microscopic changes in the cheek pouches of lathyritic animals were observed. BAPN caused an increase in the *M* saline-soluble collagen fraction or in the citrate-soluble collagen fraction of the cheek pouches. An increase in the *M* NaCl-soluble fraction was evident after only 1 day on 0.1% BAPN fumarate. This increase in the *M* NaCl fraction may be of value in rapid screening of potential lathyrogens. No 0.2 *M* NaCl-soluble collagen fraction could be found in pouches from either normal or lathyritic hamsters. Its absence may be associated with the relative resistance of the hamster to BAPN. No effect of BAPN on elastin-like fractions of the pouches could be demonstrated.

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## Inhibition of Influenza C Virus by *Hemophilus influenzae* in Embryonated Eggs.* (29765)

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Previous publications from this laboratory (1,2) have described certain aspects of the bacterial population dynamics when *Hemophilus influenzae,* type b is superimposed on an established Influenza C virus infection in embryonated eggs. Infection with the virus promotes conditions favorable to an accelerated bacterial growth rate and also provides the requirements for maintaining a high proportion of virulent members in the bacterial population. During the course of these studies it became apparent that active infection with *Hemophilus* exerted an inhibitory or depressing influence on the production of virus in a concurrent Influenza C infection. This report concerns results of one series which represents several preliminary experimental studies of this phenomenon.

*Materials and methods.* Influenza C, strain J. J. maintained in passage by intra-amniotic inoculation of 10 or 11 day embryonated eggs constituted the viral component. A pool composed of bacteria free amniotic fluids collected from the 221st passage with a HA (hemagglutination) titer of 1:512 provided the initial inoculum. *Hemophilus influenzae,* type a, American Type Culture Collection No. 9237 served as the bacterial component. Type a was chosen because it is much less lethal for embryonated eggs than types b and d. Embryonated eggs were prepared by the "window" method during the 11th incubation day. All inoculations were made by the intra-amniotic route by means of tuberculin syringes and 1½ inch long 24 gauge needles.

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At each passage groups of 16 embryonated eggs each were injected with each of the 3 types of inoculum respectively.

The combined infection was initiated by injecting 0.1 ml of a $10^{-2}$ dilution of the stock virus into the amniotic fluid during the 12th incubation day. During the 13th day 0.1 ml of a 24-hour Levinthal broth culture of *Hemophilus* was injected by the same route. Forty-eight hours later amniotic fluid was aspirated from each surviving embryo by means of a Pasteur pipette. A small sample of each fluid was tested for the growth of *Hemophilus* and for the presence of bacterial contaminants by culture on a sector of a Levinthal's agar plate. A 0.5 ml sample also was tested for the presence of virus by hemagglutination. An equal amount of each virus-positive fluid containing *Hemophilus* in pure culture was pooled. The virus content of the pool was then determined by HA.

For the second passage of the combined infection, amniotic fluid from the first passage pool was centrifuged in the horizontal at 3000 rpm for one hour at 5°C. The supernate had the hemagglutination titer of the uncentrifuged pool and approximately 1000 viable *Hemophilus* per ml. One-tenth ml of this supernate was injected into the amniotic fluid of embryonated eggs during the 12th incubation day. Seventy-two hours later amniotic fluid was aspirated from the surviving embryos. Each sample was tested as described and an equal amount of each virus-positive fluid containing *Hemophilus* in pure culture combined into a pool. The virus content of the pool was determined by HA. A third and fourth passage of the combined infection was carried out in the same manner as described for the second passage.

A control series with Influenza C virus only was maintained in parallel. Amniotic fluids were collected 72 hours after inoculation during the 12th incubation day, with 0.1 ml of a $10^{-2}$ dilution of the preceding passage. HA titers were determined at each passage on a pool composed of equal amounts of fluid harvested from individual embryonated eggs. A passage series maintained in the same manner with *Hemophilus* only was also included.

TABLE I. Hemagglutination Titers of Amniotic Fluid Pools in Combined and Single Infection with Influenza C and *Hemophilus influenzae*, type a.

| Passage No. | Influenza C only | Influenza C hemophilus | Hemophilus only |
|---|---|---|---|
| 1 | 1:512 | 1: 4 | 0 |
| 2 | 1:512 | 1: 8 | " |
| 3 | 1:512 | 1:16 | " |
| 4 | 1:512 | 1:16 | " |

*Results and observations.* Table I presents the HA titers obtained at each of the 4 successive passages on the pools of amniotic fluid from the groups of embryonated eggs injected with each type of inoculum.

It is evident from Table I that the well adapted J. J. strain of Influenza C was readily maintained in successive intra-amniotic passage at a HA titer of 1:512. In the presence of proliferating *Hemophilus* the HA titer did not exceed 1:16 indicating that in some manner there was interference with the production of virus components during the 4 successive passages. In another experimental series this effect was maintained through 10 passages without complete repression of virus production. It is also evident that *Hemophilus influenzae* does not produce hemagglutinin when propagated in the amniotic fluid of embryonated eggs.

*Effect of antibiotic on combined infection.* During the 3rd passage one-half of the eggs with combined infection received 1000 $\mu$g of streptomycin intra-amniotically 24 hours after inoculation. Forty-eight hours later the amniotic fluids collected from the treated eggs did not culture out *Hemophilus*. The HA titer of the pool of aliquots was 1:512. The fluids from untreated eggs were positive for *Hemophilus* by culture and the HA titer of the pool of aliquots was 1:16. These observations confirmed those made in several preceding similar experiments.

*Effect of* Hemophilus *on HA capacity of Influenza C.* It was necessary to determine whether the presence of *Hemophilus* had an inhibitory effect on the HA reaction and thus accounted for the low titers in the combined infection. Amniotic fluid from the 3rd passage of virus only which had a HA titer of

1:512 when diluted in saline was set up with amniotic fluid of the 4th passage with *Hemophilus* only as the diluent. In the presence of the bacteria grown in the amniotic fluid the HA titer of the pool was 1:512. The bacteria apparently have no inhibitory effect on the HA capacity of the virus *in vitro*.

*Discussion.* Further study will be required to discover and characterize the factors associated with actively proliferating *Hemophilus* which inhibit the production of Influenza C virus in combined infection in embryonated eggs. The growing bacterial population presumably creates conditions or, what is more likely, produces a substance or substances that are relatively labile which interfere with one or more stages of virus production. The low titers obtained in the combined infection represent virus produced during the first 24 hours before effective concentration of the inhibitory factor is elaborated by the bacterial population. The prompt resumption of virus production after the bacteria are killed by streptomycin suggests that there is no inactivation of virus, nor have the host cells been injured to such an extent that virus can no longer be produced. The site of action of this presumed substance or substances is not evident from the experiments performed thus far. That the virus inhibitory effect is more than likely due to a substance produced by the proliferating bacteria is apparent from the observations of Carver and Naficy(3,4). These investigators have isolated protein-like constituents from several bacterial species representing both gram-negative and gram-positive groups that are inhibitory to virus production in tissue culture systems. Each bacterial factor appears to have a definite range of action, inhibiting certain viruses but not others. Whether or not different bacterial species will have different inhibitory effects on different viruses in the embryonated eggs remains to be determined. Some may, as demonstrated by Bang(5) with *Hemophilus influenzae, var. suis,* and swine influenza exert a potentiating or virulence-enhancing effect on the virus for the embryo which Shope(6) had previously shown to take place in the naturally occurring disease.

These results are presented to indicate that the methods employed may prove of further usefulness for the study of the phenomenon of combined viral and bacterial infection.

*Summary.* Active proliferation of *Hemophilus influenzae,* type a in the amniotic fluid of embryonated eggs has a marked inhibitory but not completely suppressive effect on a concurrently established Influenza C virus infection. The nature of the inhibitory mechanism is undetermined. It is presumed that a labile substance or substances produced by the proliferating bacteria disrupt the process of production of virus constituents without appreciable damage to the host cells.

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