values decrease with an increase in the number of C atoms in the alcohol, but the R_F difference is by no means constant.

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Oxygen Uptake of Embryonated Eggs Infected with Western Equine Encephalitis Virus[1]

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During the course of an investigation designed to ascertain the effect of virus proliferation on the over-all gaseous exchange of the embryonate egg, it was observed that Newcastle's disease virus (NJ-KD) did not appear to stimulate or suppress the oxygen uptake until near the terminal stage of the experimental infection (*1*, *2*). At that time, approximately 40 hr in eggs inoculated with 1,000 MLD, a rapid reduction in the $Q^{e}_{O_2}$ (ml oxygen consumed/egg/hr) to the residual level indicative of infertile eggs occurred. Other investigators, using a group or pool technique, wherein the rates of oxygen consumption were determined not on individual eggs but rather on several in a pool, have reported a primary or initial stimulation in the oxygen uptake shortly after inoculation in the case of *Rickettsia prowazekii mooseri* (*4*) and influenza virus infections (*3*). Further experimental evidence, utilizing other viral agents, was deemed necessary in order to attempt to clarify this question; accordingly, this paper reports the results of a study of the oxygen uptake of individual embryonated eggs infected with Western equine encephalitis virus (WEE).

The reported experiments were conducted with the Olitsky strain of WEE.[3] The strain was initiated by inoculation into 10-day-old eggs and passed twice before use. It titered $10^{-7.5}$ on intracranial inoculation of 0.03 ml into Swiss mice. A 10^{-3} dilution of WEE-infected chick embryo tissue was chosen for the experiments reported here. Normal, uninfected chick embryos of the same age were likewise prepared in a saline suspension (10^{-3}). This embryo suspension served as a control on the possible effects of normal embryo proteins and metabolites on the $Q^{e}_{O_2}$ of the host system—embryonated egg.

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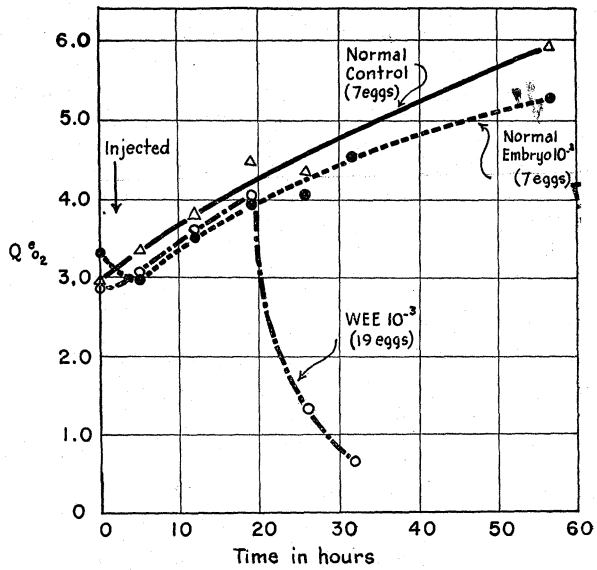

FIG. 1. Mean rates of oxygen consumption of embryonated eggs injected with WEE virus (○), normal chick embryo (●), and of normal control eggs (△).

A number of 10-day-old, trap-nested fertile eggs were divided into 3 lots. One group of 7 eggs served as a control and was not injected. This group provided a reference point against the normal mean $Q^{e}_{O_2}$ as previously established (*1*). A second control group consisted of 7 eggs injected with a 0.1 ml of a 10^{-3} suspension of normal embryo tissue. This control group, as indicated, was used to determine the effect of normal embryo constituents on the metabolic rate of the embryonated egg. A third test group was initiated by injecting 19 eggs with 0.1 ml (10^{-3}) of a chick embryo saline suspension of WEE. The eggs were injected at 4: 00 P.M. of the tenth day of incubation (37.8° C), and the rates of oxygen utilization determined at frequent intervals thereafter until all infected eggs had died, as indicated by their very low rates of respiration. All infected eggs died by the thirty-first hour. The rates of oxygen utilization of 6 eggs of the infected group were determined prior to injection. The postinoculation determinations were run at 3-, 10-, 17-, 24-, and 31-hr intervals. A final reference determination was made on both control groups 55 hr after inoculation.

It was observed that the rates of oxygen utilization of the eggs infected with WEE did not differ significantly from the normal or control series until after the seventeenth hour (Fig. 1). Thereafter, a rapid decline in the $Q^{e}_{O_2}$ of these eggs became apparent. There appeared to be no stimulation of the metabolism of the infected eggs prior to the terminal stage of the infection. This was our experience in every instance on utilizing the described virus. On the other hand, the injection of a normal embryo suspension appeared to suppress slightly the $Q^{e}_{O_2}$. Although this depressed $Q^{e}_{O_2}$ is not valid statistically, it does occur with remarkable regularity.

Data have been presented demonstrating the effect of growth and proliferation of WEE virus on the rates of

oxygen consumption of embryonated eggs. No evidence was obtained indicating that this virus induces a stimulation in the rate of oxygen uptake of such eggs during the course of the infection. Rather, it appeared that the rates of oxygen consumption followed intimately the normal value for embryonated eggs, as well as for eggs injected with an identical suspension of normal chick embryo tissue. Moreover, as the terminal stage of infection was reached, at approximately 17 hr, a marked drop in the $Q^{e}_{O_2}$ occurred. These findings are in accord with our previous observations utilizing the Newcastle's disease virus. It would appear that the growth of the virus in the embryonated egg either does not alter the metabolism until near the terminal stage, or, if the embryo metabolism is altered prior to this period, that a compensating mechanism functions in such a manner that no over-all variation in the $Q^{e}_{O_2}$ becomes apparent.

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The Biosynthesis of 17-Hydroxycorticosterone from 11-Desoxy-17-hydroxycorticosterone

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Following the demonstration by Hechter and his associates (*2*) that desoxycorticosterone was converted into corticosterone when perfused through an isolated beef adrenal gland, Hayano *et al.* (*1*) attempted to repeat the same conversion by incubating desoxycorticosterone or its glucoside with fresh adrenal slices or homogenates. They were able to demonstrate that a substance was formed which exhibited glycogenic activity in the mouse test. Although no isolation of the active component was attempted, it was assumed that oxygen had been added to desoxycorticosterone at the carbon-11 position with the formation of either corticosterone or 11-dehydrocorticosterone.

Employing a technique similar to that described by Hayano *et al.* (*1*), the authors have been able to demonstrate that 11-desoxy-17-hydroxycorticosterone (Reichstein's Compound S) can be converted into 17-hydroxycorticosterone (Kendall's Compound F).

The procedure used in these experiments was as follows: Ten g of beef adrenal gland were finely homogenated with 30 ml of a solution of .01 *M* glucose, 0.062 *M* sodium chloride, 0.02 *M* disodium phosphate, 0.025 *M* potassium chloride, 0.004 *M* magnesium sulfate, and 0.01 *M* sodium fumarate. The homogenate was incubated with 2–10 mg 11-desoxy-17-hydroxycorticosterone (Compound S), added as a fine 0.1% aqueous suspension, for 3 hr at 37° C in an atmosphere of oxygen. Following incubation, proteins were precipitated by the addition of 5 volumes of acetone. The glandular residue was removed and extracted with acetone. Both acetone solutions were combined and the solvent removed by distillation under reduced pressure. Saline was added before the final portion of the acetone was removed. The saline layer was then extracted with chloroform and the chloroform layer concentrated to approximately 1–2 ml. The sample was chromatographed on large sheets of filter paper, using the toluene-propylene glycol system described by Zaffaroni *et al.* (*4*). The sheets were developed for 48–72 hr, sufficiently long to permit good separation of 11-dehydro-17-hydroxycorticosterone and 17-hydroxycorticosterone, both of which are present as bands from chromatograms of extracts of incubated homogenates containing no added Compound S. The locations of the various compounds on the paper were detected by means of ammoniacal silver nitrate reagent and were compared with standard reference strips with Compounds E, F, and S developed at the same time.

The regions containing Compound F were then removed, and the sterol was extracted with chloroform. Upon slow evaporation of the chloroform solution, crystalline material appeared. This was collected and twice recrystallized from chloroform. The final product consisted of fine crystalline needles, which melted at 203°–204° C, with a specific rotation of 157°. The crystals gave the characteristic green fluorescence of Compound F when treated with concentrated sulfuric acid. The physical properties of the crystalline material were compared with a highly purified sample of natural Compound F[1] and found to be almost identical.

The procedure described above resulted in approximately 50% of the added Compound S being converted into Compound F, as determined by the biological activity of the material present in the final chloroform solution. During this process the remaining portion of the Compound S, not accounted for in the formation of Compound F, was converted into other substances that have not as yet been characterized.

Incubation of Compound S with heat-inactivated homogenates or in an atmosphere of nitrogen resulted in no significant conversion of this compound into other substances.

A detailed report of the specific conditions necessary for maximum conversion of Compound S to Compound F and the nature of other products that are formed is now being prepared and will be published elsewhere.

Since this work was completed, Hechter *et al.* (*3*) have shown that Compound S is converted into Compound F by perfusion of the steroid through surviving adrenal glands.

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[1] Kindly supplied by J. J. Pfiffner.