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Multiplication of Foot and Mouth Disease Virus in Adult Kidney, and Embryonic Lung and Heart Bovine Tissue Cultures. (23333)

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(Introduced by J. F. Enders)
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The multiplication of foot and mouth disease virus in tissue culture has been recently described by Seller(1), Bachrach(2), and Mazzaracchio(3) by using cultures of both bovine and pig kidney cells. The relatively large scale of virus production obtained with these methods seems to prove that tissue culture technics are, presently, the most promising way for mass production of virus and a useful technic for the general assay of its activity. In our laboratory, we have succeeded in multiplying our 3 types of f. and m. virus, by using cultures of adult bovine kidney cells, and foetal bovine lung and heart tissues.

Methods. At the beginning we used the Dulbecco and Vogt method modified by Youngner(4), in slant tubes. After the work by Bodian(5) we started the setting up of Roux flasks of trypsinized cells with the advantage that its massive proliferation has also been used as source of cells for slant tubes. The cultures of bovine foetal lung and heart tissues have been made by planting minced tissue in plasma clot in Roux flasks. A good outgrowth of cells is obtained after 5 to 6 days in stationary incubation at 37°C. The nutrient medium for both types of cultures consisted in bicarbonated Hanks solution, with 0.5% lactalbumin hydrolysate, 20% horse serum and the usual antibiotics. Previously we had used the Enders' medium, but we changed due to our difficulties in obtaining regularly the cow amniotic fluid. The virus used has been our 3 types stock strains of f. and m. virus. For the first virus inoculation, suspensions of cattle epithelium of fresh bovine passages were inoculated in the tubes. After 2 hours standing at 37°C the fluid was removed, and fresh nutrient medium was added. Ten passages were made of the 3 types of virus by using as inoculum a 10^{-3} virus dilution, before performing a T.C. titration of its activity.

Results. Two of the types of virus used gave the characteristic cytopathogenic picture at first passage. One strain of the O type required 2 blind passages before any cytopathogenic activity was detected. Virus was harvested in all cases at 18-20 hours after inoculation, and stored at −25°C, in aliquot parts with 10% peptone saline.

The virus multiplied in bovine kidney cells was titrated at the 10th passage in slant tubes of the same origin. Titres obtained showed that a definite multiplication has occurred in the cultures, as shown by titres of $10^{-6.5}$; $10^{-6.5}$; $10^{-5.5}$ and $10^{-6.5}$ ID/ml, for the strains C, C_2, A and O respectively.

The kidney cell cultures are now being used for typing field strains of virus and for titrations of guinea pig and bovine antisera.

Infected fluid from tissue culture has been used for the hyperimmunization of rabbits according to the method described by Ramos Alvarez and Sabin(6) and Freund and Thompson(7).

In the bovine foetal lung and heart tissue culture the same types of viruses have been

multiplied. The inoculum for this tissue has always been undiluted infected fluid from cultures of virus adapted to bovine kidney cells. The type of cytopathogenic damage in lung and heart tissue was different and apparently less severe than in kidney cells, and in no case a total destruction of the cell outgrowth was detected during 48 hours of observation. The great number of cells that remained unaltered at 12 hours, when a relatively high virus activity had been shown, led us to make several whole harvests of fluid at 6 hour intervals until 72 hours.

Each of these harvests was titrated in kidney cells, and their titers fluctuated around $10^{-6.5}$ and $10^{-4.5}$ ID/ml until 66 hours. Beyond this time the activity detected fell to 10^{-2}.

Virus of the C type from kidney cells, and heart and lung cultures has been titrated simultaneously in slant tubes, and in cattle by intralingual inoculation. No difference in titers was apparent in the 10-fold dilution method used in both titrations. The usefulness of the heart and lung tissue culture, as a source of f. and m. virus for vaccination purposes is now being tested. A detailed investigation of the development of cytopathogenic activity of the f. and m. virus in bovine kidney cell cultures is now also under way.

Summary. Foot and mouth disease virus has been multiplied in bovine tissue cultures. When embryonic heart and lung bovine tissue cultures were inoculated with foot and mouth disease virus, it was possible to collect virus at 6 hour intervals until 66 hours without great decrease of virus activity.

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Potassium Acetate Inhibition of *Lactobacillus casei* and Its Reversal by Lithium, Sodium and Fatty Acids.* (23334)

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In a previous communication from this laboratory it was reported that although *Lactobacillus casei* 280-16A fails to grow in conventional D-α-hydroxy acid-free media(1,2), its growth in the absence of a D-α-hydroxy fatty acid supplement may be promoted either by reducing the potassium content of the medium or by supplementing it with either lithium or (at considerably higher concentrations) sodium ion(3). Similarly, it was reported(3) that growth of *L. casei* 280-16B, which normally does not require a D-α-hydroxy fatty acid supplement(4), is inhibited by potassium in D-α-hydroxy acid-free media, but not in D-α-hydroxy fatty acid-supplemented media, and that the inhibition may be reversed by either lithium or sodium. The parent strain of *L. casei* (strains 280-16A and 280-16B are mutants) was not inhibited by potassium under these conditions(3), presumably because it (but not the mutants) is able to elaborate D-lactic acid, which serves as an adequate D-α-hydroxy fatty acid source, but it has now been found that this organism is markedly inhibited by potassium when acetate is simultaneously supplied at elevated concentrations. This synergistic inhibition of *L.*

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