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In the present case, one might have speculated upon the withdrawal of barbiturates as an accentuating factor, although the long delay of eight days would argue against this. Thus, if the cortex were left in a hyperexcitable state, there might be less inhibition from higher areas to counteract whatever the subcortical effect of trifluoperazine might be.

In the treatment of such a condition, paradoxically, both a cerebral stimulant (namely caffeine) and cerebral depressants (namely barbiturates) have been claimed to give good results. Anti-parkinsonian drugs seem to be the best available remedy, and we wish to stress the lability and reversibility of this rather frightening condition which may simulate tetanus. With increased use of high-potency phenothiazines, such syndromes may cause confusion and may result, as in this case, from the inadvertent prescription of a higher dosage than usual.

My thanks are due to Dr. C. H. McCuaig, medical superintendent of the Ontario Hospital, Kingston, and to Dr. R. Bruce Sloane for his advice in the preparation of this article.

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SHORT COMMUNICATION

ISOLATION OF A HÆMADSORPTION VIRUS FROM THE RECENT OUTBREAK OF RESPIRATORY ILLNESS IN ONTARIO*

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DURING the recent outbreak of influenza-like illness among school children as well as adults, a hæmadsorption virus was isolated. This non-influenza virus was recovered from 78 out of 175 specimens submitted from different parts of Ontario. Judging from the dates of receipt of specimens, the outbreak began about the middle of January 1959, reached its peak during March, and, gradually subsiding, ended about the end of April. In at least one community 30% of the population were affected by this illness. In addition to the usual influenza-like symptoms, a considerable number of patients had cervical adenitis. A detailed clinical picture of this illness will be reported separately.[1] The present brief communication deals with the methods of isolation, identification and some of the characteristics of this virus.

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ISOLATION OF VIRUS

Throat washings, collected in most cases within three days after onset of symptoms, were delivered to the laboratory in the frozen state. Each specimen was treated with 1000 units of penicillin and 1000 μg. of streptomycin per ml. and inoculated into monolayer monkey-kidney tissue cultures, HeLa cells and developing chick embryos.

Monkey-kidney tissue cultures were maintained in Medium HB597[2] at 37° C. No cytopathogenic changes were observed in the inoculated cultures even after 10 days of incubation, and the presence of the virus was demonstrable only by hæmadsorption technique[3] and by hæmagglutination with the fluid phase of tissue culture, using either guinea pig or human type O erythrocytes. Even continuous serial passage of the virus did not result in production of specific cytopathogenic changes. On initial isolation the maximum hæmadsorption was observed five days after inoculation. On subsequent passage hæmadsorption was detectable as early as 48 hours after inoculation, reaching its maximum by the end of 72 hours. Attempts at propagation of this virus in HeLa cell tissue cultures were unsuccessful both with the original specimens and with monkey-kidney passage virus. Even after four blind passages neither hæmadsorption nor hæmagglutination with HeLa tissue culture fluids was evident.

Embryonated eggs at various stages of development (7 to 11 days of incubation) were inoculated into amniotic sacs in triplicate series and each series incubated at different temperatures (34° C., 35° C. and 37° C.). In this manner only 13 isolations were made from 44 specimens found to be positive by tissue culture methods. Neither age of the embryos nor the different temperatures had any apparent effect on the results of attempts at isolation. Growth of the virus in eggs under the various conditions tried was rather poor. Hæmagglutination technique was used for the detection of the virus in amniotic fluid. Antiserum prepared for one of the chick embryo isolates neutralized and inhibited hæmadsorption of several tissue culture isolates that failed to grow in the chick embryo, thus indicating that isolates from both sources were antigenically the same.

SOME OF THE CHARACTERISTICS OF THE ISOLATED VIRUS

Sufficient evidence has been obtained to demonstrate that the isolated agent is a virus. This is shown by its ability to pass through a bacteria-tight filter (Fritted Disc, ultra-fine porosity) and its failure to grow on artificial media (tryptose broth, tryptose agar, blood agar, MacConkey's and brain broth) under various conditions. Further, no organisms were demonstrable in the stained smears of infected tissue culture fluids.

The virus, which henceforth will be referred to as 433 virus, grew quite readily in monkey-kidney tissue cultures. The usual infective titre ($TCID_{50}$) of the fluids was found to be 10^{-8}. The virus, while quite stable at 4° C., was inactivated at 58° C. in 30 min. without destruction of hæmagglutinating activity. The virus was also inactivated by 20% ether within 20 min. Both guinea pig and human type O erythrocytes were equally suited for

hæmadsorption and hæmagglutination reactions. The latter reaction took place equally well at 24° C. and 4° C. The virus was adsorbed by and readily eluted from erythrocytes. Heating at 58° C., however, altered it, so that while still adsorbable it could not be eluted.

RELATIONSHIP TO OTHER VIRUSES

To establish the relationship of 433 virus to other viruses, neutralization, hæmadsorption inhibition and hæmagglutination inhibition tests were performed. In hæmagglutination inhibition, either untreated infected tissue culture fluid or virus concentrated by adsorption on red blood cells and subsequent elution was used. All sera for this test were treated with potassium periodate.[4] Hæmadsorption and hæmagglutination were not inhibited and the virus was not neutralized by various influenza antisera, NDV, mumps, Copenhagen 222 or hæmadsorption Type II[5] high-titre antisera.*

Hæmadsorption Type I[5] antiserum, however, did inhibit four hæmagglutinating units of 433 virus in the dilution up to 1:160, but the same antiserum diluted 1:10 failed to inhibit hæmadsorption and failed to neutralize 433 virus. Hæmadsorption inhibition titre of this antiserum with homologous virus was 1:640.

When human type O erythrocytes after adsorption and elution with 433 virus were treated with 1% trypsin for one hour, they lost their ability to agglutinate in the presence of 433 virus and hæmadsorption Type I and Type II viruses but still were agglutinable by influenza A/Asian virus. This indicates that the receptor for 433 virus is different from that for influenza, but apparently is the same as that for the two types of hæmadsorption viruses.

RESULTS WITH PATIENTS' SERA

To establish the etiological significance of 433 virus, acute and convalescent phase sera of patients from whom the virus was isolated were tested by hæmadsorption and hæmagglutination inhibition techniques. In all the paired sera tested the titre, by both methods, was at least four times as great in the convalescent as in the acute phase sera.

COMMENTS

On the basis of the evidence obtained the isolated agent must be considered a virus. The virus grows well in monkey-kidney tissue culture and is readily isolated from throat washings.

So far this virus has failed to produce specific cytopathogenic changes in monkey-kidney tissue cultures. However, this is subject to further investigation in the light of the observation made by Pelon *et al.*[6] that cellular changes in case of 2060 virus were affected by the fluid phase of the tissue cultures. It has been established that 433 virus is not related to any of the influenza viruses, NDV, mumps, Copenhagen 222 or hæmadsorption Type II viruses. On the other hand, preliminary work indicates that an antigenic relationship but not identity may exist between 433 virus and hæmadsorption Type I virus. Further investigation of this point is in progress. If the preliminary results are confirmed and further evidence of antigenic relation is obtained, perhaps it would be justifiable to call this virus hæmadsorption Type III, at least for the time being.

Demonstration of antibody response and isolation made from numerous specimens submitted from various parts of the province would seem to exclude a simian origin for 433 virus and to indicate that it played an etiological role in this rather extensive outbreak of influenza-like illness.

It is of interest to note that in one case the virus was isolated on two different occasions, three weeks apart, from a patient whose convalescence was slow.

Detailed study of this virus is in progress and the results will be reported at a later date.

SUMMARY

During the recent outbreak of influenza-like illness in Ontario, a hæmadsorption virus was isolated. This virus was found to be different from any of the influenza viruses, NDV, mumps, Copenhagen 222 and hæmadsorption Type II viruses. Further, it has been established that it is serologically related to but not identical with hæmadsorption Type I virus.

The authors wish to express their thanks to many physicians, medical officers of health and members of the medical units of the armed forces for their co-operation in supplying specimens, sera and clinical data.

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*Various specific antisera were kindly supplied by the following investigators: Influenza antisera for PR8, FMI, A/Asian Japan 305, Lee, Swine and NDV by Dr. J. Crawley of the Connaught Medical Research Laboratories; antisera for influenza C and D/Sendai and mumps by Dr. F. P. Nagler of Dominion Virus Laboratories; antiserum for Copenhagen 222 by Dr. K. B. Petersen of the Statens Serumins.itut and antisera for hæmadsorption Type I and II viruses by Dr. R. J. Huebner of the National Institute of Health.

THE COMMON BILE DUCT

The common bile duct was measured with calipers in 112 patients operated upon at the Mayo Clinic for disease of the biliary tract. The diameter of common ducts containing no pathological changes varied from 4 to 17 mm. and averaged 8.85 mm. Ducts containing stones varied in diameter from 7 to 17 mm. and averaged 10.90 mm. The largest duct sizes were noted in cases of obstructive jaundice due to carcinoma of the ampulla of Vater or pancreas. It is concluded that duct size alone is not a reliable sign as to whether or not the common bile duct contains stones.—D. O. Ferris and J. C. Vibert, *Ann. Surg.*, 149: 249, 1959.