raminidase resulted in the appearance of two intermediate hydrolysis products. One of these products demonstrated thin-layer chromatographic properties similar to HG-4, while the other product migrated slower than HG-4 in the *n*-propanol solvent systems. The final product was resistant to further action of the enzyme and migrated with properties identical to HG-1. No other degradation products were visible by over-spraying the plates with sulfuric acid.

These data indicate that the ganglioside HG-6 is a trisialo molecule which is isomeric with the trisialo ganglioside (4-G) reported by JOHNSON AND MCCLUER[1,2]. Further characterization of this ganglioside (HG-6) must be based upon more extensive structural studies than the amount of material available at the present time allows.

This work was supported in part by Grant No. NB 05222-01 from the National Institutes of Health, United States Public Health Service.

Departments of Physiological Chemistry and Psychiatry, R. J. PENICK
Ohio State University, Columbus, Ohio (U.S.A.) R. H. MCCLUER

1 G. A. JOHNSON AND R. H. MCCLUER, *Biochim. Biophys. Acta*, 70 (1963) 487.
2 G. A. JOHNSON AND R. H. MCCLUER, *Biochim. Biophys. Acta*, 84 (1964) 587.
3 L. SVENNERHOLM, *Biochim. Biophys. Acta*, 24 (1957) 604.
4 T. MIETTINEN AND I. T. TAKKI-LUUKKAINEN, *Acta Chem. Scand.*, 13 (1959) 856.
5 R. H. MCCLUER, E. H. CORAM AND H. S. LEE, *J. Lipid Res.*, 3 (1962) 269.
6 L. SVENNERHOLM, *Acta Soc. Med. Upsaliensis*, 61 (1956) 287.
7 J. HODGE AND B. HOFREITER in R. WHISTLER AND M. WOLFROM, *Methods in Carbohydrate Chemistry*, Vol. 1, Academic, New York and London, 1962, p. 388.
8 R. J. PENICK AND R. H. MCCLUER, to be published.
9 C. J. LAUTER AND E. G. TRAMS, *J. Lipid Res.*, 3 (1962) 136.
10 R. KUHN AND H. WIEGANDT, *Chem. Ber.*, 96 (1963) 866.
11 R. KUHN AND H. WIEGANDT, *Z. Naturforsch.*, 19b (1964) 256.
12 L. SVENNERHOLM, *J. Neurochem.*, 10 (1963) 613.
13 G. TETTAMANTI, L. BERTONA AND V. ZAMBOTTI, *Biochim. Biophys. Acta*, 84 (1964) 756.
14 R. KUHN, H. WIEGANDT AND H. EGGE, *Angew. Chem.*, 73 (1961) 580.

Received June 11th, 1965

Biochim. Biophys. Acta, 106 (1965) 435–438

PN 51034

On the phospholipids of *Bacillus megaterium*

Information on the chemical identity of the phospholipids from *Bacillus megaterium* appears to be rather incomplete. WEIBULL[1] reported that cells of *B. megaterium* M contain mainly phospholipids devoid of nitrogen. On the other hand, YUDKIN[2] found that in *B. megaterium* strain KM phosphatidyl ethanolamine accounted for about 97% of the phospholipids. HAVERKATE *et al.*[3] observed that phosphatidyl glycerol was a major phospholipid from this bacterium. HUNTER *et al.*[4] reported on the occurrence of lipo-aminoacids, and in the light of the studies of MACFARLANE[5,6] and HOUTSMULLER AND VAN DEENEN[7,8] on the amino acid esters of phosphatidylglycerol from several gram positive bacteria, it became likely that these phospholipids occur in *B. megaterium* as well[7,9].

In the present study, *B. megaterium* (strain MK 10D) was cultured in the following media: (A) containing 10 g of peptone (Difco), 10 g of yeast extract (Difco), 5 g of sodium chloride, 400 mg sodium phosphate and 300 μC [^{32}P]orthophosphate per l of water; the pH was adjusted to 7.2. (B) This medium contained in addition 20 g of glucose and 2 g of ammonium sulfate per l. The cells were harvested in the stationary phase of growth, the pH of medium A being 7.0–7.2, while that of medium B was 4.9–5.3.

The lipid extracts[10] were subjected to chromatography on silica-impregnated paper[11] and the autoradiograms revealed some conspicuous differences in the nature and composition of the bacterial phospholipids from the two cultures (Fig. 1). The cells harvested from medium A were found to contain at least the following four phospholipids. Compound 1, present in low quantities only, exhibited chromatographic properties similar to those of synthetic diphosphatidylglycerol, but its identity requires further investigations. Compound 2, accounting for 36–45% (10 experiments) of the total phospholipids, was identical to phosphatidylethanolamine. After isolation by chromatography on silica columns and alkaline hydrolysis, glycerophosphorylethanolamine was the only phosphodiester detectable. Compound 3 constituted 35–45% of the phospholipids of the bacteria cultivated in medium A; its chromatographic behaviour and staining properties (ninhydrin, negative; periodate-Schiff, positive) were identical to those of synthetic phosphatidylglycerol. Alkaline hydrolysis of the isolated compound furnished glycerophosphorylglycerol only. Compound 4, reacting with ninhydrin, represented 8–14% of the bacterial phospholipids. Its chromatographic behaviour was similar to that of the lysyl ester of phosphatidyl glycerol isolated from *Staphylococcus aureus*[12]. On alkaline hydrolysis, the purified compound yielded glycerophosphorylglycerol, while lysine was the only ninhydrin-positive compound detectable on paper chromatograms of the products resulting from acidic hydrolysis.

All of the phospholipids mentioned were also present in the cells harvested from medium B (Fig. 1). However, the amount of phosphatidylglycerol was only 5–10% (8 Expts.) of the total phospholipids, which is very low compared with the corresponding amount in cells harvested from medium A. This decrease in phosphatidylglycerol appeared to be largely counterbalanced by the occurrence of a phospholipid which was not detectable in cells grown in the former broth (Fig. 1). This phospholipid (compound 5) accounted for 30–35% of the total phospholipids and appeared to contain amino groups, but no free vicinal hydroxyl groups. Separation of compounds 4 and 5, and 4 and 2, though in principle possible by chromatography on silica columns, was achieved by preparative thin-layer chromatography on silicagel G with chloroform–methanol–acetic acid–water (250:74:19:3, v/v) (ref. 13) as developer. The purity of the preparations obtained is demonstrated by the autoradiograms reproduced in Fig. 1. Compound 4 revealed a phosphorus–aminonitrogen–total nitrogen ratio of 1:2.1:2. The lysine constituent was completely converted by L-lysine carboxy-lyase (EC 4.1.1.18), thus confirming that this phospholipid is identical to the L-lysyl ester of phosphatidylglycerol. As regards the identity of the unknown phospholipid 5, it may be concluded that a derivative of phosphatidylglycerol is involved, since mild alkaline hydrolysis furnished glycerophosphorylglycerol. The compound was treated with 6 N HCl at 100° for 16 h and the water-soluble hydrolysis products were investigated by paper chromatography using as developing solvents

butanol–acetic acid–water (6:1:2, v/v), *n*-propanol–ammonia–water (6:3:1, v/v) and phenol–water (5:2, v/v). Only one ninhydrin-positive component was detectable and this coincided with [^{14}C]glucosamine. No other sugar could be detected. A detailed study on the chemical structure of this phospholipid is in progress.

The significant differences in phospholipids between cells of *B. megaterium* cultivated in media A and B respectively could also be induced, at least qualitatively,

Fig. 1. Autoradiograms of phospholipids from *B. megaterium*. Paper chromatograms were developed on silica-impregnated paper with diisobutyl ketone–acetic acid–water (40:25:5, v/v) (ref. 11). a, phospholipids of cells harvested from medium A (see text). b, phospholipids of cells harvested from medium B. c, compound 4 isolated by column chromatography and thin-layer chromatography from the mixture shown under b. d, compound 5 isolated from the mixture shown under b. The compounds are: 1, unidentified polyglycerolphospholipid; 2, phosphatidylethanolamine; 3, phosphatidylglycerol; 4, L-lysine ester of phosphatidylglycerol; 5, glucosamine derivative of phosphatidylglycerol.

by lowering the pH of a culture growing in medium A to 5.0 by the addition of hydrochloric acid. Under such conditions the relative quantity of phosphatidylglycerol was reduced and the glucosamine-containing phospholipid, which was virtually absent at pH 7, now amounted to about 15% of the total phospholipids. It would appear, therefore, that the acidity of the medium is involved, although a com-

parison with the analytical data on the phospholipids from cells grown in medium B indicates that the pH is probably not exclusively responsible for such substantial accumulation of the glucosamine derivative of phosphatidylglycerol.

It is worth noting that the phospholipids from cells of *B. megaterium* grown in media A and B, contained 50 and 90% of amino groups, respectively. The protoplast membrane fractions exhibited similar differences in phospholipid composition as recorded above for the total cells. Preliminary experiments demonstrated that the protoplasts prepared from cells cultured in medium B were more stable to lysis in 0.1 M sucrose in 0.06 M phosphate buffer (pH 6.2), as compared with those of cells harvested from medium A, but it remains to be established whether this distinction is related to the differences in phospholipid composition. The different protoplasts manifested quite different shapes under the phase-contrast microscope and their ultrastructure is now under investigation by Dr. W. VAN ITERSON (University of Amsterdam).

The investigations were supported (in part) by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

Department of Biochemistry, J. A. F. OP DEN KAMP
Laboratory of Organic Chemistry, State University, U. M. T. HOUTSMULLER
Utrecht (The Netherlands) L. L. M. VAN DEENEN

1 C. WEIBULL, *Acta Chem. Scand.*, 11 (1957) 881.
2 M. D. YUDKIN, *Biochem. J.*, 82 (1962) 40 P.
3 F. HAVERKATE, U. M. T. HOUTSMULLER AND L. L. VAN DEENEN, *Biochim. Biophys. Acta*, 63 (1962) 547.
4 G. D. HUNTER AND R. A. GOODSALL, *Biochem. J.*, 78 (1961) 564.
5 M. G. MACFARLANE, *Nature*, 196 (1962) 136.
6 M. G. MACFARLANE, in R. M. C. DAWSON AND D. N. RHODES, *Metabolism and Physiological Significance of Lipids*, Wiley, London, 1964, p. 399.
7 U. M. T. HOUTSMULLER AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 70 (1963) 211.
8 U. M. T. HOUTSMULLER AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 84 (1964) 96.
9 G. D. HUNTER AND A. T. JAMES, *Nature*, 198 (1963) 789.
10 E. G. BLIGH AND W. J. DYER, *Can. J. Biochem. Physiol.*, 37 (1959) 911.
11 G. V. MARINETTI, J. ERBLAND AND J. KOCHEN, *Federation Proc.*, 16 (1957) 837.
12 U. M. T. HOUTSMULLER AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, in the press.
13 D. ABRAMSON AND M. BLECHER, *J. Lipid Res.*, 5 (1964) 628.

Received July 20th, 1965

Biochim. Biophys. Acta, 106 (1965) 438–441

PN 51032

Inhibition of hyperlipogenesis with puromycin or actinomycin D*

The elevation in activity of the liver enzymes catalyzing the synthesis of saturated, long-chain fatty acids which is observed in rats maintained on a fat-free diet after a period of starvation[1–4] may be attributed to: (a) dimunition in the cellular

* Portions of this paper are from a thesis by one of the authors (S. E. H.) submitted to the Graduate School of Indiana University in partial fulfillment of the requirements for the Ph.D. degree-