in sheep (Ashton and Ferguson, unpublished work) and five in cattle[6], it is very likely that further examples will be found in pigs.

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MICROBIOLOGY

L Forms of Bacteria as Contaminants in Tissue Culture

The occurrence of pleuropneumonia-like organism as contaminants in tissue culture is a common experience in many laboratories[1–5]. Such infections may be inapparent or may cause progressive degeneration of the cells. The origin of this type of contamination has never been satisfactorily explained, and although the serum used in tissue culture media is often suspected we have never succeeded in isolating pleuropneumonia-like organisms from many samples of fresh human, bovine, horse and rabbit sera. Our results suggest that at least some of the pleuropneumonia-like organisms found in tissue culture are *L* forms of contaminating bacteria. This belief is based on the following observations.

Twenty cell lines carried in our laboratory yielded minute colonies when the medium in which they had grown was plated on soft meat digest agar containing 20 per cent heated horse serum. A complete minute colony derived from a HeLa culture was inoculated into 5 ml. of meat digest broth containing 20 per cent horse serum, 10 per cent yeast extract, and 100 units of penicillin per ml. This was sub-cultured and when slightly turbid a loopful was stroked out on the solid medium used for the initial isolations. Tiny colonies appeared following incubation for 2 days at 37° C. On the third day most of the small translucent colonies had produced further dense opaque outgrowths. These were found to be composed of a penicillin-sensitive *Corynebacterium*. It was clear that these *Corynebacteria* were derived from the small colonies. Pure cultures of the *Corynebacterium* and its *L* form were maintained in 20 per cent horse serum broth. Penicillin was always added to the *L* form cultures to prevent reversion to the bacterial form.

In order to test the hypothesis that the *Corynebacterium* gave rise to its *L* form in tissue culture, HeLa cultures were cured of *L* form infection by growing them for several sub-cultures in medium containing 200 units of neomycin ('Mycifradin sulphate', Upjohn Co.) per ml. Preliminary experiments had shown that the *L* forms from several cell lines were very sensitive to the tetracycline group of antibiotics and neomycin.

Terramycin, chloramphenicol and aureomycin were found to be toxic for the cells at effective concentrations. However, Hearn *et al.*[4] have used aureomycin at toxic levels to free tissue cultures from pleuropneumonia-like organism contaminants. This hazardous procedure was avoidable since neomycin was effective and had no observable toxic effect on HeLa cells at a concentration of 200 units/ml. At each sub-culture during neomycin treatment some cultures were grown without antibiotics for several sub-cultures with frequent changes of medium. When repeated tests for *L* forms in the medium were negative, the cells were deemed to have been cured. Up to 14 days treatment was necessary in some cases. It is of interest that the cells had to be maintained in medium containing 200 units of neomycin per ml. for several sub-cultures before *L* forms became undetectable, even although the organisms were susceptible to <10 units per ml. of the antibiotic in agar medium. The necessity for this high concentration in tissue culture may be due to poor penetration of the antibiotic into the cell or to some other factor related to the intracellular situation of the *L* form.

Cultures freed from *L* forms were then returned to the original medium with 100 units of penicillin and streptomycin per ml. Some cultures were infected with *Corynebacteria* and others with its *L* form. Although the cells infected with the *L* forms did not appear to be different from the uninfected controls when viewed by low-power microscopy, they had apparently become chronically infected and *L* forms could be isolated regularly from them. In the cultures infected with the *Corynebacterium*, this agent was undetectable in the medium of the infected cultures within 2 days, but persisted intracellularly for 9 days. *L* forms were first detected in these cultures at 6 days and regularly thereafter.

These experiments indicate that contamination of tissue cultures containing penicillin with penicillin-susceptible bacteria may cause an inapparent intracellular infection with the *L* form of the bacterium. This could lead to confusion in antigenic analysis of cells, and in metabolic studies the contribution of the commensal *L* form to the host cells' biochemical activity may be significant and should be excluded.

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VIROLOGY

Reactivation of Poxviruses by a Non-Genetic Mechanism

It has recently been shown that poxviruses inactivated by heat are reactivated in cells in which another poxvirus multiplies[1]. The virus yield from cells infected with inactivated virus of one strain and active virus of another strain contains infectious particles of both strains, and also, if the viruses are sufficiently closely related, recombinants[2]. Inactivation by heat, therefore, does not inactivate the deoxyribonucleic acid of the virus particles, since all the known genetic markers are preserved. Since proteins