Influence of Glucose on the Multiplication of Ornithosis Virus *in vitro*

The reproduction of rickettsiae was reported to take place in the host cells when the cell metabolism is rather low[1]. Since viruses of Psittacosis-LGV group have similarities to rickettsiae, the experiments were set up to test the influence of glucose on the multiplication of ornithosis virus *in vitro* in comparison with *Rickettsia tsutsugamushi*.

Carrel or Erlenmeyer flasks of 4·5 cm diameter were used for tissue culture work. Virus suspension was always prepared from the mouse brain infected with the KAM strain[2] by using glucose-free Tyrode solution and inoculum for tissue culture contained about 10^4 LD 50 virus. To a series of culture flasks 2·7 ml of culture fluid, 0·1 ml of minced tissue and 0·3 ml of virus suspension were added. Each flask was sealed with a rubber stopper and put in 37°C incubator. The contents of each flask was taken out at varying times after virus inoculation and after light centrifugation, LD 50 of the supernatant (10^{-1}) was measured by intracerebral titration in the mouse as described before[3].

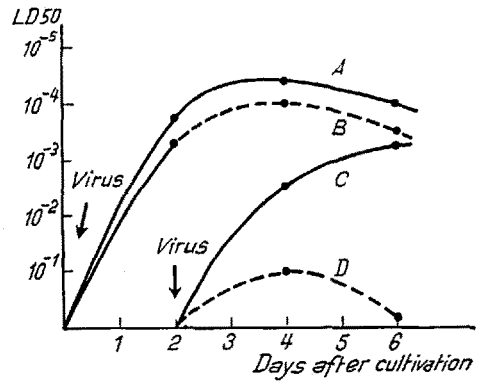

Fig. 1.–Multiplication of ornithosis virus in chick embryonic tissues *in vitro*.

When normal Tyrode solution with 100 mg/dl glucose was used as a culture medium, the propagation of the ornithosis virus obviously occurred and serial cultivation of the virus was successful either in embryo or yolk sac from a 9–11 day chick embryo. In the medium with 10 mg/dl glucose the virus gradually decreased and no propagation of the virus was observed in glucose-free medium. In the glucose-containing culture medium minus cells the ornithosis virus lost its infectivity within 24 h at 37°C. Various amino acids or folic acid could not substitute glucose to support virus multiplication. Attempts were also made to propagate *Rickettsia tsutsugamushi* in embryo or yolk sac in either glucose-containing or glucose-free fluid, but failed.

Influence of glucose on the later stage of virus cultivation was examined. Virus suspension was added to the two series of flasks (Fig. 1, *A* and *B*) at the beginning of tissue culture and on the second day of cultivation the culture medium of *A* flasks was substituted with Tyrode solution with 100 mg/dl glucose and in *B* the medium was substituted with glucose-free Tyrode solution. As shown in Figure 1, even when culture medium was substituted with glucose-free Tyrode solution, multiplication of the virus proceeded just as in glucose-containing medium. At the same time, the embryo was cultivated in culture medium with 100 mg/dl glucose and on the second day, in one series of flasks (*C*) the medium was substituted with the solution with 100 mg/dl glucose and in another series of flasks the medium was substituted with glucose-free solution (*D*). To both series of flasks the virus suspension was added next. In this instance, the tissue permitted reproduction of the virus in glucose-containing medium but did not in glucose-free medium. Thus, the need for glucose as a source of energy for the growth of ornithosis virus is similar to that of influenza virus[1].

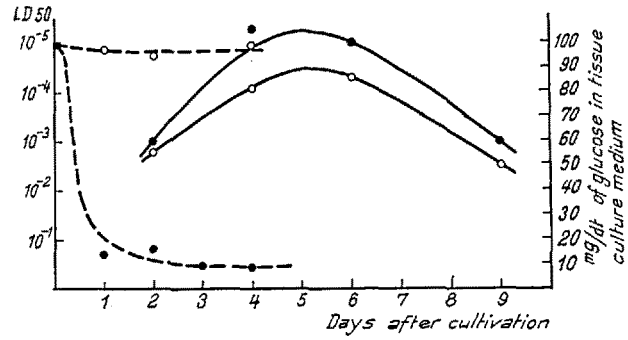

Fig. 2.–Multiplication of ornithosis virus and sugar consumption in chick embryonic tissues.

●——● virus titer
●·····● amount of glucose
} in embryo-cultured media.

○——○ virus titer
○·····○ amount of glucose
} in yolk sac-cultured media.

The ornithosis virus was cultivated using chick embryo and yolk sac in Tyrode solution with 100 mg/dl glucose and multiplication of the virus and sugar consumption by virus-infected tissues was measured successively in a series of culture flasks. The amount of glucose in culture fluid was determined by the method of SOMOGYI[2]. In the virus-infected embryo most of the glucose was utilized 24 h after cultivation, namely, multiplication of the virus attained the maximum after complete sugar consumption. On the other hand, the virus propagated in the yolk sac as well as in the embryo, but no appreciable sugar consumption was observed in this instance. This result is in accord with the experiments reported by MOULDER *et al.*[3] using different methods. It is conceivable that the yolk sac utilizes glucose as does the embryo, but other reducing substances derived from the yolk sac cause such results, as if there occurred no glucose utilization in the yolk sac. According to MOULDER *et al.*, the yolk sac has the ability to accumulate a high concentration of free glucose without being able to oxidize it. So far as we have determined, there were no significant differences in glucose utilization between normal and virus-infected tissues.

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Zusammenfassung

Glukose ist unbedingte Voraussetzung zur Synthese von Ornithosis-Virus sowohl beim Embryo wie beim Dottersack *in vitro*. Nach erfolgter Infektion vermehrt sich das Virus auch ohne Glukose. Die beiden Glukosestoffwechsel unterscheiden sich deutlich.

[1] H. ZINSSER and E. B. SCHOENBACH, J. Exp. Med. *66*, 207 (1937).
[2] T. KUWATA, Science *114*, 640 (1951).
[3] T. KUWATA, Naturwiss. *41*, 43 (1954).
[1] M. O. EATON, Arch. f. Virusforsch. *5*, 53 (1952).
[2] M. SOMOGYI, J. Biol. Chem. *160*, 19 (1945).
[3] J. W. MOULDER, B. R. S. MCCORMACK, and M. K. ITATANI, J. Infect. Dis. *93*, 140 (1953).

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