galactose and minor amounts of glucose and mannose were detected in the hydrolysate of the precipitate whereas a pure glucomannan was left in solution. The presence of a galacto-arabo-xylan may therefore be suspected. However, the xylose residues in fraction I could also be parts of a very acidic xylan. The hemicellulose fraction II (*cf.* Table 1) which had not precipitated with barium hydroxide and which accounted for nearly two thirds of the recovered material was shown to be mainly an arabo-xylan with an arabinose : xylose ratio of 1:8.5 and an uronic acid content of 19 %. Even with a high excess of barium hydroxide (more than 0.2 M) no precipitation occurred. Two explanations might be possible. Either the barium salt of this acidic arabo-xylan is soluble in barium hydroxide concentrations up to 0.2 M or the branches of arabinose residues sterically hinder salt formation of neighbouring chains.

It is evident from these preliminary experiments that the use of barium hydroxide as a precipitant will provide a complement to previous methods of fractionating polysaccharides even if the scope and limitation of the method have not yet been fully explored.

Experimental. The hemicelluloses were dissolved in water or in dilute sodium hydroxide and then saturated barium hydroxide solution was added. If a precipitate formed it was centrifuged and was purified by dissolving it in water and reprecipitating it with barium hydroxide. After centrifugation the precipitate was dissolved in 2 N acetic acid and from this solution the hemicelluloses were precipita-

ted with ethanol and were washed successively with ethanol/water, with ethanol and with ether.

The quantitative paper chromatography of the samples was carried out according to Saeman *et al.*[2] using the solvent systems ethyl acetate-acetic acid-water (3:1:3) and, for the separation of glucose and galactose, ethyl acetate-pyridine-water (2:1:2).

1. Scott, J. E. *Chem & Ind. London* **1955** 168.
2. Saeman, J. F., Moore, W. E., Mitchell, R. L. and Millet, M. A. *Tappi* **38** (1954) 336.

Received January 4, 1958.

Preparation of Thiotaurine (Aminoethanethiosulfonic Acid)

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Recently [1] an enzymatic transsulfuration product from β-mercaptopyruvate and hypotaurine (2-aminoethanesulfinic acid) was identified as 2-aminoethanethiosulfonic acid, for which the shorter name thiotaurine was suggested. Thiotaurine has now been synthesized with taurine as starting material. Taurine was first transformed into phthalimidoethanesulfonyl chloride [2] and the latter then converted to hypotaurine by a novel method. The sulfonyl chloride was first reacted with phenylhydrazine to give phthalimidoethanesulfonyl phenylhydrazide which was not isolated but instead heated with an excess of phenylhydrazine and tri-*n*-butylamine in ethanol, in order to remove the phthaloyl group [3]. During this treatment it was found that the sulfonylphenylhydrazide decomposed to the corresponding sulfinic acid [4]. Hypotaurine was thus formed (yield 80 %) and precipitated with methyl ethyl ketone. Thiotaurine was then obtained from hypotaurine through treatment of the latter with ammonium polysulfide [5] and crystallized from ethanol-water (yield 40 %). Colourless crystals, m.p. about 213° (decomp.), were obtained and found to contain 22.5 % cyanide labile sulfur [6] (calc. for $NH_2C_2H_4S_2O_3H$: 22.7 %). Paper chromatography of the compound in 4 different solvent systems demonstrated only the presence of one single component. It was previously suggested that thiotaurine might be identical with an unknown sulfur containing compound from human blood, first detected by Smith and Tuller [7] by paper chromatography and reported to behave as taurine. This possibility could now be excluded, as the synthetic thiotaurine was found to be clearly resolved from taurine in the solvent system used by Smith and Tuller.

Details of this work will be published later.

1. Sörbo, B. *Biochim. et Biophys. Acta* **24** (1957) 324.
2. Winterbottom, R., Clapp, J. W., Miller, W. H., English, J. P. and Roblin, R. O. Jr. *J. Am. Chem. Soc.* **69** (1947) 1393.
3. Schumann, I. and Boissonas, R. A. *Helv. Chim. Acta* **35** (1952) 2235.
4. Escales, R. *Chem. Ber.* **18** (1885) 893.
5. Limpricht, H. *Ann.* **278** (1894) 239.
6. Sörbo, B. H. *Acta Chem. Scand.* **7** (1953) 1137.
7. Smith, E. L. and Tuller, E. F. *Arch. Biochem. Biophys.* **54** (1955) 114.

Received January 8, 1958.