Konzentration des «densitometrischen Zylinders» c_d aus und es gilt c_d = konst.

Die Verfasser wählten Albumin als Standardprotein. Die densitometrischen Kurven verschieden konzentrierter Lösungen sind in Figur 2 dargestellt. Die Versuchswerte sowie die berechneten Werte für die Konstruktion der Kalibrierkurve in Figur 3 sind in der Tabelle I zusammengefasst. Die Werte h wurden mittels einer Millimeterskala bestimmt, an welcher 30 mm 10 Strichen der Densitätsskala entsprechen.

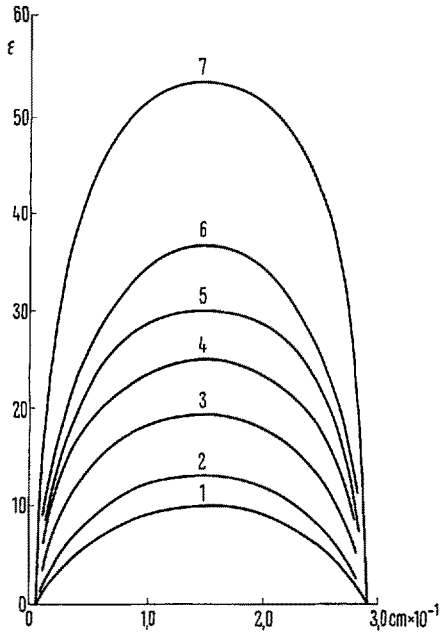

Fig. 5. Densitometrische Kurven der Teste verschieden konzentrierter Albuminlösungen. (1)–(6) wie in Figur 2 angeführt, (7) = 2,2 × 10^{-3} g/ml.

Tabelle II führt als Beispiel die Ergebnisse der Bestimmungen des gesamten Proteingehaltes in Extrakten eines peripheren Nervs, nervus ischiadicus, an, die durch Extraktion von 28 mg des operativ entnommenen Nervs in 2 ml physiologischer Kochsalzlösung dargestellt worden waren. Aus einem Volumen von 0,8 ml des entfetteten Extraktes wurden einerseits 5 Parallelmessungen mittels der oben beschriebenen Methode ausgeführt, andererseits wurde eine Bestimmung nach FOLIN[2] unternommen. Der durch densitometrische Messungen gewonnene Durchschnittswert (c_x 1,49 × 10^{-3} g/ml) stimmt sehr gut mit dem kolorimetrisch bestimmten Wert (1,78 × 10^{-3} g/ml) überein. Die Genauigkeit der Messung wurde anhand der Standardabweichung beurteilt, die in diesem Falle 0,0136 × 10^{-3} beträgt.

Summary. An analytical method of quantitative determination of proteins in small volumes is described. The method is based upon the densitometric evaluation of colour tests in agar-gel. The quantity of proteins is determined by graphical integration of densitometric curves and, in the arrangement described, it is possible to assay proteins in volumes of the order 10^{-3} ml.

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16. Mai 1967.

[2] H. LOWRY, N. J. ROSEBROUGH, A. Z. FARR und R. J. RANDALL, J. biol. Chem. *193*, 263 (1951).

Marking of Chloroplast Galactolipids with Tritium

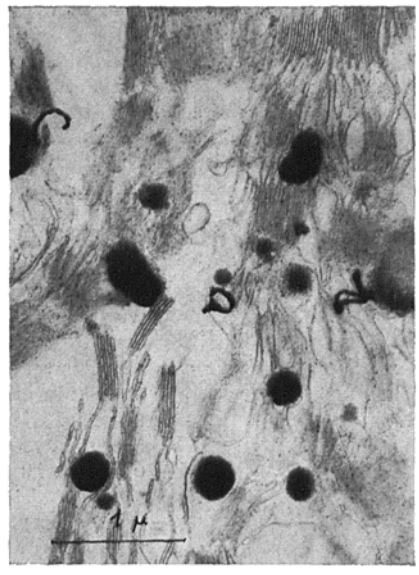
Galactose oxidase (D-galactose: O_2 oxidoreductase, EC 1.1.3.9) acts on D-galactose, 2-D-glucosamine and many galactosides, to form D-galactohexodialdose or aldosides[1]. The aldehyde group formed by the action of this enzyme can be subsequently reduced back to reform the initial galactoside. Use of tritiated borohydride for this reduction was suggested[2] as a procedure for the preparation of ^{3}H-labelled galactose and galactosides such as oligosaccharides[2], glycoproteins[3] and cerebrosides[4]. The same enzyme reaction was also employed in a histochemical staining method to show presence of terminal galactose and galactosamine units in mucus-secreting cells[5].

We have recently attempted to apply the galactose oxidase reaction in studies of ultrastructure as a means for the identification of galactosides in various biological membranes. For this purpose, the enzyme-oxidized structures were reduced by NaB^3H_4 and then studied by radioautography in the electron-microscope.

It is well known that galactolipids constitute a major portion of the lipid fraction of the chloroplast membrane[6]. In the present report the use of the galactose oxidaes reaction is suggested as a possible procedure for their ultrastructural analysis and detection. We feel that the method as described here, or in other variations which can be applied, provides a useful tool for studies on the appearance, turnover and other metabolic aspects of galactolipids in the chloroplast membrane.

Experimental[7]. Washed Swiss chard leaves were disintegrated in a Waring blendor for 15 sec in 0.2 M phosphate buffer pH 7.0, containing 0.4 M sucrose. The fraction containing chloroplasts[8] was collected by centrifugation between 200 and 1000 g. The chloroplasts were thrice washed in the same sucrose buffer solution, dispersed in it to 15% (v/v) suspension, then treated with a glutaraldehyde solution (4% final concentration) prepared in 0.2 M phosphate buffer pH 7.0. After 160 min at 4 °C, the chloroplasts were washed 5 times with 20 volumes of buffer to remove excess aldehyde, then suspended in 2% $NaBH_4$ solution at room temperature. Two h later the chloroplasts were washed again with the same buffer and dispersed to about 15% (v/v) suspension in a solution containing 200 units galactose oxidase and 450 units catalase/ml in 0.2 M phosphate buffer, pH 7.0. After 5 h at 25°C, NaB^3H_4 (100 μc/μmole) was added to a final con-

A



B

Radioautograph of stained thin sections of chloroplasts which were oxidized with galactose-oxidase and then reduced with NaB^3H_4. (A) Intact chloroplast. (B) Disintegrated chloroplast.

centration of 0.1 mg/ml, and after a further incubation of 1 h, chloroplasts were washed with a similar buffer to remove all soluble radioactivity. Samples of the tritium-labelled chloroplast suspension were collected on Millipore HA ultrafilters and placed in scintillation liquid for counting[9]. Activity found was 1.6×10^5 dpm/mg dry chloroplast weight. Acid hydrolysis of the preparation (1 *N* HCl; 100 °C for 3 h) and chromatographic analysis[2,3] of the concentrated hydrolyzate indicated that the tritium resided in a hexose with a chromatographic behaviour expected for galactose.

Tritium-labelled chloroplasts were treated with OsO_4 fixative solution and embedded in Epon[10]. Thin sections (500–700 Å) were covered with Ilford L-4 emulsion[11], stored 60 days in the dark at 4 °C and developed with Kodak Microdol-X. After staining with uranyl acetate[10] and lead citrate[12] solutions (3 min in each case), the sections were examined in the electron microscope (Figure). In the preparation described above, 1–4 silver grains were observed for each section of a chloroplast. The grains were associated with the lamellar structure and predominantly found in peripheral areas of the particles. It could be roughly estimated that only less than 2% of the total galactoside moieties in the chloroplast[13] were labelled by tritium in the experiment described. It is suggested that oxidizing thin sections rather than whole chloroplasts as well as employing tritiated borohydride of a much higher specific activity, would provide a more efficient procedure for detecting galactolipids by radioautography.

Résumé. Une technique pour le marquage des galactolipids du chloroplast par tritium est décrit. Cette technique utilise l'oxidation du galactoside par le galactose-oxidase suivi par une réduction des aldehydes formées avec borohydride tritié. L'application de cette technique pour l'étude ultrastructurelle de chloroplasts est montré.

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9th June 1967.

[1] G. AVIGAD, C. ASENSIO, D. AMARAL and B. L. HORECKER, Biochem. biophys. Res. Commun. *4*, 474 (1961); J. biol. Chem. *237*, 2736 (1962).

[2] G. AVIGAD, Israel J. Chem. *4*, 91p (1966); Carbohydrate Res. *3*, 430 (1967).

[3] A. G. MORELL, C. J. A. VAN DEN HAMER, I. H. SCHEINBERG and G. ASHWELL, J. biol. Chem. *241*, 3745 (1966).

[4] A. K. HAFRA, D. M. BOWEN, Y. KISHIMOTO and N. S. RADIN, J. Lipid Res. *7*, 379 (1966).

[5] G. P. ROBERTS and S. K. GUPTA, Nature *207*, 425 (1965).

[6] A. A. BENSON, Adv. in Lipid Res. *1*, 387 (1963); A. Rev. Pl. Physiol. *15*, 1 (1964). – A. FREY-WYSSLING and K. MUHLETHALER, *Ultrastructural Plant Cytology* (Elsevier Publishing Co., Amsterdam 1965). – C. F. ALLEN and P. GOOD, J. Am. Oil Chem. Soc. *42*, 610 (1965). – R. B. PARK, in *Plant Biochemistry* (Ed. J. BONNER and J. E. VARNER; Academic Press, New York 1965), p. 124. – A. A. BENSON and T. E. WEIER, in *Biochemistry of Chloroplasts* (Ed. T. W. GOODWIN; Academic Press, New York 1966), vol. I, p. 91. – C. F. ALLEN, O. HIRAYAMA and P. GOOD, in *Biochemistry of Chloroplasts* (Ed. T. W. GOODWIN; Academic Press, New York 1966), vol. I, p. 195. – A. ROSENBERG, J. Lipid Res. *7*, 733 (1966). – C. R. SMITH and I. A. WOLFF, Lipids *7*, 123 (1966). – A. ROSENBERG and J. GOUAX, J. Lipid Res. *8*, 80 (1967).

[7] Reagents used were: Purified galactose-oxidase preparation obtained as described in ref. [1]; crystalline catalase from Boehringer und Söhne, GmbH, Mannheim; tritiated sodium borohydride from New England Nuclear Corp., Boston, Mass. Reagents for radioautography and electron microscopy were as those described in the respective references. A RCA EMU-3G electron microscope and a Porter-Blum MT 1 ultramicrotome (Ivan Sorvall Inc., Norwalk, Conn.) were employed.

[8] D. A. ARNON, M. B. ALLEN and F. R. WHATLEY, Biochim. biophys. Acta *20*, 449 (1956).

[9] Tritium was counted in a Packard Tri-Carb scintillation spectrophotometer with 20% efficiency using 0.3% 2,5-diphenyloxazole (PPO) and 0.01% 2,2-*p*-phenylenebis-5-phenyloxazole (POPOP) in toluene as the scintillator liquid. ^{3}H-toluene was employed for internal standards. Samples containing chloroplasts were counted 70 h after they had been put into the vials with the scintillator liquid. This allowed the complete disappearance of some initial, blank phosphorescence of unclear provenance. Some preliminary examinations suggest that this phosphorescence is associated with an effect of tritium on the chloroplast pigments.

[10] D. C. PEASE, *Histological Techniques for Electron Microscopy*, 2nd edn (Academic Press, New York 1964). – E. H. MERCER and M. S. C. BIRBECK, *Electron Microscopy, A Handbook for Biologists*, 2nd edn (Blackwell, Oxford 1966).

[11] L. C. CARO and R. P. VAN TUBERGEN, J. Cell Biol. *15*, 173 (1962).

[12] J. H. VENABLE and R. COGGESHALL, J. Cell Biol. *25*, 407 (1965).

[13] H. K. LICHTENTHALER and R. B. PARK, Nature *198*, 1070 (1963).