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KINETICS OF PHOSPHOLIPID EXTRACTION FROM *COXIELLA BURNETII*

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Summary. — Kinetics of phospholipid extraction from purified suspensions of *Coxiella burnetii* in phase I by various chloroform—methanol mixtures at various temperatures were evaluated based on the amount of phosphorus extracted. Extraction by a boiling (53 °C) 2 : 1 chloroform : methanol mixture proved to be the most efficient.

Key words: Coxiella burnetii; phospholipids; extraction; chloroform; methanol

The cell wall of *Coxiella burnetii* resembles that of Gram-negative bacteria. Its outer membrane is bound by lipoproteins to a peptidoglycan layer and its main components are a lipopolysaccharide, a protein and a phospholipid (Lugtenberg and Vanalphen, 1983). The phospholipids are localized mainly on the inner side of the outer membrane, this covert position preventing them from being involved in antibody production. In chlamydiae, such antigenic determinants can be uncovered upon hydrolysis (Brade *et al.*, 1986). Removal of phospholipids leads to changes in the properties of *C. burnetii* cells (Williams and Cantrell, 1982). After lipid extraction by a chloroform-methanol (C+M) mixture, the killed *C. burnetii* cells caused no hepatosplenomegaly in mice but retained their protective activities (Kazár *et al.*, 1983).

C. burnetii cells extracted by a C + M mixture were used as a vaccine against Q fever which induced no immunopathological reactions in vaccinees (Williams *et al.*, 1986).

In the present study we attempted to determine extraction conditions that would ensure the highest yield of phospholipids, paying attention to the optimal ratio of the solvents and to optimal extraction temperature and time.

The Nine Mile strain of *C. burnetii* in phase I was used. Purified lyophilized cells were extracted by 2 : 1 and 4 : 1 C+M mixtures and by chloroform and methanol each alone at various temperatures (see Fig. 1). The experiments were done on 30 mg *C. burnetii* in 30 ml of the given solvent. Two-ml samples were taken at time 0 (immediately after reaching the extraction temperature) and then after 30 min and 1, 2, 3, 4, 5, 6 and 7 hr and filtered through Whatman No. 1 filter paper. Phosphorus was determined spectrophotometrically at 820 nm in mineralized samples (Lowry *et al.*, 1954), following evaporation of the solvent in a stream of nitrogen.

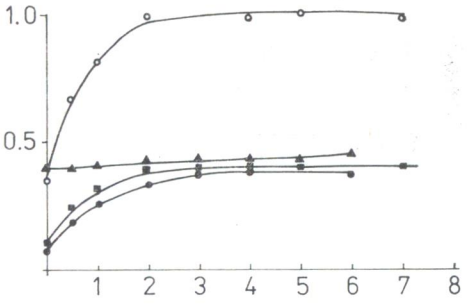

Fig. 1.
Phospholipid extraction from *C. burnetii* by various solvents at various temperatures
Abscissa: P %; ordinate: time (hr) of extraction
○ Boiling 2 : 1 C+M mixture (53 °C)
■ 2 : 1 C+M mixture at 37 °C
● Boiling 4 : 1 C+M mixture (54 °C)
▲ Boiling methanol (65 °C)

The extraction kinetics of phospolipids from *C. burnetii* under the conditions used are illustrated in Fig. 1. In each experimental series, the maximal amount of phosphorus was extracted after 2—3 hr. Only traces of phosphorus were extracted by chloroform alone (not shown).

Extraction by methanol at 65 °C, a 2 : 1 C+M mixture at 37 °C and by boling 4 : 1 C+M mixture (at 54 °C) yielded similar results. The greatest amount of phosphorus was found in samples extracted by a boiling 2 : 1 C+M mixture (approx. at 53 °C).

To determine residual phospholipids in the suspension after extraction with a boiling 2 : 1 C+M mixture, extraction with a fresh C+M mixture was repeated for 2 hr. The resulting sample was treated as after the first extraction, the amount of phosphorus was determined and the procedure was repeated once again. The results presented in Table 1 indicate that no phosphorus was detected in the third extract.

We found that under all conditions used in the present study 2—3 hr of extraction were sufficient to reach a state of equilibrium. Additional phospholipids could be extracted only with fresh solvent. Extraction with a boiling 2 : 1 C+M mixture yielded the best results. C+M mixtures have become widely used for extraction of phospholipids from biological materials (Marinetti, 1962; Nichols *et al.*, 1985; Kolarovic *et al.*, 1986). Methanol and ether and C+M mixtures were also used long ago (Folch *et al.*, 1957; Marinetti,

Table 1. Phosphorus contents of extracts from C. burnetii after repeated extractions with fresh 2 : 1 C+M mixtures at 53 °C

Extraction	Extraction time (hr)	% P in the extract
1st	2—8	1
2nd	2	0.85
3rd	2	0

P content of *C. burnetii*: 2. 58%

1962). Usually, long extraction times were used which, however, are not as efficient as several times repeated short extractions.

Increased temperature during extraction could lead to lipid desctruction, especially oxidation. Thus the effects of temperature should be subjected to a detailed study.

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