SURVIVAL OF RICKETTSIA PROWAZEKI IN DIFFERENT DILUENTS

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At the present time very little information is available about the survival of typhus rickettsiae in fluid media. Data on this point are of value, however, in the solution of a number of problems which arise. The data presented in this report were not collected with the idea of making a detailed investigation of various media but simply to assess the value of media in common use for work in experimental typhus.

Most of the data available in the literature are of very limited value in a comparative way. Topping (1940) has reported that skim milk, used as the suspending medium, prolonged the survival of rickettsiae of several species when preserved in the dried state. He also has recommended its use in work on the toxin (confidential report). Other authors have reported that rickettsiae will survive for varied periods of time in different media. (Laigret and Durand, 1933; Medical Research Council, 1930.)

EXPERIMENTAL

Methods. Eastern cotton rats (*Sigmodon hispidus hispidus*) of between 2 and 4 weeks of age were inoculated intraperitoneally with 0.5 ml of 2×10^{-1} dilutional passage cotton rat liver suspension, containing from 200 to 700 fifty per cent mortality doses, as determined by intracardial titration. Four to 6 animals were employed to obtain the suspensions used in each experiment. Previous experiments had determined that about 72 hours after inoculation was the optimum time for obtaining the maximum yield of rickettsiae with this dose. At this time the rats were killed with ether. The skin was removed from the ventral surface of the abdomen and thorax, and a small incision was made through the peritoneal wall over the lower edge of the liver in such a manner that when the lower end of the incision was clasped with a forceps and gently elevated, the peritoneal cavity became a sac which could be easily washed and scraped. A test tube containing 10 ml of distilled water was cooled in an ice bath during the time of the washing. Small portions of the water (1 to 1.5 ml) were removed and placed in the peritoneal cavity. While still containing the water, the cavity was scraped, after which the water was removed and mixed with the original cooled water. This process was repeated 2 or 3 times for each rat. The same wash water was used for each of the succeeding rats. (If a capillary pipette is employed to add and remove the fluid, the capillary portion should not be drawn too thin, as the large amount of fibrin present readily occludes it. A thin glass rod that has had the end roughened on an emery wheel is useful as a scraping instrument and has been found easier to work with than a curette.) Following the washing, the suspension

was centrifuged at 1500 to 2000 r.p.m. for 15 minutes, which removed most of the fibrin and resulted in a supernatant containing relatively few cells. Smears of this fluid revealed very large numbers of rickettsiae. One ml of the supernatant was added to 5 ml of the medium under test, and the test suspensions were allowed to stand at room temperature (26 to 28 C). At various intervals after the rickettsiae were added to the medium, 1 ml portions were removed, and each cotton rat of groups of 4 was inoculated with 0.2 ml intracardially. The rats were observed for the next 8 days, the number of dead animals and the day of death being noted in each group.[1] The Breinl strain and another strain of the epidemic type, originally isolated in Bogotá, Colombia, were used in these experiments.

The following media have been tested by the method described above:

1. Distilled water.
2. Physiological saline.
3. Sterile skim milk.
4. Thioglycolate medium.
5. Plain broth.
6. Allantoic fluid from 10-day-old chick embryos.
7. Chick juice prepared by homogenizing, in a Waring blendor, an equal weight of 8-day-old chick embryos and distilled water, followed by centrifugation at 10,240 r.p.m. for 1 hour in an angle centrifuge. The supernatant was used in the tests.
8. Tyrode's solution, as well as Tyrode's solution variously modified by the addition of serum from rabbits, guinea pigs, or cotton rats to a concentration of 10 per cent.
9. Glutathione in 1 per cent concentration in saline, distilled water, Tyrode's solution, or serum–Tyrode's solution.
10. 20 per cent normal yolk sac in plain broth.

Results. In the majority of the tests approximately 4 to 16 fifty per cent mortality doses were contained in 0.2 ml of the inoculum after dilution in the test medium. On the basis of these results the various media can be divided into 3 groups: those media in which rickettsiae survive poorly for a period of 6 hours; those in which the organisms survive well for 6 hours but do not survive well for 24 hours; and those media in which the organisms survive well for a period greater than 24 hours. Distilled water and saline were found to be the most deleterious media; while chick juice, yolk sac, and skim milk were the most innocuous media. The other media comprised the intermediate group. Glutathione in 1 per cent concentration had no beneficial effect. The solutions of this compound in distilled water and saline behaved as did distilled water and saline alone, and the solution in the Tyrode medium behaved as did this medium alone.

Table I shows the result of a test which included 2 of the best media, 2 of intermediate effect, and 1 with the most harmful effect on the viability of rickettsiae. The Breinl strain was used and the technique was that described under *Methods*.

As can be seen, skim milk is the best medium for preserving the viability of

[1] Aseptic technique must be used throughout, and with care contamination is unusual.

rickettsiae; the 20 per cent suspension of normal yolk sac is better than the remaining media; 10 per cent guinea pig serum–Tyrode's solution, and ordinary Tyrode's solution are of equal efficiency; and distilled water is the poorest of all.

In all the experiments skim milk was used as one of the controls, while distilled water was generally used as the other. By this means it was possible to obtain some degree of comparison between individual tests.

TABLE I

Influence of various media on the survival of Rickettsia prowazeki

MEDIUM	TIME OF SAMPLING			
	2 hrs	6 hrs	24 hrs	48 hrs
Skim milk	0/4*	0/4	0/4	2/4
Plain broth	0/4	0/4	4/4	4/4
Distilled water	2/4	3/4	4/4	4/4
20 per cent normal yolk sac	0/4	0/4	1/4	4/4
Tyrode's solution	0/4	0/4	4/4	4/4
Guinea pig serum—Tyrode's solution	0/4	0/4	4/4	4/4

* The numerator represents the number of cotton rats that survived the infection; the denominator, the number of rats used in the test.

TABLE II

Comparison of the effect of different media on the infectivity end point of a suspension of cotton rat liver

IMMUNIZING DOSE	MEDIA USED FOR DILUTIONS		
	Saline	Broth	Skin milk
10^{-3}	5/5*		
10^{-4}	4/5		
10^{-5}	3/5		
10^{-6}	0/5	3/4	4/4
10^{-7}	0/4	5/5	4/4
10^{-8}		2/3	2/4
10^{-9}		2/5	1/4
10^{-10}		0/5	0/5

* The numerator represents the number of cotton rats that survived the infection; the denominator, the number of rats used in the test.

In order to check the validity of these results another experiment was performed, again using the Breinl strain. A homogenized suspension of infected cotton rat liver, in broth, was titrated in tenfold dilutions in 3 different media in order to determine the infectivity end point. The dilutions were done at 0 C, and only an hour elapsed between the thawing of the ampoule and the time when the inoculations were finished. The immunizing dose was given intraperitoneally. Three weeks after the immunizing dose the animals were challenged with the same liver suspension, which was part of a large pool stored at −76 C, by inoculating 7 fifty per cent mortality doses intracardially. Control animals of the

same age and weight were included to determine the number of 50 per cent mortality doses received by the immunized animals. Table II presents the result of the challenge dose given to the immunized animals.

The result of this experiment verifies the data obtained by the other method. The difference in titer between distilled water and broth or skim milk used as diluting media is about a thousandfold. There is essentially no difference between the titers in skim milk and broth, probably because of the lower temperature and the short time interval between the preparation of the dilutions and their inoculation.

DISCUSSION

Occasionally this laboratory has been faced with problems concerning the survival of rickettsiae in the suspending media, particularly in regard to protection tests and to determining the infectivity of suspensions. It is also obvious that the more sensitive the laboratory methods, the more valuable the information.

Singer (1941) reported the beneficial effect of 1 per cent glutathione in his media when studying the growth of rickettsiae in artificial media. Glutathione, under the experimental conditions described in the present report, did not show any such effect.

The method described lends itself to more accurate quantitative work if the suspensions are titrated each time and samples withdrawn at more frequent intervals. Little manipulation is required in order to obtain suspensions with few cells so that possibilities of some multiplication do not have to be seriously considered. In addition to the advantages of being able to work with both murine and epidemic strains, it is also possible to prepare the stock suspension in a poor medium such as distilled water or saline, thereby overcoming the necessity of having to take into consideration the effect of dilution of a good medium in other media of varied effect.

SUMMARY

A method is described for the determination of the influence of various fluid media on the survival of rickettsiae, and the results of tests on some of them are reported. *Rickettsia prowazeki* was found to survive longest in sterile skim milk.

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