treated here. The main purpose of this study has been to point out *the existence of a very strong adsorption of both high- and low-molecular molecules on the outer parts of the Sephadex gels.*

The two possible procedures which can be employed in gel filtration of humic acids seem to be, either to elute with a salt eluent (as done in Refs. 4, 6, 8, 9, 11, 13, and 14) and discard the strongly adsorbed part (containing both high- and low-molecular components) or to elute dialyzed samples with distilled water [3] (obtaining a less efficient separation). The main rule must be to avoid ionic strength gradients during the separation. This is also emphasized by Eaker and Porath.[17]

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1. Bailly, J. R. *Compt. Rend.* **264** (1967) 564.
2. Dell'Agnola, G., Ferrari, G. and Maggioni A. *Ric. Sci. Suppl.* **34** (1964) 347.
3. Dell'Agnola, G. and Maggioni, A. *Boll. Sci. Fac. Chim. Ind. Bologna* **23** (1965) 321.
4. Ferrari, G. and Dell'Agnola, G. *Soil Sci.* **96** (1963) 418.
5. Gjessing, E. T. *Nature* **208** (1965) 1091.
6. Koter, M., Chodan, B., Chodan, J. and Panak, H. *Zezyty Nauk Wyzszej Szkoly Rolniczej Wroclawia Melioracja* **15** (1963) 343.
7. Ladd, J. N. and Butler, J. H. A. *Australian J. Soil Res.* **4** (1966) 41.
8. Mathur, S. P. and Paul, E. A. *Can. J. Microbiol.* **13** (1967) 573.
9. Mehta, N. C., Dubach, P. and Deuel, H. *Z. Pflanzenernähr. Düng. Bodenk.* **102** (1963) 128.
10. Obenaus, R. and Neumann, H. J. *Naturwiss.* **52** (1965) 131.
11. Passera, C. and Maggioni, A. *Ric. Sci. Suppl.* **36** (1966) 652.
12. Posner, A. M. *Nature* **198** (1963) 1161.
13. Robert-Gero, M., Hardisson, C., Le Borgne, L. and Pignaud, G. *Ann. Inst. Pasteur* **111** (1966) 751.
14. Roulet, N., Mehta, N. C., Dubach, P. and Deuel, H. *Z. Pflanzenernähr. Düng. Bodenk.* **103** (1963) 1.
15. Soukop, M. *Collection Czech. Chem. Commun.* **29** (1964) 3182.
16. Wildenlain, W. and Henseke, G. *Z. anal. Chem.* **229** (1967) 271.
17. Eaker, D. and Porath, J. *Separation Sci. In press.*

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Studies on Sphingosines

15. Degradation of Phytosphingosine to Hydroxy Fatty Acid and Ethanolamine by the Yeast *Hansenula ciferrii*

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The first experimental evidence for an enzymatic degradation of sphingolipid long chain bases was presented by Korey and Stein,[1] who studied an enzyme system of rat brain, capable of degrading gangliosides stepwise, including the long chain bases. Very recently indications for fatty acids as catabolites of long chain bases were given.[2-4] Phytosphingosine (1,3,4-trihydroxy-2-amino-octadecane) was degraded to hydroxy fatty acid.[4] Formally, the remaining part is 2-aminoethanol. Alternatively, a two-step one carbon degradation, possibly preceded by a deamination, should be considered. The present communication gives evidence for ethanolamine as a catabolite of phytosphingosine in the yeast.

Hansenula ciferrii (F-60−10) [5] was grown for 4 days at 25°C in yeast maintenance broth with ^{14}C-phytosphingosine,[4] prepared from the yeast given 3-^{14}C-serine. The incubation mixture was extracted with chloroform-methanol 2:1, v/v, and the insoluble cell residues were filtered off. The extract was partitioned against water (chloroform-methanol-water 8:4:3, v/v/v). The upper (I) and lower phases were separately taken to dryness. The upper phase (I) was hydrolyzed for 13 h in 1 M HCl in water and evaporated. The lower, lipid phase was hydrolyzed for 6 h in 2 M HCl in water and partitioned as described above. The upper phase (II) was evaporated. Fatty acids and long chain bases in the lower phase were separated and purified using silicic acid column and thin layer chromatography (Table 1).[4]

The two upper phases (I and II) were separately subjected to dinitrophenyl (DNP) synthesis [6] and the products extracted with diethyl ether before (a) and after (b) acidification with hydrochloric acid. In II all radio-

Table 1.

Substrate:	Radio-activity (cpm)
^{14}C-Phytosphingosine with 21 % ^{14}C in C_4-C_{18}	145 000
Products:	
Phytosphingosine	60 000
Normal fatty acids	140
Hydroxy fatty acids	1 440
DNP-ethanolamine from upper phase I	1 100
DNP-ethanolamine from upper phase II	370

activity was found in the alkaline extract (a). This was subjected to preparative thin layer chromatography and except for a small amount of DNP-phytosphingosine the only radioactive compound found was DNP-ethanolamine.

Ia and Ib were separately fractionated by silicic acid column chromatography, the conditions modified from an earlier described procedure.[7] Seven fractions were collected, the first eluted with formic acid-ethyl acetate-hexane 2:8:90, v/v/v, and the next five fractions with a stepwise increase of polarity. The last fraction was eluted with acetic acid until practically all the yellow colour had left the columns. The fractions were subjected to analytical thin layer chromatography [8] using authentic samples as references. The following DNP-derivatives were identified: DNP-glycine, DNP-alanine, DNP-serine, DNP-valine and DNP-ethanolamine. Radioactivity was found only in fractions containing DNP-ethanolamine (Ia, fractions 3–5). These fractions were further purified by column and thin layer chromatography. DNP-ethanolamine from I and II had an activity ratio of about 3:1 with similar specific activities.

The two DNP-ethanolamine fractions (I and II) were put together and repeatedly chromatographed on thin layers of silicic acid and on reversed phase paper chromatography.[9] In this way, the chromatographically pure DNP-ethanolamine was shown to retain its radioactivity.

The results in the present paper give evidence for a direct enzymatic splitting of phytosphingosine between carbon atoms 2 and 3. The tetraacetylphytosphingosine, found as the major sphingolipid of this mutant yeast,[5] may be the immediate precursor. The cleavage of the C–C bond may thus be facilitated by an electron withdrawal to the acetyl groups. The initial products may be *N*,*O*-diacetylethanolamine and a mixed anhydride of acetic acid and 2-acetoxy palmitic acid. The presence of ethanolamine in the first lower, lipid phase may be due to the presence of the less polar diacetyl derivative, later degraded in the acid hydrolysis. This possible role of the acetyl derivatives, as well as the specificity of the enzyme involved, are subjects for further studies in this laboratory.

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1. Korey, S. R. and Stein, A. In Folch-Pi, J. and Bauer, H., (Eds.), *Brain Lipids and Lipoproteins and the Leucodystrophies*, Elsevier, Amsterdam 1963, p. 71.
2. Barenholz, Y. and Gatt, S. *Biochem. Biophys. Res. Commun.* **27** (1967) 319.
3. Stoffel, W. and Sticht, G. *Z. physiol. Chem.* **348** (1967) 941.
4. Karlsson, K.-A., Samuelsson, B. E. and Steen, G. O. *To be published.*
5. Wickerham, L. J. and Stodola, F. H. *J. Bacteriol.* **80** (1960) 484.
6. Ghuysen, J. -M., Tipper, D. J. and Strominger, J. L. In Neufeld, E. F. and Ginsburg, V., (Eds.), *Methods in Enzymology*, Academic, New York 1966, Vol. 8, p. 685.
7. Green, F. C. and Kay, L. M. *Anal. Chem.* **24** (1952) 726.
8. Brenner, M., Niederwieser, A. and Pataki, G. In Stahl, E., (Ed.), *Dünnschichtchromatographie*, Springer, Berlin 1962, p. 403.
9. Karlsson, K.-A. *Nature* **188** (1960) 312.

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