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Authors: P. Fiset and R. Silberman, School of Medicine,  
University of Maryland, Baltimore, Maryland

Title: Purification of rickettsia by means of ion exchangers  
(Purification des rickettsies au moyen d'échangeurs  
d'ions).

Journal: Arch. Inst. Pasteur de Tunis 43: 231-236 (1966).

September 1969

Numerous methods for the extraction and purification of rickettsia from the vitelline sac of chick embryos have been described in the past. In general, these methods employ several cycles of differential centrifugation, extraction with ether (2,9), infusorial earth, such as celite for the purpose of adsorbing and sedimenting cellular debris (3,10), or repeated washings with hypertonic saline solutions (6). Quite recently, sucrose density gradient centrifugation has been shown to be very useful (7). When these are employed together, these differential methods result in preparations of high purity. Depending on the method, various limitations are encountered. For example, extraction with ether or with hypertonic solutions inactivates the rickettsia and, in addition, the ether liberates their "soluble" antigens. Celite offers an efficient procedure for obtaining viable and relatively pure preparations. However, the electron microscopic examination of such preparations reveals the presence of cellular debris (4) and, in addition, one finds at least in the case of the psittacosis agent, that a high percentage of the organisms are lost during adsorption (8). Centrifugation in a sucrose gradient does not lend itself readily to the production of antigen on a large scale.

Hoyer and collaborators, 1958 (4), demonstrated that it is possible to obtain preparations of viruses and rickettsia of high purity by means of chromatography on columns of DEAE-cellulose and ECTEOLA-cellulose. In the case of rickettsia, adsorption takes place in 0.02 M phosphate buffer. Later, elution can be carried out using a solution of 0.02 M phosphate buffer in

0.1 M NaCl. These preparations are very pure, but the total yield by this method is quite small probably as a result of the mechanical retention of the rickettsia in the cellulose column.

The modification of this method described by Silberman and Fiset, 1963 (8), for the purification of the psittacosis agent, has been adapted to the purification of rickettsia of the exanthematic group and of Q fever. In principle, this method is very simple. In place of using a chromatographic column, the cellulose is added to a crude suspension of the rickettsia. Under appropriate conditions of pH and molarity, the cellular debris is adsorbed onto the cellulose leaving the rickettsia free in the supernatant after light centrifugation,

#### MATERIALS

##### Solutions:

1. Sorensen buffer: 0.013 M phosphate and 0.15 M NaCl at pH 7.0.
2. 10 % solution of sucrose in Sorensen buffer, pH 7.0.

These solutions are filtered through Millipore filters (0.45  $\mu$  porosity) immediately before use and 0.2 % formalin is added.

##### Rickettsial strains

1. R. prowasekii: The Breinl strain was used. In our laboratory, this strain has undergone 152 consecutive passages in the vitelline sac of chick embryos. The previous history is not known to us.
2. R. mooseri: We have employed the Wilmington strain. In our laboratory, it has undergone 42 successive passages in chick

embryos. The previous history of the strain is not known to us.

3. Coxiella burneti:

a. Nine Mile Strain, phase 1: this strain was obtained from the Rocky Mountain Laboratory, Hamilton, Montana. It has undergone 307 consecutive passages in guinea pigs and 3 passages in chick embryos.

b. Nine Mile Strain, phase 2: This strain was also obtained from the Rocky Mountain Laboratory. It has undergone an unknown number of passages in guinea pigs and 90 successive passages in chick embryos.

Ion Exchangers

The anion exchangers, DEAE (Cellex-D with an exchange capacity of 0.86 meq/gram) and ECTEOLA (Cellex-E, with an exchange capacity of 0.84 meq/gram) were used (Bio-Rad Products, distributed by Calbiochem, Los Angeles, California). These two products have comparable performances and can be sterilized by autoclaving with danger of inactivation.

METHODS

Six-day old chick embryos were inoculated by the vitelline sac method with 0.2 ml of a suspension of rickettsia at a dose capable of killing 50 % of the embryos in 7 to 8 days. The vitelline sacs were harvested and diluted to a concentration of 20 % in formalized phosphate buffer. After homogenization, the suspension was held in the cold for 24 to 48 hours in order to permit inactivation of the rickettsia. If one desires viable organisms, then the formalin can be excluded. In the case of subsequent manipulations, aseptic techniques may be used.

The material is centrifuged at top speed (16,000 x g) for one hour in a Sorvall type GSA angle rotar or at 29,000 x g for 30 minutes in a Sorvall type SS34 angle rotor. The supernatants are decanted off and the deposits are resuspended in the phosphate buffer-sucrose solution and again centrifuged at top speed. The washings with the sucrose solution is repeated until one obtains an almost clear supernatant. Four to six washings are usually sufficient for this purposed.

After this preliminary removal of gross impurities, the residue is resuspended in phosphate buffer and restored to the original volume. Cellulose is added on the basis of one gram per 100 ml of suspension. After homogenization, the mixture is centrifuged at low speed (1,000 rpm) for 15 minutes in an horizontal rotor. The supernatants are carefully decanted off. One can recover the rickettsia which are dispersed in the cellulose pellet by a simple extraction with phosphate buffer followed by low-speed centrifugation. This supernatant is added to the first one and they are both centrifuged at top speed. The sediments are resuspended in phosphate buffer to a volume one-tenth that of the original. It is often necessary at this point to centrifuge at low speed in order to eliminate completely cellulose fibers.

At this point, the preparations are ready to be employed. They can be standardized by serological methods using reference immune sera, or, as we prefer, with regards to the absolute concentration of rickettsia. In our laboratory, these preparations are standardized so that 1 ml of suspension contains 1 mg (dry weight) of rickettsia.

RESULTS

The following results have been obtained with the rickettsial strains described above:

1. R. prowasekii:

160 gm of vitelline sacs.

total yield: 60 mg of rickettsia, or 0.27 mg/gm

2. R. mooseri:

100 gm of vitelline sacs.

total yield: 130 mg of rickettsia, or 1.3 mg/gm.

3. C. burneti, phase 1:

Lot 1: 355 gm of vitelline sacs.

620 mg of rickettsia, or 1.7 mg/gm.

Lot 2: 376 gm of vitelline sacs.

765 mg of rickettsia or 2.26 mg/gm.

4. C. burneti, phase 2:

393 gm of vitelline sacs.

total yield: 100 mg of rickettsia, or 0.49 mg/gm.

DISCUSSION

DEAE and ECTEOLA ion exchangers allow one to obtain rickettsial suspensions of high purity. Since this method of extraction is very mild, it is possible to obtain suspensions of viable organisms which are probably intact from an antigenic point of view.

We have not been able to explain satisfactorily the differences in yield that we have observed with the rickettsial strains studied. It is quite possible that these differences are the result of seasonal differences in the quality of the chick embryos. Since other authors have observed that with C. burneti in phase 2, one



consistently obtains far less organisms from chick embryos than with C. burneti in phase 1 (1,5), it is possible that in the case of the rickettsia which are well adapted to the chick embryo, a much smaller number of organisms are necessary to induce death of the egg. This phenomenon merits a more systematic study.

At the end of purification, if the suspensions of R. prowasekii and R. mooseri are extracted with ether, the "soluble" antigen of the group is liberated. These preparations of soluble antigen are completely clear and exhibit excellent activity in the complement inhibition test. The rickettsia obtained after this treatment demonstrated a marked specificity particularly in the agglutination reaction.

#### SUMMARY

A modification of the method of Silberman and Fiset (8) has been described for the extraction and purification of rickettsiae from yolk sacs of chick embryos using anion exchange cellulose, BEAE or ECTEOLA. The method is simple and gentle and yields highly purified preparations.

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