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Regeneration of Virus Myxomatosum (Sanarelli) in the Presence of Cells of Exudates Surviving in Vitro.

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For a number of years the relation of viruses to cells has been under investigation in our laboratory. In pursuance of this study, attempts to cultivate the virus of infectious myxomatosis of rabbits in different kinds of tissue cultures were made. At the beginning of the work, bits of rabbit testicle suspended in a mixture of rabbit serum (1 part) and Tyrode's solution (3 parts) were used as a medium. Such preparations, however, proved unsuitable for the *in vitro* cultivation of the virus. Previous observations indicated that large mononuclear wandering cells are involved in this disease. Therefore, it seemed not unlikely that the inadequacy of the above methods of cultivation was referable to the failure of mobilization of these cells in excised tissues. To test this hypothesis, attempts to cultivate the virus in the presence of mononuclear cells obtained according to the method of Gay and Clark[1] were made.

A sterile mixture (6 cc.) of beef extract and gum acacia was injected into the right pleural cavity of a normal rabbit. Seventy-two hours later the cavity was opened aseptically and the fluid contents were aspirated. Heparin (1 cc. of a 1:1000 solution for each 10 cc. of pleural fluid) was added to the exudate to prevent clotting and the mixture was centrifuged for 5 minutes at low speed. The sediment was resuspended in Tyrode's solution and again centrifuged. Finally, the cells from 5 to 10 cc. of exudate were suspended in 2 cc. amounts of a mixture of rabbit serum (1 part) and Tyrode's solution (3 parts) and placed in 3 cm. Carrel flasks. To each flask with its 2 cc. of medium, 0.2 cc. of sterile tissue juice containing myxoma virus was added. The cultures were then incu-

[1] Gay, F. P., and Clark, A. R., *Arch. Path.*, 1926, **1**, 847.

bated at 37.5°C. for 3 or 4 days. Subcultures were accomplished by the transfer of 0.2 cc. of the incubated material to flasks containing 2 cc. of a fresh medium. In this manner the active agent has been carried through 20 subcultures. The titre of the virus in the first culture before incubation was 1:100. After incubation it was 1:10,000. In subsequent cultures the titre of the virus at the end of the period of incubation varied between 1:100,000 and 1:1,000,000.

In view of the fact that normal testicular tissue suspended in a mixture of serum and Tyrode's solution did not support the multiplication of the virus, a series of experiments was conducted to determine whether such tissue irritated by beef extract and gum acacia is capable of maintaining regeneration of the active agent. The irritant (1 cc.) was injected into the testicles of normal rabbits, and 72 hours later the irritated organs were removed and minced. Then bits of the minced tissue were suspended in a mixture of serum and Tyrode's solution. The medium placed in Carrel flasks was inoculated and handled as described above. In this manner the virus has been carried through 9 subcultures, and, in spite of a 10-fold dilution at the time of each transfer, the titre of the active agent at the end of each period of incubation has been between 1:100,000 and 1:1,000,000.

By means of the supravital technique, examinations of the exudates immediately after removal from the pleural cavities as well as after incubation in the cultures demonstrated that the primary cells were elements of the monocytic series. Active monocytes with small neutral red rosettes and all kinds of "stimulated" forms including the "coarse-granule" epithelioid type were regularly present in large numbers.[2] Histological studies of the irritated testicles revealed an infiltration of the interstitial tissue with large mononuclear cells which in supravital preparations were found to belong to the monocytic group.

These experiments demonstrate that the virus of infectious myxomatosis is capable of *in vitro* pullulation in the presence of cells surviving in liquid medium, and seem to indicate that cells of the monocytic series play an important rôle in the process. Experiments are now in progress to study the action of the virus in the presence of a variety of cellular elements in the hope of obtaining information regarding the specific susceptibility of different types of cells.

[2] Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 231.