of this medium.

Summary. 1. Lansing poliomyelitis virus proliferates in cultures of human embryonic brain and cord supplied with a synthetic nutrient medium. 2. The amount of glucose used by cultures that contain virus is significantly less than that used by uninoculated control cultures.

The human embryos used in these studies were obtained through the courtesy of the gynecological staff of the Toronto General Hospital. We are indebted to Dr. R. C. Parker and his colleagues for supplies of the synthetic medium.

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Cultivation of Coxsackie Virus in Embryonated Eggs and in Chick Tissue Cultures. (19497)

MYRTLE SHAW. (Introduced by Gilbert Dalldorf.)

From the Division of Laboratories and Research, New York State Department of Health, Albany.

First attempts to grow Coxsackie virus in embryonated hens' eggs were unsuccessful(1). Since then there has been only one report of adaptation to growth in eggs. Huebner(2), after alternate mouse to egg passage, carried a Group A, Type 2 strain through a number of consecutive yolk-sac passages. Similarly, there has been a report of successful cultivation in tissue culture. Slater and Syverton(3) maintained a Group A, Type 4 strain in embryonic mouse cell medium for 24 passages.

Results of the following investigation confirm the difficulty of growing the Coxsackie viruses in embryonated eggs and suggest that, while Group A, Type 2, may perhaps be more readily adapted than other types, the individual strain appears to be the most important factor.

Methods and materials. Virus strains. Thirty-one strains were used, including representatives of 10 types of Group A and 3 of Group B. In most cases, 2 or more strains of a given type were tested but only one strain each of Group A, Type 6, 7, and 9 and Group B, Type 2. Twelve strains had been received from other laboratories as muscle suspension or mouse brain; the remainder were isolated in this laboratory from human fecal specimens. Stock supplies of the virus were maintained as infected mouse brain in 50% glycerol. Infected leg tissue suspension was the usual material for egg and tissue-culture inoculation, the exceptions being 2 Group B strains, where brain tissue was used as the inoculum.

Embryonated eggs. After preliminary exploration, the following procedure was used as a screening test. Eggs were incubated for 7 days at approximately 37°C; 0.1 ml of 10% virus suspension was inoculated into the yolk sac and incubation continued for 6 days at approximately 35°C before harvesting embryos and a portion of the yolk-sac tissue. The heads, minus the eyes, of 6 to 8 embryos were weighed, ground with sterilized sand, and a 20% suspension prepared, using as a diluent 0.85% saline containing 10% beef infusion broth. The suspensions were centrifuged for

10 minutes at approximately 2500 rpm. Supernatant fluids were stored in a dry ice cabinet until mouse infectivity tests could be performed. Similar suspensions were prepared from pools of legs and wings, from the remainder of the bodies including the viscera, and from the pooled yolk-sac tissue. These suspensions were prepared to determine the localization of the virus but for passage of untested materials, a mixture of equal parts of the 4 suspensions was used. On the assumption that virus might be present only in one suspension, the use of the mixture provided for no more than a 4-fold dilution of the active material. Generally, suspensions of first passage materials were not tested in mice since positive mouse infectivity tests might be ascribed to survival of the inoculum. *Tissue cultures.* The flask culture technic was used throughout. Prior to the adaptation of any of the strains to eggs, tissue culture studies of Group A, Type 1 (No. 48249) had been made using brain or leg tissue from one-day-old mice, with indifferent success. The strain was carried through a number of passages but with steadily declining titer. The first egg-adapted strain was grown without difficulty *in vitro* on embryonic chick tissue. Other strains were then investigated. The fluid phase consisted of one part ox serum ultrafiltrate, 3 parts Simms X7 solution(4) with sufficient penicillin and streptomycin added to give a concentration of 50 units and 50 μg/ml, respectively. Five ml of the fluid were dispensed into each rubber-stoppered 125 ml Erlenmeyer flask. Chick embryos 6 to 8 days of age were minced and washed in Simms solution. Four drops of tissue were used per flask. The initial inoculum was 0.1 ml of 20% suspension of infected mouse legs or brains or a similar preparation of infected chick embryo legs and wings. Cultures were incubated at 36°C for 7 days, plated, and 0.1 ml transferred to a fresh flask of medium. Fluid from the seed culture was centrifuged for 10 minutes at approximately 2500 rpm. The supernate was stored in a dry ice cabinet until mouse infectivity tests could be performed.

Tests for determining presence of virus. Tissue culture fluids and suspensions prepared

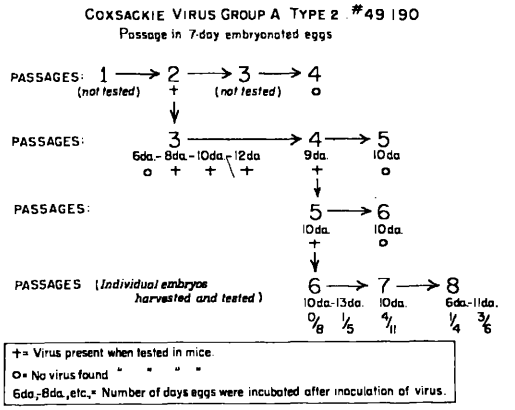

FIG. 1. Coxsackie virus, Group A, Type 2, #49190.

from infected egg or embryo tissues were tested for virus by injecting into suckling mice 1 to 3 days of age. The approximate mouse infective titer was determined by injecting serial tenfold dilutions of the materials. The specificity and identification of the egg-adapted or tissue culture strains were checked by neutralization tests using immune sera prepared in mice or hamsters.

Results. Embryonated eggs. Twenty-four strains representing 10 types of Group A virus and 5 strains which included 3 types of Group B virus were investigated. Negative results were obtained with all Group B strains and with Group A, Types 1, 3, 5, 6, 7, 8, 9, and 10. Only 5 Group A strains gave evidence of virus being present in any quantity in the second passage. One was the Type 4 strain grown by Slater and Syverton(3) in tissue culture; 2 other Type 4 strains gave negative results. The investigation of this strain was not continued. Two were Type 2 strains isolated in this laboratory. Strain No. 49190 was transferred through 8 egg passages with no enhancement of pathogenicity and no gross evidence of infection. Fig. 1 shows the history of these passages. When, with a lengthened incubation period, the strain could not be maintained in passage, it was decided to prepare and test suspensions of individual embryos. Only one embryo of 13 harvested from the 6th passage contained detectable virus; only 4 of 11 were positive in the 7th passage. Since these embryos showed no signs of infection, it was not possible to determine by gross observation which to harvest and use for

passage. The low incidence of infected embryos explains the failure of passages in which pooled tissue of a number of embryos was used. In those embryos that became infected, virus was present in fair titer (10^{-4}). Results with strain No. 50136 were similar. The 2 remaining strains (Nos. 5127 and 50376) were received as Group A, Type 5 from another laboratory. Both gave evidence of marked cross reactivity with Group A, Type 2 and the virus grown in egg and tissue culture was similar serologically to Type 2 only. Strain No. 5127 grew even less readily than those previously described but the Type 2 element of strain No. 50376 immediately adapted to cultivation in eggs and was highly pathogenic for the embryos, occasionally causing death. The infected embryos were pale and swollen, with thin fragile legs and feet curled or bent in abnormal positions. Histopathologic examination of the infected embryos revealed hyaline degeneration of the striated muscles, as is seen in infected mice (1), but no lesions of the central nervous system. This strain was carried through 8 egg passages. A suspension of the pooled legs and wings from embryos of the 7th passage was infective for mice by intraperitoneal injection (0.05 ml dose) when diluted to 10^{-7}. Virus was also present to high titer in other embryonic tissues and to a much less degree in yolk-sac tissue. Three other Type 5 strains failed to grow in eggs.

The results obtained with strain No. 50376 were sufficiently unusual to suggest the presence of another agent. However, Group A, Type 2 antiserum specifically prevented the growth of the strain in eggs. Attempts to increase the pathogenicity for eggs of another Type 2 strain by simultaneous inoculation of Type 5 virus were unsuccessful. Embryo suspensions of No. 50376, made non-infectious for eggs by the addition of Type 2 serum, did not initiate growth when added to egg inoculum of strains of Type 4 and Type 6, previously found not to grow in eggs.

Tissue cultures. Strain No. 50376, adapted to eggs, grew readily in culture on chick embryo tissue. Fluid of the 8th tissue culture passage was infective for mice in a dilution of 1:100,000. Egg passage virus No. 49190, which infected only occasional embryonated eggs, was easily cultivated *in vitro* on embryonic chick tissue. That growth in chick tissue cultures was not dependent on prior adaptation of the virus to eggs was shown by successful cultures obtained with the above strains and strains No. 50136 and No. 5127, using infected mouse leg suspension as inoculum. All attempts to cultivate No. 50376 or No. 49190 in mouse leg or mouse brain tissue culture were unsuccessful.

The Type 4 strain adapted by Slater and Syverton to mouse tissue culture grew also in chick tissue medium. Three other Group A, Type 4 strains did not. Eight additional strains representing Types 1, 3, 5, and 6 failed to grow.

Summary. Among 29 strains of Coxsackie virus tested in embryonated eggs, one strain, serologically Group A, Type 2, exhibited unusual pathogenicity for chick embryos. Of 16 strains tested, the same strain, 3 other Type 2 strains, and a Group A, Type 4 strain grew readily *in vitro* on embryo chick tissue. These results suggest that strain differences are a primary factor in adaptation to growth in embryonated eggs or tissue culture.

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