Identification of an artifact in the hydrolysis of choline-containing phospholipids

In the course of a study of the phospholipids of the South African pilchard (*Sardina occllata* Jenyns), choline was liberated by hydrolysis of the material for 48 h in 6 *N* HCl at a temperature of 115° in a sealed tube. Choline was identified by chromatography on filter paper according to the method of LEVINE AND CHARGAFF[1,2]. Two blue spots were invariably obtained, one with the mobility of choline and the other a hitherto unidentified faster moving compound.

LEVINE AND CHARGAFF[1] hydrolyzed lecithin preparations and beef brain phosphatides with 6 *N* HCl at 100° for 48 h. These authors also described the appearance of two spots on paper chromatograms, when testing for choline with phosphomolybdic acid. They quoted an R_F value of 0.43 in a solvent system of *n*-butanol–diethylene glycol–water (4:1:1) for choline. However, for the unidentified substance in the above-mentioned solvent system, two R_F values were quoted: R_F = about 0.59 (purified lecithins) and R_F = 0.53 (beef brain phosphatides).

PHILLIPS[3] reported two compounds, which stained with phosphomolybdic acid, after hydrolysis of the lecithin and sphingomyelin fractions of human serum phospholipids with 6 *N* HCl at a temperature of 110° for 18 h. One compound, with an R_F value of 0.33 in the solvent system *n*-butanol–acetic acid–water (4:1:4, upper layer), was identified as choline; the other, with an R_F value of 0.41, remained unidentified.

LOVERN, OLLEY AND WATSON[4], in a study of the phospholipids of cod, hydrolyzed the phospholipids in a sealed tube with 2 *N* HCl at 120° for 24 h. These authors observed the presence of an unidentified compound occurring with choline in only one instance. It has been suggested that this unknown substance might be an unidentified nitrogenous base present in phospholipids[1,2]. The present paper shows that choline chloride when heated with aqueous HCl, gives rise to the formation of (2-chloroethyl)-trimethylammonium chloride. This compound is identical to the unidentified base obtained by hydrolysis of pilchard phospholipids with aqueous HCl.

Experimental

It was found in this laboratory that a solution of choline chloride in 6 *N* HCl heated for 48 h at 115° in a sealed tube and chromatographed on filter paper according to LEVINE AND CHARGAFF[1] produced two spots; one corresponding to choline and the other to the unidentified compound arising from the hydrolysis of pilchard phospholipids. Similarly, the hydrolytic procedures of LEVINE AND CHARGAFF[1], of PHILLIPS[3] and of LOVERN *et al.*[4] applied to choline chloride all gave rise to the formation of the unknown substance. This substance, therefore, appeared to be an artifact produced in the hydrolysis with HCl, and was subsequently identified as (2-chloroethyl)-trimethylammonium chloride. (2-Chloroethyl)-trimethylammonium chloride was synthesized according to TAKETOMO[5]*.

* Titratable chloride (argentometric): 22.36%; calculated for $C_5H_{13}NCl_2$ (158.07): 22.43%. No specific m.p. Decomposition at 249°. TAKETOMO[5] states decomposition at 243°, and TOLBERT[6] reports a m.p. of 300° with decomposition at 240–245°. The picrate of (2-chloroethyl)-trimethylammonium chloride had a m.p. of 212–214°. TOLBERT[6] found this to be 207°.

Chromatograms with choline chloride and (2-chloroethyl)-trimethylammonium chloride as marker compounds were run in different solvent systems, as shown in Table I, and no difference between the artifact produced on heating choline chloride with HCl, (2-chloroethyl)-trimethylammonium chloride and the unidentified compound in hydrolysates of pilchard phospholipids was observed. Mixtures of these compounds did not show any separation when chromatographed on filter paper.

TABLE I

R_F VALUES OF CHOLINE CHLORIDE AND (2-CHLOROETHYL)-TRIMETHYLAMMONIUM CHLORIDE IN DIFFERENT SOLVENT SYSTEMS ON WHATMAN NO. 1 PAPER

Solvent system	R_F (20–24°)				*Remarks*
	Choline chloride	*(2-Chloroethyl)-trimethyl-ammonium chloride*	*Artifact from choline chloride and HCl*	*Compound in hydrolysate of phospholipids*	
n-Butanol–diethylene glycol–water (4:1:1)	0.45	0.58	0.60	0.59	Descending
	0.40	0.55	0.54	—	Ascending
n-Butanol–acetic acid–water (4:1:4, upper layer)	0.31	0.39	—	0.39	Ascending
Ethanol–ammonia (95:5)*	0.48	0.56	0.58	—	Ascending
n-Butanol–pyridine (4:1) saturated with water	0.14	0.21	0.21	—	Ascending

* According to BREGOFF, ROBERTS AND DELWICHE[7].

An additional proof of the identity of the unknown substance was obtained by heating choline chloride in 6 *N* HCl at 115° for 168 h. Approximately 30 mg of the mixture was chromatographed on two sheets of filter paper (20 × 40 cm) with a descending solvent system *n*-butanol–diethylene glycol–water (4:1:1). The papers were dried in air and for 0.5 h at 100°. A guide strip was cut off the papers to locate the position of the faster moving compound, and the corresponding area containing this compound was cut out. The pieces of filter paper were first washed with ethyl ether to remove diethylene glycol, and thereafter extracted with 2 × 50 ml hot ethyl alcohol. After filtration the ethyl alcohol was removed *in vacuo*, and the remaining material treated with an aqueous solution of picric acid. The crystals obtained were filtered, washed with ethyl alcohol and dried over P_2O_5 *in vacuo*. The melting point of the crystals was 212–214°, and on mixing the material with the picrate of (2-chloroethyl)-trimethylammonium chloride no depression of melting point was observed.

The hydrolytic procedure of LOVERN *et al.*[4], *viz.* in 2 *N* HCl at 120° for 24 h, applied to choline chloride, produced only very small quantities of (2-chloroethyl)-trimethylammonium chloride, as judged by the intensity of the second spot on chromatograms. This method of hydrolysis did not produce (2-chloroethyl)-trimethylammonium chloride when applied to a pilchard phospholipid fraction, and was subsequently adopted in this laboratory. The hydrolysis according to LEVINE AND CHARGAFF[1] did not give as intense a second spot as the method of PHILLIPS[3]. Preference was, however, given to the method of LOVERN *et al.*[4].

Discussion

In the hydrolysis of lecithins[1], beef brain phosphatides[1,2], human serum phospholipids[3] and cod phospholipids[4] with aqueous HCl, an unidentified nitrogenous base, staining with phosphomolybdic acid has been found. This compound is in all probability (2-chloroethyl)-trimethylammonium chloride, and is formed by reaction of HCl with choline.

Hydrolysis of phospholipids with aqueous HCl therefore causes a loss of choline. The magnitude of the error introduced in quantitative choline determinations (in particular, LEVINE AND CHARGAFF'S planimetric method[1]) was not investigated, but it appears from the intensity of the two spots produced that hydrolysis according to LOVERN *et al.*[4] and LEVINE AND CHARGAFF[1] only gives rise to the formation of small quantities of (2-chloroethyl)-trimethylammonium chloride.

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Modification of gas chromatographic columns by addition of glycerol

In this laboratory the estimation of volatile solvents normally occluded in colloidal propellants was attempted by means of gas chromatography. These solvents include methanol, ethanol, ethyl ether, acetone, ethyl acetate and water. The total content is usually about 1 %. Preliminary experiments with synthetic mixtures showed that water caused a serious interference, masking some of the constituents due to extreme "tailing".

The problem of "tailing" due to water has been encountered by many workers. The solution has generally been to use a highly polar liquid phase which retains the water until the organic constituents have been separated.

Several polar compounds, including polyethylene and polypropylene glycols supported on firebrick, in varying percentages were used in this laboratory for the