of water and appear 18 units lower; and $R_3 + 43$, a rearrangement peak containing one of the $-C^*H_2-$ groups. There are, of course, many more peaks in the otherwise complex spectrum which further aid in the interpretation. No appreciable peak is observed at M (mol. wt.) but one at M + 1,[5] arising from an ion-molecule collision; its intensity relative to others, therefore, changes with pressure and focusing conditions and is thus easily recognized. From this the sum of $R_1 + R_2 + R_3$ can be calculated.

The proposed structures of these fragments were substantiated by comparison of the mass spectra of the reduction products of N-acetyl-gly-phe-OEt and N-acetyl-leu-ala-pro-OEt with $LiAlH_4$ and $LiAlD_4$, respectively. The spectra of the two pairs showed the expected shift in mass numbers (each $-C^*H_2-$ in I becomes $-CD_2-$).

This method, if applied to partial hydrolysates of natural peptides, should be very advantageous in the elucidation of their structure owing to the inherent speed and sensitivity of mass spectrometric analysis.[6] At present we are extending our method to peptides of higher molecular weight and of other amino acids.

(5) This has been observed also with some other types of compounds; *e.g.*, aliphatic ethers (F. W. McLafferty, *Anal. Chem.*, **29**, 1782 (1957), and references therein).

(6) The reduction can be carried out with a fraction of one milligram and yields enough material for a good spectrum.

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## LIPIDS CONTAINING MONO- AND DIMETHYLETHANOLAMINE IN A MUTANT STRAIN OF *Neurospora crassa*[1]

*Sir:*

Previous studies have shown that a choline deficient mutant of *Neurospora crassa* (strain 47904) differs from the normal wild-type (strain 1A) by its accumulation of monomethylethanolamine,[2] dimethylethanolamine[3] and the phosphate esters[4] of these two amines. More recent investigations have disclosed a striking difference between the phospholipids of strain 47904 and those found in strain 1A.

The ether and alcohol extractable lipids from mycelia of strains 1A and 47904 were washed free of non-lipid contaminants by the method of Folch[5] and then subjected to prolonged acid hydrolysis. The resulting hydrolysate of the normal strain yielded choline as the predominant methylated ethanolamine. In contrast to this, the lipide hydrolysate of strain 47904 contained monomethylethanolamine, dimethylethanolamine and small amounts of choline (Table I). The methods used for the isolation and estimation of these amines have been described.[3]

TABLE I

| Amine isolated after lipid hydrolysis | Strain 47904 | Strain 1A |
|---|---|---|
| Monomethylethanolamine | 13–16[a] | ... |
| Dimethylethanolamine | 2–3 | Trace |
| Choline | 0.3–0.9 | 11–14 |

[a] Data expressed as micromoles per gram of dry tissue.

Chromatographic separation of the total lipids prior to hydrolysis was carried out by the use of silicic acid columns according to the method described by Mead and Fillerup.[6] Almost all of the lipid-bound methylated ethanolamines present in the two strains under investigation were found in the phospholipid fraction. Preliminary investigations suggest that in the choline deficient strain the phosphatidyl derivatives of monomethylethanolamine and dimethylethanolamine have replaced most of the lecithin normally found in *Neurospora crassa*. The chemical characterization of these unusual phospholipids is now in progress.

(6) J. F. Mead and D. L. Fillerup, *ibid.*, **227**, 1009 (1957).

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## THE DEPHOSPHORYLATION OF CASEIN BY ALKALIES[1]

*Sir:*

The readiness with which phosphate is cleaved by alkali from phosphoproteins such as casein and vitellin has long puzzled protein chemists. There is a considerable body of evidence that the phosphate is attached to the serine residues of these proteins by ester linkages,[2] yet phosphate esters are characteristically resistant to hydrolysis by alkali.[3] Some years ago, Mecham and Olcott[4] published evidence which suggests that the reaction is one of $\beta$-elimination rather than hydrolysis, but their results do not seem to have attracted wide attention. We have sought further evidence bearing on the $\beta$-elimination hypothesis.

Bovine casein was dephosphorylated with alkalies in water enriched with $O^{18}$, and the inorganic phosphate formed was isolated as $MgNH_4PO_4$ and converted to $KH_2PO_4$. The $KH_2PO_4$ was analyzed for $O^{18}$ by pyrolysis to $KPO_3$ and water, equilibration of the water with carbon dioxide, and analysis of the carbon dioxide in a mass spectrometer. An inspection of the results (Table I) shows that there

(1) This investigation was supported in part by a grant from the Division of Research Grants of the National Institutes of Health, Public Health Service RG-5794, and by the Cancer Research Funds of the University of California.

(2) N. H. Horowitz, *J. Biol. Chem.*, **162**, 413 (1946).

(3) B. Wolf and J. F. Nyc, *Biochim. et Biophys. Acta*, **31**, 208 (1959).

(4) B. Wolf and J. F. Nyc, *J. Biol. Chem.*, **234**, in press (1959).

(5) J. Folch, I. Ascoli, M. Lees, J. A. Meath and F. N. Le Baron, *ibid.*, **191**, 833 (1951).

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(2) Gertrude E. Perlmann, *Advances in Protein Chem.*, **10**, 1 (1955). The question as to whether phosphodiester and pyrophosphate-(diester) linkages occur in casein, in addition to the phosphomonoester groups, is still moot, but is not relevant here.

(3) Phosphodiesters of vicinal glycols (not possible in proteins) and phosphotriesters are exceptions to this rule, but they give monoesters and diesters, respectively, not inorganic phosphate.

(4) D. K. Mecham and H. S. Olcott, THIS JOURNAL, **71**, 3670 (1949).