TABLE I

MEAN RECOVERY OF ASPARTIC AND GLUTAMIC ACID ON THE BASIS OF ORDER OF ANALYSIS RELATIVE TO NORLEUCINE

Acid	*1st* ($n = 12$)	*2nd* ($n = 4$)	*3rd* ($n = 5$)
Aspartic	0.90	0.87	0.84
Glutamic	0.86	0.78	0.73

Differences observed are significant at the 95 % confidence level.

To date, we have mostly negative information on the products formed. We have not found any positive correlation with any other peak elsewhere in the chromatogram.

It has been suggested that the color loss we have observed may be due to esterification[3]. In the case of aspartic acid the observed loss may very well be due to color yield differences of the methyl esters. If simple esterification is not the answer, another possibility is the conversion of the aspartic acid to fumaric or maleic acids. The ammonia released by this reaction might not be detected by our system. The large loss of glutamic acid is best explained by its conversion to ninhydrin negative pyrrolidone carboxylic acid[4]. Work to establish the degradation products is now in progress.

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Institute of Arctic Biology, University of Alaska, College, Alaska 99701 (U.S.A.)

L. W. NAUMAN
WM. GALSTER

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Column separation of phosphatides, acyl carnitines and carnitine

The separation and isolation of carnitine derivatives has been reported[1–6]. Although individual carnitine derivatives may be isolated by these procedures, it is often desirable to separate and isolate the phosphatides, acyl carnitines and carnitine by a single procedure. This is particularly true if the starting material is insect tissue. In these organisms when radioactive carnitine is used as a substrate, the products

in vivo are phosphatidyl β-methylcholine and acyl carnitines[1,7] and *in vitro* acyl carnitines, free carnitine and an unknown[1]. Two column procedures are described which permit the separation of phosphatides, acyl carnitines and carnitine.

Experimental

Extraction of lipid-bound carnitine. The procedure used for the extraction and analysis of the compounds isolated from insects has been described previously[1,7].

Column chromatography. In previous experiments[8] column chromatography on alumina proved to be satisfactory for the quantitative fractionation and isolation of radioactive phosphatidyl choline synthesized from ^{14}C-phosphoryl choline or ^{14}C-choline. If a mixture of phosphatidyl choline, palmityl carnitine and carnitine is placed on a basic alumina (cationatropic, activity grade I, M. Woelm, Eschwege) column, the separation shown in Fig. 1 is obtained. The identity of the peaks was confirmed by thin-layer chromatography[1].

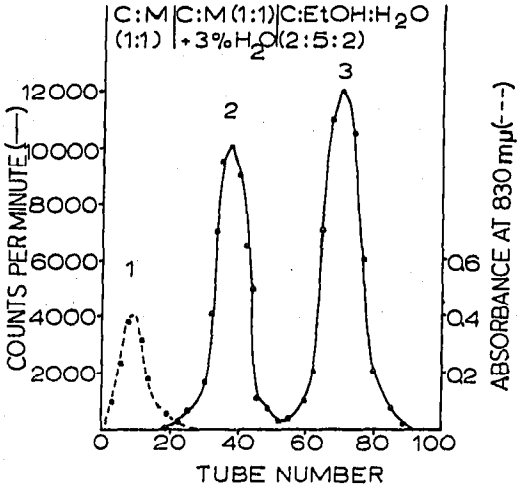

Fig. 1. Alumina separation of mixture of phosphatidyl choline, palmityl-^{14}C-carnitine and ^{14}C-carnitine. The palmityl-^{14}C-carnitine was synthesized by the method of BREMER[9]. 400 μg lipid P (phosphatidyl choline), palmityl-^{14}C-carnitine (150,000 c.p.m.) and ^{14}C-carnitine (80,000 c.p.m.) was applied on a 5 g, 1 × 10 cm alumina column. The column was then washed with 20 ml $CHCl_3$. The eluting solvent is shown on the figure. C = Chloroform; M = methanol. Five ml fractions were collected. Peaks: 1 = phosphatidyl choline; 2 = palmityl carnitine; 3 = carnitine.

When lipid-bound carnitine derivatives formed *in vitro* from either (methyl-^{14}C) or (carboxyl-^{14}C)-carnitine were fractionated on an alumina column, two peaks were found (Fig. 2b). The minor peak (peak 1) behaved similar to lecithin, but its identity is not yet determined[1]. The major peak (peak 2a) behaved similar to palmityl carnitine and was shown to be an acyl carnitine[1]. When a lipid extract from larvae reared on methyl-^{14}C-carnitine was fractionated on an alumina column (Fig. 2b), a major peak (Peak 1) was found as well as a minor peak (Peak 2). Peak 1 was identified as phosphatidyl-β-methylcholine and Peak 2 as an acyl carnitine[1]. The recovery of radioactivity was greater than 90 % on these columns.

In addition to separation on alumina, the lipid extracts were also separated on DEAE-cellulose (Matheson, Coleman and Bell) columns. Typical elution patterns are illustrated in Fig. 3. Peak 1 was shown to be phosphatidyl-β-methyl choline and

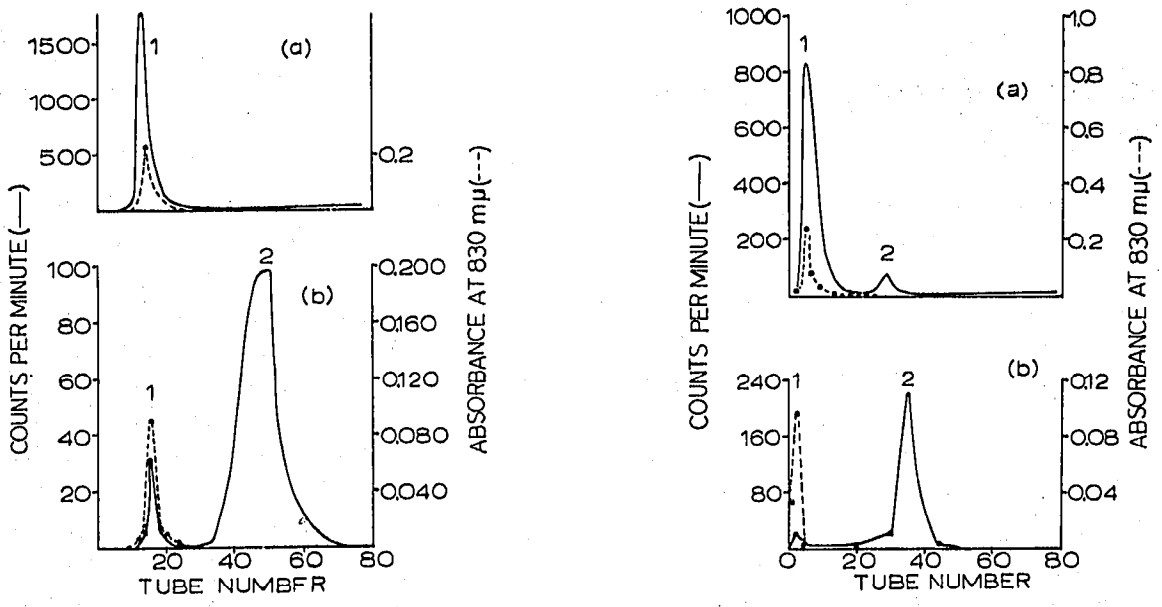

Fig. 2. Alumina separation of lipid extracts. (a) From larvae grown on a defined diet with (methyl-^{14}C)-carnitine as a choline substitute. (b) From fat body preparations of larvae incubated in the presence of (methyl-^{14}C)-carnitine. Conditions same as Fig. 1.

Fig. 3. DEAE cellulose chromatography of lipid extracts. (a) From sterile larvae grown on a defined diet with (methyl-^{14}C)-carnitine as a choline substitute. (b) From fat body preparation of larvae incubated in presence of (methyl-^{14}C)-carnitine. The extracts were applied to a 15 g, 2.2 × 25 cm DEAE-cellulose column prepared according to ROUSER *et al.*[10] The eluting solvent was $CHCl_3$–MeOH (9:1) and 5 ml fractions were collected.

Peak 2 an acyl carnitine[1]. Chromatography on DEAE-cellulose columns was qualitatively reproducible but the recovery of radioactivity was approximately 80 %. For the best recovery it is advisable to prepare new DEAE-columns for each fractionation.

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Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oreg. 97331 (U.S.A.)

C. MEZEI
R. W. NEWBURGH

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