Formation of a double salt of phosphatidylcholine and zinc chloride

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ZnCl₂ forms a 1:1 double salt with phosphatidylcholine. This compound resembles the long-known double salt of CdCl₂ and phosphatidylcholine except that the latter has the composition (CdCl₂)₃( phosphatidylcholine)₂. Treatment of phosphatidylcholine with a mixture of equimolar amounts of ZnCl₂ and CdCl₂ yields the ZnCl₂ double salt. The ZnCl₂ double salt can be obtained as an amorphous flocculent precipitate and as small spherules. These results are discussed in relation to the toxic action of cadmium salts on the mammalian testis and to the protection afforded by zinc salts. It is suggested that membrane phospholipids are essential components of specific sites for reversible binding of Zn²⁺ and Cd²⁺.

Boursnell et al. (1977, 1978) obtained evidence that the zinc in boar seminal plasma was immobilized by added phosphatidylcholine. Thus the transfer of zinc from seminal plasma to spermatozoa, which occurs when the semen is cooled to 4°C, was inhibited by ovophosphatidylcholine. This observation has led to the preparation of a zinc analogue of the well-known double salt of phosphatidylcholine and CdCl₂. The latter compound, discovered by Strecker (1968), became the basis of procedures for the purification of lecithin from animal sources [See, for example, Pangborn (1951)].

Materials and methods

Anhydrous granular ZnCl₂, CdCl₂, 2.5H₂O and lecithin ('egg grade I', as a solution in chloroform/methanol) were obtained from B.D.H. Chemicals, Poole, Dorset, U.K. Ethanol, methanol and peroxide-free diethyl ether were redistilled.

Determinations of all elements except C and H were carried out on solutions of phosphatidylcholine derivatives in 1M-acetic acid. Zinc and cadmium were assayed by atomic-absorption spectrophotometry. Nitrogen was determined by a micro-Kjeldahl method and chloride by means of Diagnostic Kit 830 from Sigma (London) Chemical Co. Total phosphorus was converted into orthophosphate (Umbreit et al., 1957) and this was determined by the procedure of Chen et al. (1956). The elemental composition of phosphatidylcholine was calculated on the assumption that its acyl content consisted of equimolar proportions of esterified stearic, palmitic, oleic and linoleic acids. Melting points were measured on a Koffler stage.

Phosphatidylcholine (250mg) was freed from solvents in a stream of N₂ at room temperature and dissolved in ethanol to a volume of 10ml to give a 33 mM solution. Centrifuging was at 1000g and all operations were at 0–4°C.

Products obtained by adding ZnCl₂ and CdCl₂ to equimolar amounts of lecithin

ZnCl₂ and CdCl₂ were made up as 33 mM solutions in 95% (v/v) methanol.

Experiment 1. To 3 ml of lecithin solution was added 3 ml of ZnCl₂ solution. A bulky amorphous precipitate was centrifuged down 1 h later, washed with 95% methanol and freed from solvents in a stream of N₂ (fraction 1a). The supernatant and washed fluids were stored overnight when small translucent spherules appeared in both. The spherules were firmly attached to the glass walls of the containers and were washed by decantation with 95% methanol, dried and combined (fraction 1s) (m.p. 177–179°C; found: C, 56.6; H, 9.0; N, 1.7; P, 3.6; Zn, 7.9; Cl, 8.3; C₄₅H₉₃O₈₇NP·ZnCl₂ requires C, 56.8; H, 9.2; N, 1.5; P, 3.4; Zn, 7.2; Cl, 7.8%). Thus the spherular fraction was an equimolar double salt of phosphatidylcholine and ZnCl₂. The spherules were not birefringent under a polarizing microscope.

Experiment 2. This differed from Expt. 1 in that the ZnCl₂ was dissolved in diethyl ether. An amor-
phous precipitate appeared slowly and was collected the next day (fraction 2a). This was analytically identical with fractions 1a and 1s (Table 1). No spherular fraction was obtained.

*Experiment 3.* Repeating Exp. 1, but with CdCl₂ replacing ZnCl₂, we repeatedly obtained amorphous and spherular products. However, the molar ratio of CdCl₂/phosphatidylcholine varied in a random manner within the limits 1.0–1.4.

*Experiment 4.* To 3 ml of lecithin solution was added a mixture of 3 ml each of ZnCl₂ and CdCl₂ solutions. No amorphous material appeared but by the next day a spherule fraction (4s) had formed. It proved to be the ZnCl₂ compound. Cadmium was not detected in a 0.6% (w/v) solution of the product. This means that the atomic ratio Cd/Zn was less than 0.001. Tests with standard solutions containing high ratios of ZnCl₂/CdCl₂ gave no evidence that excess of zinc suppressed the spectrophotometer signal given by cadmium.

**Table 1. Composition of double-salt fractions obtained from phosphatidylcholine and ZnCl₂**

<table>
<thead>
<tr>
<th>Fraction number</th>
<th>Yield (%)</th>
<th>N</th>
<th>P</th>
<th>Zn</th>
<th>Cl⁻</th>
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<tbody>
<tr>
<td>1a</td>
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<td>0.99</td>
<td>1.10</td>
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<tr>
<td>1s</td>
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<td>1.03</td>
<td>1.00</td>
<td>1.96</td>
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<td>0.97</td>
<td>1.01</td>
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<tr>
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</tr>
<tr>
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<td>1.02</td>
<td>1.01</td>
<td>1.63</td>
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<td></td>
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<td>2.05</td>
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</table>

**Results and discussion**

**Comparison of ZnCl₂ and CdCl₂ double salts of phosphatidylcholine**

Thudichum (1844) concluded that CdCl₂ combined with phosphatidylcholine in equimolar ratio, but later authors (e.g. Erlandsen, 1907; Eppler, 1913) found ratios nearer to 1.5 than 1.0. Thudichum also obtained CdCl₂-phosphatidylcholine as spherules, which he assumed to be crystal aggregates. However, lack of birefringence of spherular ZnCl₂-phosphatidylcholine argues against its crystallinity. Phospholipids other than phosphatidylcholine also combine with CdCl₂. Though such double salts are more soluble than the phosphatidylcholine derivative they tend to be adsorbed by precipitates of the latter. Lack of agreement on the composition of CdCl₂-phosphatidylcholine during the 80 years after its discovery (Wittcoff, 1951) is therefore not surprising. The problem was resolved when Baer & Kates (1950) treated three synthetic lecithins with 2.5 molar proportions of CdCl₂ and obtained three double salts with the composition (phosphatidylcholine)₂(CdCl₂)₃. In Expt. 3 the products were presumably co-precipitates of the 2:3 double salt and phosphatidylcholine.

From equimolar amounts of ZnCl₂ and phosphatidylcholine we invariably obtained a double salt containing the two components in a 1:1 ratio (Table 1). When ZnCl₂ was in excess spherular fractions consisted of the same double salt, but some amorphous fractions contained excess of zinc over phosphatidylcholine though no excess of chloride. This can be explained by the very high affinity of ZnCl₂ for water and its resulting hydrolysis. Hot methanolic solutions of either form of the ZnCl₂ derivative deposit spherules when slowly cooled. The appearance of insoluble ZnCl₂-phosphatidylcholine in Expt. 4 suggests that phosphatidylcholine has a higher affinity for ZnCl₂ than for CdCl₂. Thus in absence of the ZnCl₂ the CdCl₂ double salt would have been deposited. All results in Table 1 were obtained with freshly prepared phosphatidylcholine solutions. Stored solutions tended to give gummy non-stoichiometric products.

It is interesting that CdCl₂ and glycerophosphocholine yield a 3:2 double salt that dissociates to a 1:1 salt on recrystallization from aqueous ethanol (Baer & Kates, 1948).

**Competition between Zn²⁺ and Cd²⁺ in vivo: phospholipids as possible mediators**

Pařízek (1957, 1960) showed that subcutaneous injection of rats with 0.04 mmol of CdCl₂/kg body wt. caused regressive changes in the spermatogenic epithelium within a few hours. At 24–48 h all testicular tissue was severely necrosed. Simultaneous injection of an excess of zinc acetate gave complete...
protection against this cadmium necrosis. During a histochemical study of rat testis Timm & Schulz (1966) found high concentrations of membrane-bound zinc in the germ cells and interstitial cells. But when rats were injected with CdCl₂ the bound zinc of the Leydig cells and Sertoli cells was rapidly replaced by bound cadmium.

In an attempt to correlate the information in the present paper we put forward the hypothesis that some membrane phospholipids can form double salts incorporating Zn²⁺. Such phospholipids would be essential components of membrane binding sites (and possibly of translocation systems) for this metal. The same sites would also have affinity for cadmium and perhaps for other heavy-metal ions.

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References