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Capponi, M. and Keldar, I. : Cultivation of Coxiella burneti on KB cells and preservation of its pathogenicity.

As we saw in an earlier work (2), R. burneti is one of the only rickettsias that does not appear greatly modified by its culture in vitro be it on cells of rootstock or on cells of first explantation or on a gelose in the tissues.

One knows, actually, how much each rickettsia is sensible to a change of surroundings or medium, that it consists of cultivating it on the embryonate egg, on the animal or on the cells in vitro. Examples would be easy to choose: such a method of inoculation that suits R. prowazeki is not favorable to R. orientalis or to R. conori; those cells which suit very well R. burneti are bad support for the other pathogenic rickettsias.

Certain authors, while proving the measurable growth of R. burneti in vitro, were able to notice a light weakening of its pathogenic power, however. (6). This is what we wanted to verify in the course of some passages on the KB cells, controlling each time the guinea pigs, the embryonate eggs, and the KB cells, the conservation of the virulence of the rickettic rootstock.

The passages were made on the third day from the culture in vitro at 36° (this temperature being better suited than the temperature of 37°). Actually, one discovers a visible augmentation of the number of rickettsias after the second day, which can increase the chances of conservation of the pathogenic power by avoiding to take elements too transformed or old. However, the passages made in the earlier attempts had been on the eighth day or ninth day, without the results having been sensibly different (2).

The inoculum was prepared from four vitellin membranes, rich in rickettsias of rootstock C. 9 of R. burneti, membranes kept for 15 days at -30°, thawed out, washed in physiologically

sterile water, centrifuged after grinding in the grinder with ball bearings of steel, and suspended in 10 ml. of Hanks liquid. Some KB cells freshly trypsined, placed at the rate of 50. 000 cells to the mililiter, in a medium with hydrolysat of casein at 10% of colt serum, were mixed with the inoculum at the rate of 0 ml. 4 of this one for 2 ml. of medium by Leighton's tube, after a preliminary agitation of the mixture at a very slow speed. Control experiments were made without the inoculum, others with an inoculum composed of normal vitellin membranes, washed, ground and centrifuged, in order to appreciate the poisonousness of the egg on the KB cells. Finally, a control was made at the departure, both in order to appreciate the sterility of the inoculum and its pathogenic powers: the absence of bacteria and the death of the innoculated eggs from the 8th to the 11th day with numerous rickettsias were thus able to be proved.

A daily sample and a daily examination with coloration of at least a lamella were made at the same time for the innoculated tubes and the control tubes. The rickettsias, first of all rare in the cytoplasms, localized themselves next in the vesicles, close to the nuclei, as it had already been observed earlier. But it does not seem that the mixture of cells freshly trypsined to the inoculum is more favorable to the fixation of rickettsias on the cells than the procedure that we habitually employ and which consists of inoculating, while changing the medium, the cells already fixed since three or four days.

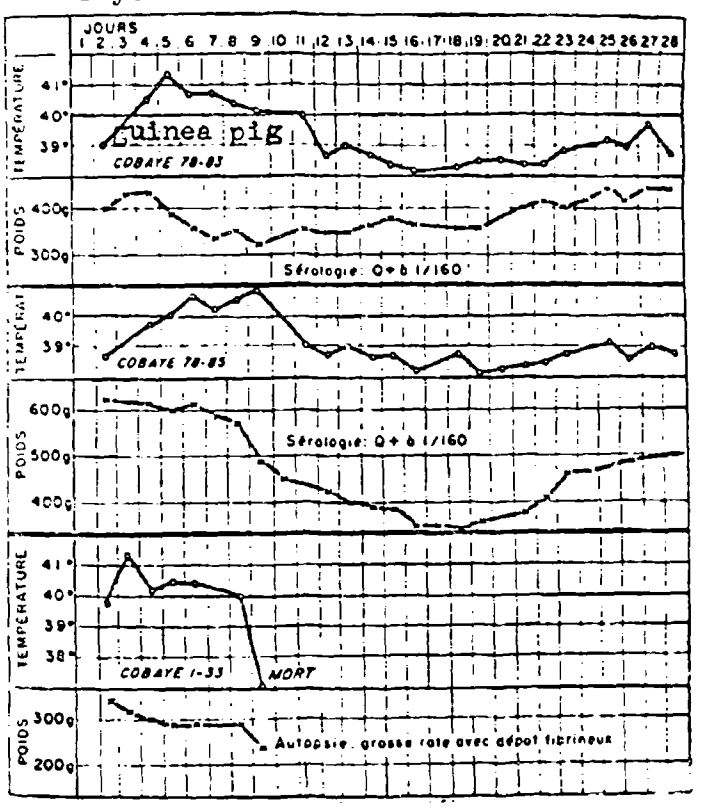
On the third day of the culture in the oven, a guinea pig was inoculated by the intra-peritoneal method with 2 ml. of the medium of a tube, a second guinea pig with 2 ml. of the concentrated suspension of cells of the same tube detached by scraping with a lancet; finally a control guinea pig was inoculated with a suspension of vitelline membranes not infected. At the same time, incubated eggs of seven days were inoculated by the intra-vitelline method with the same suspensions and the same inoculum mixed with freshly trypsined KB cells and were put in Leighton tubes. At each passage, the guinea pigs were followed one month, the eggs right until the death of the embryo with a daily mirage and the tubes during 10 days with some samples and some daily colorations.

Four passages were thus made and permitted the following remarks. The guinea pigs had a sickness that one can consider as typical in the first days, both with the cellular suspension as well as with the medium contained in the inoculated tubes. Their temperature rose brusquely on the fourth or fifth day of their experimental sickness, to 41o 4; it lowered then in order to normalize itself towards the tenth day approximately. The curve of weight had an inverse curve: the weight loss was sometimes considerable and went close to 300 g. for one of the guinea pigs: it returned to normal or more or less rose again

after the thermic fall. Of course, these animals were isolated in a room reserved for these inoculations, under a constant irradiation by U. V. rays; and a special thermometer was used and reserved for these guinea pigs with an extremely careful purifying between each temperature taking. These are indispensable precautions with such a rootstock as the R. burneti.

We have wanted to compare on rabbits and a guinea pig used as a control piece the conservation of pathological power of this rootstock, since if originally it killed the guinea pig and the rabbit, it could have lost its virulence. A first rabbit lost considerable weight, but resisted: on the other hand a second rabbit died around the tenth day and the autopsy showed a rate very augmented of volume, friable and redressed with a fibrinous coating. The guinea pig whose thermic curve is joined here lost considerable weight himself, and after having presented a curve with two bells, one at 41° 3, the other at 40° 4, died on the ninth day with a recovered rate of an analagous coating.

Days



mort (dead)
poids (weight)

Autopsy--gross rate with fibrinous deposit

(The following three notes correspond to the preceding diagram):

1. Temperature curve and weight curve of guinea pig 78-83 inoculated by intra-peritoneal method with 2 ml. of tubes 51-52 medium on the 3rd day of the culture.

2. Same curves of guinea pig 78-85 inoculated with the cells from tubes 51-52 on the 3rd day of the culture.

3. Test guinea pig 1-33 inoculated with 2 ml. of broyat of vitellin membrane infected by the C. $_9$ rootstock of R. burneti: same curves.

The guinea pigs that had received intra-peritoneal injections of infected cells or of a corresponding medium had on the thirtieth day and even from the twentieth day some antibodies against R. burneti up to a rate of 1/160, which, in micro-agglutination and with this rootstock, is an elevated rate. These antibodies decreased eventually and none of the guinea pigs died, which can make one think that there is important conservation, but not total, of pathogenic power.

For the embryonated eggs, inoculated at the same time as the guinea pigs, the embyros died from the eighth to the eleventh day, be it with the infected medium alone or with the cells in suspension, except at the fourth passage where the embryos died on the thirteenth day, thus with a small retardation, but with some vitelline membranes very rich in rickettsias. This is somewhat comparable to that which we had already obtained with our earlier passages (2) on some cells of chicken embryos or on the KB cells or on a gelose with tissues.

Along with these diverse examinations, researches of rickettsias were also practiced by the indirect method of Coons and the colorations of cells infected by the orange-colored acridine. But these examinations with the U. V. rays simply confirmed the presence of rickettsias in the culture tubes, without bringing any better information than the routine examination at May-Grünwald-Giemsa.

In conclusion, as certain authors had declared (5, 6), one could envisage the maintenance of rootstock of R. burneti on some rootstock cells as on certain cells of first explantation. Actually, if one proves a small weakening of the pathogenic power (since the guinea pigs for example, despite a severe experimental sickness, do not die) one can find again very quickly on the egg or the animal the initial virulence, as there is no mutation on this artificial medium, but a simple transitory adaptation. Thus, in a laboratory that would not have use of an incubator or of a sufficient breeding, the maintenance of the stock-root of R. burneti in vitro would be

envisageable. However, it is not useless to say that the method of Barykine and of Cox of culture on the vitelline membrane method that goes back to 1938, rests, when one can practice it, the best mode of maintenance of the rootstock and the best mode of culture, if one is careful to divide from time to time the lyophilized rootstocks in case of failing always possible in incubated eggs, and if one disposes it evidentally of a congealment permitting to conserve at -30° the removed infected membranes.

In resume a rootstock of R. burneti can keep a very great part of its pathogenic power after a prolonged culture in vitro.

Summary

R. burneti ~~can~~ keep its pathogenicity after many weeks of culture on KB cells or on ~~other~~ cells like chick fibroblasts.

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