single entity on paper and $CaCO_3$-column chromatography. After decarboxylation[13], only coproporphyrin III was found by the 2,6-lutidine chromatography[14]. Its methyl ester crystallized as coproporphyrin III methyl ester, melting at 138–141°.

Cu and Zn complexes of the methyl ester were prepared, as described previously[1]. Melting points and absorption maxima of the methyl ester and their Cu and Zn complexes are recorded in Table I. Melting-point determinations were carried out with a Koeffler micro melting-point apparatus. Spectrophotometric measurements were made with a DU. Beckman spectrophotometer.

These experimental results indicate strongly the identity of this biosynthetic porphyrin with "porphyrin 208".

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Enzymic oxidation of cerebrosides: studies on Gaucher's disease

An abnormal lipid which accumulates in the spleen of patients with Gaucher's disease has been isolated and identified as glucocerebroside[1,2]. There have been several reports describing in addition the presence in such spleens of varying amounts of galactocerebroside[3–5], a normal constituent of brain. While it is possible that there is more than one molecular form of the clinical disease, the question arises as to the possible loss of the galactose-containing component during purification or hydrolysis of the cerebroside. Previously available techniques have employed hydrolysis of the cerebroside, followed by chromatographic separation of sugars[2,5] or by enzymic

removal of glucose and chemical estimation of remaining sugar[1,4,5]. The present communication describes an enzymic oxidation of intact galactocerebroside, which forms the basis of a highly sensitive and specific method. These experiments were prompted by the finding that galactose oxidase from *Polyporus circinatus* oxidizes the 6-position of galactose to form a dialdehyde and that 1-substituted galactosides may serve as substrates[6,7]. While the enzyme did not react with aqueous suspensions

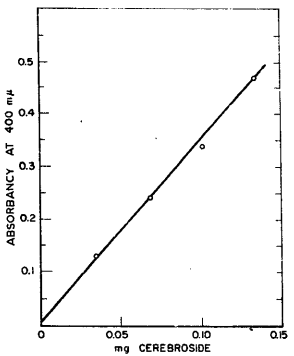

Fig. 1. Enzymic oxidation of galactocerebroside. Galactocerebroside was dissolved in tetrahydrofuran and measured amounts were pipetted into 12 × 100 mm tubes and carefully taken to dryness with N_2. To each tube as well as to a substrate blank were added 0.75 ml of buffered peroxidase–*o*-dianisidine system* containing 11 μg of galactose oxidase**, 0.25 ml of water, and 1.0 ml of tetrahydrofuran, freshly distilled from $Fe(NH_4)_2SO_4 \cdot 6H_2O$. After incubation for 80 min at room temperature, 0.1 ml of 3 *N* HCl and 1.0 ml of tetrahydrofuran – water (1:1, v/v) were added. Absorbancy was measured in a Beckman DU Spectrophotometer.

* "Glucostat" without glucose oxidase, prepared by Worthington Biochemical Company, Freehold, N.J. (U.S.A.).

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TABLE I

DETERMINATION OF GALACTOCEREBROSIDES

Glucocerebroside A was from the spleen of a 35-year old female, and B was from a 10-month old female. Incubations were as described in Fig. 1. All samples were also run with buffered peroxidase–*o*-dianisidine system, but without galactose oxidase and minor corrections were made for the amounts of color produced in each sample.

Substrate	*Absorbancy*	*% Galactocerebroside**
102 μg galactocerebroside	0.340	
102 μg galactocerebroside + 447 μg cerebroside A	0.332	
447 μg cerebroside A	0.009	0.63
620 μg cerebroside B	0.021	0.94

* Maximal values, corrected for 4.9 % apparent inhibition of galactocerebroside color development in the presence of glucocerebroside observed above.

of galactocerebrosides, the enzyme system was fully active in a solvent mixture containing dissolved cerebrosides (Fig. 1). This method was applied to cerebroside preparations obtained by chromatography[8] from spleens removed from patients with Gaucher's disease (Table I). It may be concluded that in the two cases studied here, the cerebroside contains less than 1 % galactocerebroside.

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