Virus Interference by Serially Passed Hodgkin's Disease Extracts in Chicken Eggs.* (17958)

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Many workers have joined in the search for an etiologic agent in Hodgkin's Disease (H.D.) and many biological and chemical technics have been tried without solving the problem. An infectious agent has long been suspected as being concerned in the process, and ever since Gordon's(1) report, repeated suggestions of a possible virus etiology have appeared; however, no significant or confirmed evidence of its presence has been reported.

Extensive studies of H.D. by the use of the fertile chicken egg has been made by Bostick(2) in efforts to detect any distinguishing characteristic that might be present. A slight lethal effect of serially passed H.D. extracts on chicken eggs was noted; so the experiments were continued in order to detect other distinguishing characteristics.

Experimental procedures. Fresh H.D. lymph nodes were ground with sterile sand, filtered through a Seitz filter if contaminated, and inoculated into the amniotic sac of 7 to 20 fertile chicken eggs, which had been incubated at 37.5°C for 7 days. After inoculation they were incubated at 35.5°C. The amniotic fluids (Am. F) of some live eggs and all of those dying between 2 and 9 days later were harvested and pooled. After culturing both aerobically and anaerobically, these pooled fluids were inoculated into another series of 7 to 20 eggs. If bacterial contamination occurred, the fluid was Seitz filtered before being passed on. All material was filtered on at least one occasion and had been serially passed through at least 4 (and usually 10) groups of eggs before being used in any interference experiments. Identical but separate serial passages were carried on with non-H.D. cancer-laden lymph node extracts, and this was used as control material. These control tissues were obtained from 4 separate patients. The H.D. lymph nodes were originally obtained from 9 separate patients. They were carried separately in serial passages and pooled with each other from time to time as indicated in the interference technics below.

Interference technics. Groups of 8 to 20 fertile eggs were inoculated with 0.1 cc of Am. F. in the manner described above. One group received Am. F. from control serial passages and the other from H.D. serial passages. After 72 hours incubation, all of the eggs were inoculated into the amniotic sac with 0.02 cc of Lee virus having a hemagglutination titre of 5 units. The Lee virus used was an egg adapted strain that had been transferred in the amniotic sac at intervals over many months. For the test, the Lee Am. F. was harvested 24 hours after inoculation and its hemagglutination titre was determined by the method of Salk(3). The fluids with a titre of 1:10,240 or over were pooled, retitred and then frozen in the ice box at −18°C until used. Usually the Lee was stored in separate vials, and only one of them at a time was thawed and used as needed. After the inoculation of the Lee virus the eggs were incubated at 35.5°C for 18 hours, whereupon all of the live eggs were placed in the ice box at 8°C for 3 hours. Then each was opened, the Am. F. cleanly pipetted off and a smear made to demonstrate the absence of bacterial contaminants. The Am. F. of each egg was tested for the amount of Lee virus present, as demonstrated by its hemagglutination titre.

Results. The technic described above is the result of many months of experimental probing. Before it was evolved the experiments

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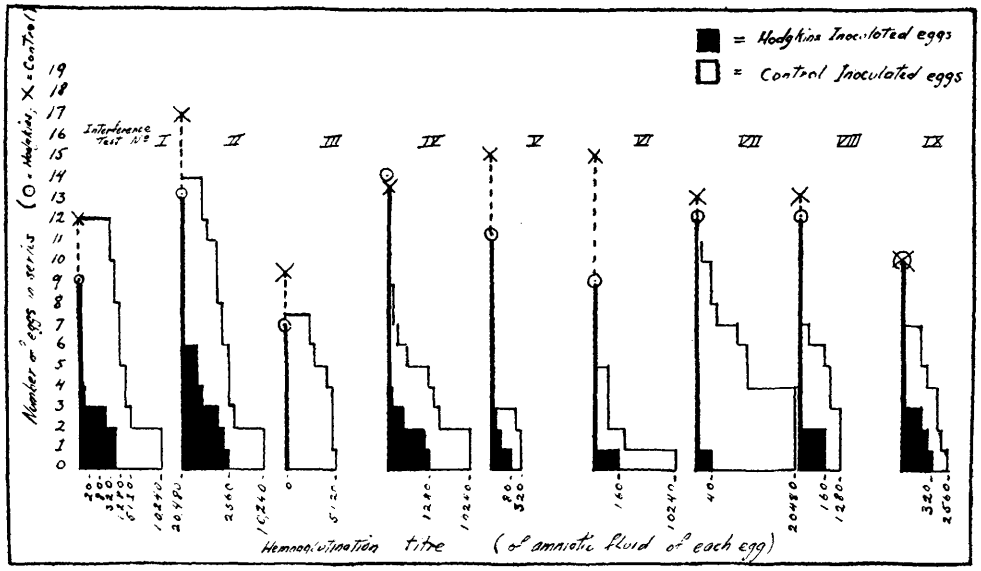

FIG. 1.
Contrast of influenza interference effects by Hodgkin's disease and control amniotic fluid as shown by comparison of their respective hemagglutinative titres.

did not show interference. However, after it was established, essentially all H.D. Am. F. showed virus interference capacity. The H.D. material was first tested for interference activity by using pooled Am. F. derived from 9 patients. When that revealed interference the tests were made on pooled Am. F. from 3 patients, then from 2 patients, and finally on the Am. F. derived from single H.D. cases. The results of the tests are indicated in Fig. 1.

Discussion. The capacity of Am. F. from H.D. inoculated eggs to interfere with the growth of Lee virus in fertile eggs was demonstrated repeatedly. This characteristic was possessed by material from multiple cases of H.D. The factor concerned must possess the capacity to reproduce in fertile chicken eggs since it was present in Am. F. after more than 10 serial passes in chicken eggs. By the 10th serial passage many months had passed since the original inoculation, and the dilution of the original H.D. tissue extract was at least 10^{-20}. Also the factor is Seitz filterable, since all Am. F.s were so filtered on at least one occasion, and usually on several, before being used in these experiments.

All of the non-H.D. control material was treated exactly like all H.D. material, and it represented the extracts of lymph nodes from 4 separate patients. Duplicate experiments designed to demonstrate that a parallelism existed between the hemagglutination titres and the infectivity titres of the Lee virus were not done at this phase of the study. Since interference activity was demonstrated in all H.D. Am. F. tested, it seems most unlikely that these results could be explained on the basis of having picked up a vicarious virus that perchance existed in human tissues. It may well be that this characteristic of H.D. extracts may be of greatest importance, for at last a difference between H.D. and other tissues has been demonstrated. Numerous obvious technics of biochemical, serological and immunological type can be focused on the problem so as to directly give, if possible, this factor etiologic significance. Similar technics, when applied to studies in other types of malignant lymphomas and reticuloendotheliosis, might be most revealing in an etiological sense.

Conclusions. The Am. F. harvested from series of fertile chicken eggs originally inoculated with H.D. tissue extracts showed virus interference capacities. Upon inoculation into fertile chicken eggs they were able to interfere with or to completely inhibit the growth of Lee virus subsequently inoculated into these

eggs. This factor was encountered in all H.D. Am. F.s tested and may be of etiologic significance in the disease. This factor is filterable, doubtless has the capacity to reproduce itself and probably has some slight lethal effect on chicken embryos.

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Neutralization of Pit Viper Venom by King Snake Serum.* (17959)

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There is considerable published evidence that certain venomous and non-venomous animals show some degree of tolerance to animal venoms(1-4). Numerous authentic incidents are recorded in the literature of snakes being bitten by other venomous snakes resulting in some instances in death and sometimes in survival(5). The fact that death may occur indicates that tolerance is limited; but, when the approximate amount of venom injected by the strike is considered on the basis of dosage per unit of body weight, the tolerated dose in the snake must be very large in comparison with that in man in order to permit any survivals. It has also been shown experimentally that some homologous and non-homologous serums exert a limited antivenin action(1,6,7).

The present work was suggested by the common belief in rural areas of this region that the king snake is immune to the bite of American vipers and by the report of Ditmars(8) of the survival of a king snake *(Lampropeltis getulus)* bitten by a water moccasin of much larger size. The investigation involved primarily a study of the degree of neutralization of viper venom by king snake serum as determined by the lethal action of the venom in mice. Experiments are also presented showing the low degree of toxicity of heat-detoxified king snake serum. With the technic used in our experiments it has been possible to show a higher degree of protection than, to our knowledge, has hitherto been reported for reptilian sera. An abstract of this work has already appeared (9).

Materials and methods. The chain king snake *(Lampropeltis getulus* Floridans) and the spotted king snake *(Lampropeltis getulus* Holbroki) obtained from Ross Allen's Institute, Fla. and from local collectors were used in these experiments. Blood was collected by severing portions of the tails following which it was allowed to clot and the serum separated by centrifugation. The serum was detoxified by heating it in test tubes in a water bath at 56°C for 30 minutes,

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