determined (Table 2). Lysine, MML and DML accounted for 90–95 per cent of the total radioactivity. No radioactivity was present in histidine 3-MH, or ammonia. Only 5–10 per cent of the total radioactivity was recovered in the neutral and acidic amino-acids. The high specific radioactivities of MML and DML support their identifications and establish their synthesis by the amoebae. Some of the differences in specific radioactivities of the three amino-acids are undoubtedly attributable to unavoidable errors in the measurements of small amounts of amino-acids and of low levels of radioactivity in a highly quenched system.

Table 2. RADIOACTIVE AMINO-ACIDS IN ACTIN FROM AMOEBAE GROWN IN THE PRESENCE OF ^{3}H-LYSINE

	Protein (c.p.m./mg)	Lysine	MML	DML	TML
		(c.p.m./μmole)			
Crude amoeba extract	79,000	120,000	130,000	110,000	96,000
Purified G-actin	53,000	83,000	112,000	67,200	—

Crude amoeba extract and purified G-actin from amoebae grown in the presence of 10 mCi of ^{3}H-lysine were hydrolysed in 6 M HCl for 72 h at 110° C. The basic amino-acids were separated by system B of Kuehl and Adelstein[9] and the effluent from the photometer of the Beckman '120-B' amino-acid analyser was collected in fractions of 3·3 ml. Aliquots of 1 ml. were added to 15 ml. of a solution of 10 per cent naphthalene and 0·5 per cent diphenyloxazole in dioxane. The precipitates that formed were allowed to settle and the supernatant solutions were decanted. The precipitates were dissolved by heating for 1–2 h at 60° C in a solution of 4 per cent naphthalene, 0·2 per cent diphenyloxazole, and 20 per cent water in dioxane to which had been added 10–20 μl. of concentrated HCl. The samples were analysed for radioactivity in a Beckman model 'LS-250' scintillation counter and were corrected for quenching by use of the external standard. The specific radioactivities of the crude amoeba extract have been adjusted to compensate for the addition of non-radioactive carrier protein (see text). Essentially, no counts were detected in the fraction of the actin hydrolysate with the elution time of TML.

Finally, one should consider whether the methyllysines of amoeba actin might be derived from a contaminating protein even though the isolation procedure[1] is based on the unique properties of actin and the final product seems to be essentially homogeneous by sensitive physicochemical and biochemical criteria[1,2]. Histones (but not from all species) are the only other proteins known to contain DML and these proteins also have a very high content of lysine, histidine and arginine[4,14]. Were the DML of amoeba actin derived from a contaminating histone with the highest content of DML yet reported[4,5,14], the contaminating protein would also account for between 50 and 200 per cent of the lysine, histidine and arginine. This seems highly unlikely especially because the compositions of the basic amino-acids of amoeba actin and muscle actin are nearly identical[1]. Moreover, MML, DML and TML occur in histone[5] in the ratio of 1 : 2 : 1 which is very different from that observed for amoeba actin. We conclude that amoeba actin contains 1 mole of DML and 1 mole of 3-MH per mole of protein of molecular weight about 42,000. The data for MML and TML are not conclusive. TML is present in greater concentration in the "Sephadex-excluded" proteins and its presence in purified amoeba actin might represent a contaminating protein. But if our estimate of at least 90 per cent purity for the amoeba actin is correct, such a contaminating protein would have to contain a very high percentage of methyllysines. On the other hand, amoeba actin may be molecularly heterogeneous, with certain lysine residues in various stages of methylation. These questions, and the mechanism of methylation, will only be resolved by further experimentation.

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Phthalic Acid in the Deep Sea Jellyfish *Atolla*

DURING an analysis of the lipids of the deep sea jellyfish *Atolla*, an unusually large percentage of phthalic acid was found in the fatty acids of the saponifiable fraction. The identification was based on gas liquid chromatographic (GLC) analysis on three columns (Table 1).

Table 1. GLC OF PHTHALIC ACID FOUND IN *Atolla*

	t_R of Standard Me 1,2 phthalate	t_R of unknown
5 per cent/9'/NPGS at 175° C	0·424	0·430
3 per cent/5'/Apiezon L at 200° C	0·077	0·077
20 per cent/5'/DEGS at 175° C	1·40	1·37

Retention times are relative to 18:1 standard oleic acid.

GLC analysis was carried out on the total Me esters, hydrogenated Me esters and on the non-urea complexed esters[1]. Mass spectrometry confirmed the identification of phthalic acid and indicated the 1,2 acid as the principal isomer present[2].

The specimens of *Atolla* and other zooplankton were taken from deep net hauls (1,000–0 m) in the North Atlantic (33° N 14° W) for lipid analysis. All material was kept in specially cleaned glass jars under nitrogen at −25° C until analysed in the laboratory. Analyses of the other animals from the deep hauls revealed no significant amounts of phthalic acid, and so contamination during the netting and storage of *Atolla* seems unlikely.

No plastic came into contact with the extract during the preparative work-up and analysis of the *Atolla* lipids, which was one of a series of similar analyses. The same glassware was used as in the other analyses, which revealed no contamination by phthalic acid. All solvents were redistilled analar reagents. Thus there is unlikely to have been any contamination during the analysis.

I have concluded that the phthalic acid was present in the animal before capture. Although it is present as only 0·01 per cent of the total animal wet weight this represents 13 per cent of the total lipid weight and 26 per cent of the total fatty acid weight. It is most unlikely that phthalic acid is present as a normal metabolite and so it seems to have been assimilated and stored by the animal.

Phthalic acid is in fairly widespread use, chiefly as a plasticizer in the plastics industry, but the occurrence of such large amounts in a deep living oceanic animal, such as *Atolla*, seems remarkable.

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