A PLEUROPNEUMONIA-LIKE ORGANISM ASSOCIATED WITH INFECTIOUS ATROPHIC RHINITIS OF SWINE

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Recently McKay and Carter (1, 2) reported the isolation of L type organisms from the nasal cavities of swine affected with atrophic rhinitis and infectious rabbit subcutis derived according to the technique referred to previously (2). These L type cultures were considered to be the pleuro-pneumonia-like phase of *S. necrophorus*, although the identity of all such cultures had not been completely established. The difficulty of distinguishing the L forms of bacteria, which do not revert readily to bacilli, from pleuropneumonia-like organisms (PPLO) is well known.

That some of the L type cultures referred to in previous reports were in reality pleuropneumonia-like organisms was suggested by the fact that many of them did not revert to the bacillary phase or show any evidence of gas production. Ten strains in all were isolated. Eight were recovered from each of eight pigs with rhinitis and two were isolated from 1st and 5th passage rabbit subcutis. Three strains were propagated in 8-day chicken embryos when thioglycollate cultures were injected via the yolk sac. However, as a means of cultivation, the embryonated egg offered no particular advantage over serum-enriched thioglycollate broth (Difco). In the frozen state, the viability of the three strains in egg fluids was maintained for a period longer than three months.

The morphology and growth characteristics of these three strains have been compared with a pleuropneumonia-like organism, recovered from a filtrate obtained according to the technique outlined by Switzer (3) — i.e. with the 02 Selas candle — from a 10-week-old pig with marked atrophic rhinitis. The filtrate was inoculated into the special thioglycollate medium in 1.0 ml amounts and good growth was obtained within 24 hours. Growth could also be demonstrated, with somewhat more difficulty, in 8-day embryonated eggs inoculated via the yolk sac after incubation for 4—6 days.

If the organisms present in the filtrate were derived from *S. necrophorus*—*S. necrophorus* is almost invariably associated with atropic rhinitis — one would expect that such organisms would be recoverable from necrotic processes with which *S. necrophorus* is associated. Filtrations were made through 02 Selas candles of the following material: 1. Two samples of pus from bovine liver abscesses; 2. Necrotic rabbit subcutis initiated by the subcutaneous insertion of necrotic material from bovine footrot; 3. Two samples of rabbit

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subcutis, displaying marked necrosis as a result of the inoculation of *S. necrophorus* and *Pasteurella multocida* isolated from a pig with rhinitis. The filtrates were inoculated into tubes of thioglycollate broth. Examinations of tubes after varying periods of incubation to 96 hours revealed no evidence of growth.

The technique employed for the isolation of all L or pleuropneumonia-like strains, except the one recovered from the filtrate, has been outlined previously (1,2). The primary growth on anaerobic blood agar was poor and could just be discerned with x85 and x100 magnifications. However, the colonies were often so numerous from both rabbit subcutis and diseased nasal structures that sections of agar, removed from areas containing no macroscopic bacterial colonies and dropped in thioglycollate broth, very frequently yielded growth, characteristic of the pleuropneumonia group.

Hanging drops stained with a small amount of methylene blue offered a rapid and effective method of demonstrating the fragile forms of the L or pleuropneumonia-like organisms. It is the authors' belief, after considerable study, that the L forms of *S. necrophorus* can be distinguished culturally and morphologically from the pleuropneumonia-like organisms. The former produced a flocculent and somewhat more turbid growth than the latter. Turbidity in the primary broth cultures of the PPLO's was hardly discernable although a definite diffuse turbidity was apparent after the 3rd subculture. The L cultures of *S. necrophorus* produced many bulbous forms and large vegetative appearing filaments. Rods of varying length containing dark staining spherical bodies could also be discerned. On solid media, colonies of the kind described by Klieneberger-Nobel (4) could be seen. The large vegeta-

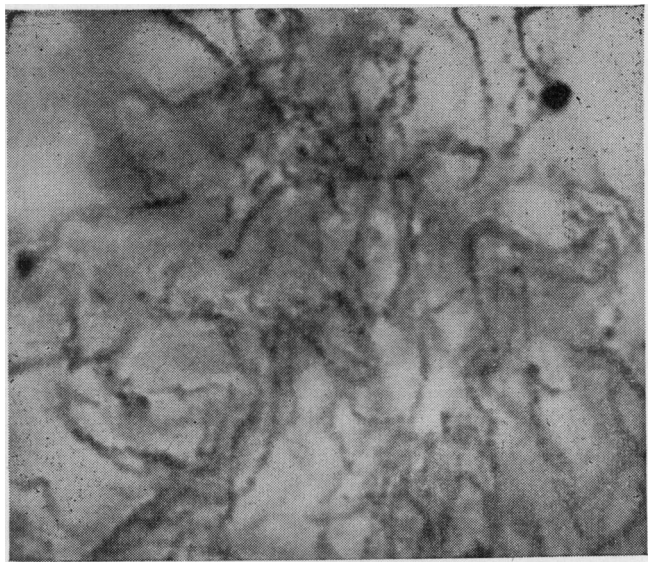

Fig. 1

Primary culture of the PPLO showing elementary bodies in chain formation. Hanging-drop preparation stained with methylene blue, X1000 approximately.

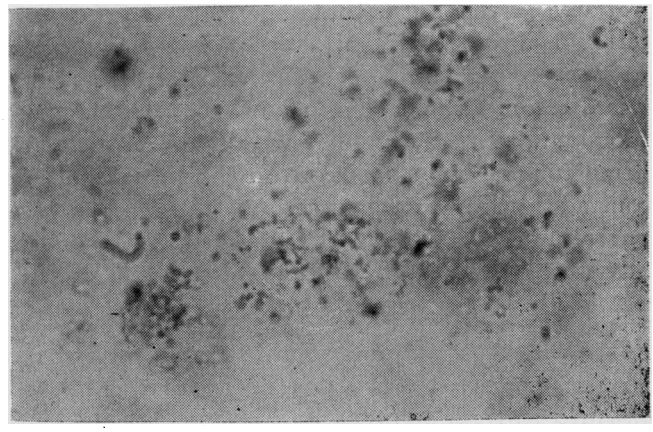

Fig. 2

Third subculture of the PPLO displaying elementary bodies, granules, conidioids and spheroids. Hanging-drop stained with methylene blue, X1000 approximately.

tive forms have not been seen in the cultures of the PPLO's and the latter produced minute burrowing colonies on serum-enriched infusion agar. A photomicrograph of one of these burrowing colonies was shown in an earlier report Fig. 11 (1), in which it was considered to be derived from *S. necrophorus*. Since many of this type of colony have been recovered from the strains now considered to be PPLO's, the original identification of the colony shown in Fig. 11 (1) is considered erroneous.

On initial isolation after 24 to 48 hours incubation in thioglycollate the PPLO's appeared in the form of filaments containing varying numbers of darker staining spheres and chains of larger spheres resembling, somewhat, streptococci. This latter morphology, somewhat blurred due to the hanging drop, is shown in Fig. 1. After further incubation and subculturing the predominant forms were what Wilson and Miles (5) called elementary bodies, granules, conidioids and spheroids (See Figs. 2 and 3). The capacity to produce long chains of elementary bodies appeared to be lost in later cultures.

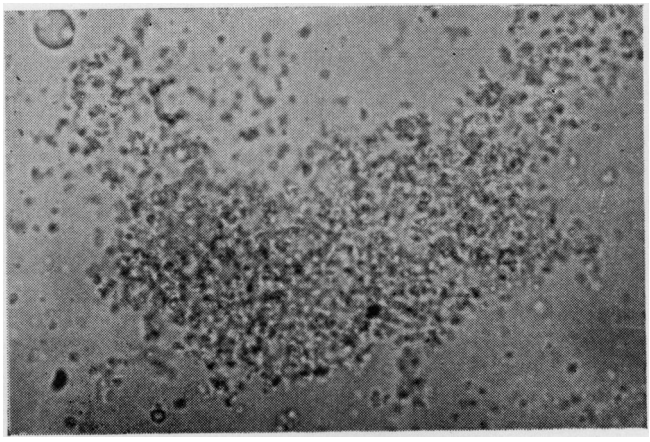

Fig. 3

Colony of the PPLO after 48 hours incubation on serum agar. Colony mount stained with methylene blue and azure (Dienes), X1000 approximately.

On infusion agar containing 20% horse serum, the best growth was obtained anaerobically. Only very sparse growth could be obtained aerobically in primary cultures. In subcultures beyond the third, small numbers of minute raised colonies could be seen with the dissecting microscope (x30) on aerobic blood and serum agar. They were characteristic of the pleuropneumonia group, being round, somewhat raised in the centre and possessing a rough surface. In contradistinction to this organism, the L phase of *S. necrophorus* grows only anaerobically. A variety of solid media has been employed and it was found advantageous to use fresh meat infusions in preference to the dehydrated preparations.

These observations, admittedly incomplete, have been published in order that the earlier observations of McKay and Carter might be more readily oriented to those recorded by Switzer. The association of PPLO's with infectious rabbit subcutis and filtrate suggest that they have etiological significance. Experiments involving instillations of these organisms and *S. necrophorus* are in progress.

ACKNOWLEDGMENT

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