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Survival of Vaccine Virus Separated from Living Host Cells by Collodion Membranes.

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The fact that vaccine virus is capable of multiplication in the presence of living host cells[1,2] cultivated *in vitro* has been known for a number of years. Reports concerning the growth of the virus on lifeless media have not been substantiated. Although it has, as yet, been impossible to cultivate the virus of vaccinia in the absence of living cells, no one definitely knows what function these cells serve in the cultures. Recently, Maitland and Maitland[3] demonstrated that vaccine virus increased in a medium consisting of minced kidney suspended in a mixture of serum and Tyrode's solution. The development of this fluid medium, in which living cells persist for at least 5 days,[4] has made possible experiments designed for an intensive study of the relation of vaccine virus to host cells.

Collodion sacs, prepared according to the method of Northrop and Kunitz,[5] were placed over the lower ends of small open tubes that fitted into larger test tubes. The small inner tubes with their attached sacs and the larger outer tubes were sterilized separately in an autoclave, after which the former were placed in the latter, thus forming 2 compartments separated at the lower level by the membrane alone.

Levaditi neurovaccine prepared in the testicles of rabbits was used. Emulsions of the infected testicles, from which cells were thoroughly removed, were diluted with rabbit serum and Tyrode's solution and then placed on one side of the collodion membrane, while a mixture of minced normal kidney, normal serum, and Tyrode's solution was placed on the other side. Vaccine virus under these conditions, although separated from living cells, survived a 4-day incubation at 37°C., while the virus in controls—a mixture of virus, serum, and Tyrode's solution incubated alone in test tubes—became inactive.

Kidney tissue, killed by freezing and thawing, mixed with serum

[1] Harde, E. S., *Ann. Inst. Pasteur*, 1916, xxx, 299.

[2] Parker, F., Jr., and Nye, R. N., *Am. J. Path.*, 1925, i, 325.

[3] Maitland, H. B., and Maitland, M. C., *Lancet*, 1928, ii, 596.

[4] Rivers, T. M., Haagen, E., and Muckenfuss, R. S., *J. Exp. Med.*, 1929, l, 181.

[5] Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1925-26, ix, 351.

and Tyrode's solution did not support the survival of vaccine virus at 37°C. for many days. If, however, a mixture of vaccine virus, kidney tissue killed by freezing and thawing, serum, and Tyrode's solution was placed on one side of a collodion sac, and a preparation of living minced kidney tissue, serum, and Tyrode's solution was put on the other side, the survival of the virus was far better than that in the first set of experiments described above. In fact, there seemed to be complete survival, if not multiplication of the virus. The results were so striking that further work is in progress to ascertain the nature of the factors responsible for the survival of the virus, and to determine if multiplication of the virus can take place under such conditions.

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Observations Suggesting a Local Factor in Pathogenesis and Healing of Rickets.

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The pathogenesis of rickets is a moot question. By many it is believed that this disorder is entirely of systemic origin and that the local lesions at the epiphyses are secondary in nature. This point of view has been fortified by the demonstration that a low concentration of inorganic phosphorus in the blood is one of the most constant phenomena of rickets in infants and in animals. There can be no doubt of the participation of the systemic factor. However, as we have pointed out from time to time, rickets may come about occasionally in infants and in animals in spite of the fact that the calcium and the phosphorus in the blood have remained at normal levels. In rats this relationship was noted most often when about 10% dried milk was added to the standard rickets-producing ration. Furthermore, it was observed that the lesions of rickets may fail to become manifest in some infants and animals which are poorly nourished, although the phosphorus in the blood is definitely below the non-rachitic level. This incongruity was noted in some instances among rats which had been fed the Sherman-Pappenheimer diet, which is deficient in several nutritional factors.

Last year this subject was again brought to our attention by the occurrence of a mild degree of rickets in some infants, notwithstand-