INFLUENZA

II. EFFECT OF INFLUENZA VIRUS MULTIPLICATION ON THE OXYGEN CONSUMPTION CURVES OF EMBRYONATED EGGS INCUBATED AT DIFFERENT TEMPERATURES*

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In previous experiments (1) the multiplication of typhus rickettsiae in embryonated eggs was found to cause an initial increase in oxygen uptake (as compared with non-infected control eggs) followed by a prolonged decrease terminating in embryonic death. Preliminary experiments (unreported) showed an essentially similar effect of influenza virus A infection on the oxygen consumption of eggs.

In 1948, Parodi and his coworkers (2) reported in detail the results of experiments of this type. These workers found that influenza virus infection caused a marked decrease in oxygen consumption in fertile eggs. Their figures show an initial increase in oxygen consumption, preceding the more definite drop, although they did not attach significance to this fact. Our confirmation of this initial increase of oxygen consumption seems to indicate that it is a constant phenomenon, comparable to that observed in the case of rickettsial infection.

In the experiments to be reported here, coincident determinations of infectivity and oxygen consumption have been made at intervals during a growth period of 96 hours, and certain relationships between the variations in infectivity and in the rates of oxygen uptake have become apparent.

Material and Methods

Embryonated eggs in groups of 30 were infected intra-allantoically with the PR8 strain of influenza A virus by the method described in the preceding paper, and maintained at 34°, 37.5°, and 40°C. The methods used for determining the rates of oxygen consumption, and for the statistical evaluation of the results, have been described previously (1). The oxygen consumption curves of groups of uninfected control eggs were also determined.

Experimental Results

Fig. 1 shows the relatively uniform increase in the rate of oxygen consumption obtained characteristically when groups of normal eggs were studied.

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This chart also shows the effect of the three environmental temperatures on the rate of increase.

In Fig. 2, periodic increases and decreases in the rates of oxygen uptake of infected eggs at different temperatures are seen. At 37.5°C and at 40°C, the development of infectivity titres of $10^{-4.5}$ and $10^{-4.5}$ respectively during the first 10 hours of virus growth coincided with definite interruption of the normal increase in oxygen consumption. At all three temperatures, the oxygen uptake curves showed significant peaks 24 or 25 hours after inoculation. Corresponding peaks in infectivity titres also occurred at this time. During the next 11 or 12 hours, decreases in oxygen consumption rates were recorded, and again there were corresponding drops in infectivity titres. Thereafter, a relatively constant titre of infectivity was maintained at 37.5°C, while the oxygen consumption rose to a peak at 60 hours and then fell rapidly. At 34°C, the oxygen consumption rose between the 36th and 48th hours, and thereafter remained constant, while the infectivity titre fell somewhat and then...
rose slowly. At 40°C., three distinct peaks in infectivity titre are to be noted, and three corresponding peaks in oxygen utilization, although the general trend in oxygen consumption was upward. The lethal effect on the embryos was greatest at 37.5°C., and the highest and best sustained infectivity titres were noted at this temperature. The lethal effect on the embryos was least marked at 40°C., and the lowest and most variable infectivity titres were found at this temperature.

**DISCUSSION**

Evidence is introduced in the following paper which indicates that a toxic substance associated with influenza virus multiplication increases the oxygen consumption of embryonated eggs when present in certain concentrations, but decreases the oxygen consumption when a fourfold increase in its concentration occurs. It seems probable that the periodic alterations in oxygen usage...
consumption shown in Fig. 2 are the result of periodic variations in the concentration of this toxin. These cyclic changes may be caused by the periodic accumulation and degradation of inactive virus particles which inhibit the multiplication of live virus (3-6).

CONCLUSIONS

In addition to the cycles of growth shown by the influenza A virus during the first 24 hours of its residence in the fertile egg, cycles separated by longer time intervals have been noted between the 24th and 96th hours. These longer cycles are best seen when the eggs are incubated at 40°C.

Corresponding fairly accurately with these cycles of growth of the virus, wide cyclic variations in the rates of increase in oxygen consumption of the infected eggs have been found to occur. These variations are in striking contrast to the uniformity of increase noted in uninfected eggs.

The variations in infectivity may be caused by periodic interference with virus multiplication by accumulated inactive virus particles. The variations in oxygen consumption probably are correlated with variations in the concentration of virus toxins.

BIBLIOGRAPHY