THE GROWTH OF THE REITER STRAIN OF TREPONEMA PALLIDUM IN THE CHICK EMBRYO

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Attempts to cultivate the virulent *Treponema pallidum* in the developing hen's egg have yielded no conclusive proof that reproduction has occurred in the embryo, but indicate rather that the spirochetes have merely survived for the periods involved (Calloway and Sharp, 1941). Wile and Snow (1941) and Wile and Johnson (1944) have shown approximately 25 per cent of inoculated embryos to be infectious for rabbits after 8 days' incubation, but the lack of microscopically demonstrable spirochetes at that time, however, led them to suggest that the organism survived in an ultramicroscopic phase.

Since positive evidence for an ultramicroscopic form is lacking, the failure of the virulent spirochetes to grow in chick embryos may be due to either an unsuitable host or the lack of anaerobiosis in the embryo. The fact that the so-called Reiter strain of *Treponema pallidum* is grown easily on a lifeless medium under anaerobic conditions suggested an approach to the question of the role of anaerobiosis in the embryonic culture. In the following experiments the influence of the presence or absence of developing tissue and anaerobiosis on the growth of this organism in chick embryos was studied.

MATERIALS AND METHODS

The inoculum used was the sediment of centrifuged 4-day-old cultures of the spirochetes grown in Brewer's thioglycolate medium enriched with 10 per cent human serum. Ten-day-old embryos were injected with varying quantities of the inoculum—up to 0.5 ml. The routes of injection were the chorioallantoic membrane, allantoic fluid, fetal circulation, and the yolk sac. In order to determine whether reproducing cells or oxygen tension was the limiting factor, 10-day-old embryos were held at 4 C, 20 C, and −47 C for the periods indicated in table 1 and treated in the manner described above, and other embryos were inoculated and immediately coated with paraffin. Other treated and untreated embryos were similarly inoculated and immediately thereafter were further injected with varying quantities of sterile Brewer's thioglycolate medium and sodium thioglycolate with the hope of initiating growth. All embryos were incubated at 37.5 C. At intervals, the yolk sac, chorioallantoic membranes, embryos, allantoic fluid, and albumin were removed aseptically and incubated in Brewer's thioglycolate medium with 10 per cent serum for at least 2 weeks. At the same time, microscopic dark-field examination of these tissues was made. Three serial transfers via the chorioallantoic membrane were made.

RESULTS

Paraffin coating these eggs killed the embryos in several hours. The temperature-treated embryos survived for 1 to 4 days, as reported by Rabinowitz *et al.*

(1948), who have shown that such tissues contained living cells and would support the growth of certain rickettsiae. Lahella and Horsfall (1949) reported similar results using influenza virus.

As noted in table 1, the Reiter strain failed to grow or survive in the untreated, developing chick embryos, and in this respect it behaved like the virulent treponemes. In the case of the Reiter strain this could have been due to its well-known lack of any parasitic capacity, to the lack of thioglycolate with the essential SH groups, or to the lack of anaerobiosis necessary for its growth. The first of these suggestions failed as an explanation in view of the results presented in table 1 on the temperature-treated eggs in which, although the embryos were dead, the spirochetes failed to grow or survive. The second suggested explanation was also insufficient in view of the failure of the organisms to grow or survive in developing or temperature-treated embryos to which Brewer's thioglycolate medium or sodium thioglycolate had been added.

On the other hand, the paraffin-coated eggs showed numerous treponemes by the seventh day after inoculation in all parts of the embryo regardless of the

TABLE 1

Growth of the Reiter treponeme in treated and untreated eggs

NO. OF EGGS	TREATMENT OF EGGS	FATE OF EMBRYO	GROWTH OF TREPONEMES
12	None	Normal development	None
52	Held at 4 C for 1 to 14 days	Death after 2 days	None
52	Held at 20 C for 1 to 4 days	Death after 4 days	None
12	Held at −47 C 1 day	Death	None
12	Paraffin-coated	Death	100%

route of injection or the presence or absence of thioglycolate. In all cases these treponemes were morphologically typical and could be cultivated in thioglycolate medium in the usual manner. Three transfers from one egg to another have been made to eliminate the possibility of minute amounts of the original culture medium supplying nutritional requirements. Thus, the essential factor for the Reiter treponeme in the chick embryo would seem to be anaerobiosis (or its effects), a fact reported while this work was in progress by Newcomer and Haanes (1949), who employed a technique utilizing a mixture of hydrogen and carbon dioxide in a Brewer jar. However, they left unanswered the question dealt with here as to the effect of the anaerobiosis on the spirochete or on the embryo. In addition, they cited the difficulties inherent in their procedure, which because of the high humidity favors the germination of spores of bacteria and molds adherent to the shell, leading to an excessive amount of contamination. The use of paraffin-coated eggs seems to overcome these difficulties since no contamination was noted in any of the experiments reported here.

DISCUSSION

It seems evident from these experiments that the Reiter strain of *Treponema pallidum* can be cultivated in chick embryos if anaerobic conditions are provided.

Although such embryos are killed by the procedure, the failure of the organisms to grow in dead embryos unless the latter are protected from air indicates that growth occurs as a direct effect of the lowered oxygen tension on the spirochete and not because of the lethal effect on the embryo. Since virulent *Treponema pallidum* will not grow in the developing chick embryo and is also anaerobic, it is suggested that the lack of anaerobiosis is the limiting factor. However, with some isolated exceptions, these organisms have not been cultivated on lifeless media even under anaerobic conditions, and it also seems from a limited number of observations that the continued development of chick embryos in the presence of an oxygen tension sufficiently low for growth of the spirochetes may not be possible. It seems probable, however, that the technique presented here may have some value in the study of the requirements of virulent *Treponema pallidum*.

SUMMARY

The Reiter strain of *Treponema pallidum* was cultivated in the embryos of paraffin-coated hens' eggs. The results indicated that the effect of the procedure was to establish the anaerobic conditions necessary for the reproduction of the spirochetes.

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