Biological and Serological Properties of Frater Virus — a Cytopathogenic Agent Associated with Aseptic Meningitis

By

Ian B. R. Duncan*

With 2 Figures

(Received February 13, 1961)

During the late summer and autumn of 1959 an epidemic of aseptic meningitis took place in Scotland due to a cytopathogenic agent which could not be identified as any known virus. Sixty nine cases were found which showed definite evidence of infection with the virus and a typical clinical picture of aseptic meningitis (1, 2). Increases in antibodies to the virus during the course of the illness in the sera of these patients, together with a much higher rate of isolation of the virus from aseptic meningitis patients than from control patients, strongly suggested that the virus did cause the disease. The virus is meantime being referred to as Frater virus after the type strain. In a short preliminary account of the epidemic and of the virus (1) brief reference was made to the biological and serological properties of Frater virus which indicated that it probably belonged to the ECHO group. These properties are now reported in full, and in the light of the findings the classification of Frater virus is discussed.

Materials and Methods

Virus strains. Seventy five strains of Frater virus were examined; all were isolated in human thyroid, human amnion, or monkey kidney tissue cultures from specimens of faeces examined in this laboratory. Strains 1 to 6, and 1 and 2 in particular, were studied most intensively. Strain 1 was isolated from an infant with a disease clinically typical of paralytic poliomyelitis, who showed no virological evidence of infection with poliovirus. The purity of this strain was ensured by making a series of 3 terminal dilutions. Sixty two strains including strains 2, 3, 4 and 6 were from patients with aseptic meningitis, but as 2 of them were obtained from the same patient they really repre-

* Present address: St. Joseph’s Hospital, London, Ontario, Canada.
sent only 61 different strains. The remaining 12, including strain 5, were from patients treated in hospital for diseases other than aseptic meningitis. The strains of polioviruses and Coxsackie and ECHO viruses were the same as those used in previous work (3) except that the Brunenders strain of polio-virus type 1 was used instead of the Mahoney strain.

**Sera.** An antiserum to each of strains 1, 2, 3, 4, 5 and 6 of Frater virus was prepared by injecting a guinea pig intra-muscularly at 4-day intervals with 1 ml. of virus seed in a course of 7 injections. Antisera to polioviruses types 1, 2, and 3 were hyperimmune monkey sera obtained from the Virus Reference Laboratory, Colindale, England. Antisera to Coxsackie viruses A 9, B 1, 2, 3, 4, and 5 and ECHO viruses 1 to 20 except 10 and 16 were prepared in this laboratory by immunising rabbits.

**Pathogenicity tests.**

a) **Animals.** 0.03 ml. of 10th tissue-culture-pass seed of Frater virus strain 1 was inoculated intracerebrally into each of 6 adult mice which were observed for 4 weeks. Tissue culture fluid of between the 6th and 10th passage of each of 12 strains of Frater virus was injected intra-peritoneally into a family of suckling mice in a dosage of 0.03 ml. per mouse. With 3 strains the mice were given 0.01 ml. of virus seed intra-cerebrally in addition to the 0.03 ml. intra-peritoneally. Aliquots of 23 faecal extracts from which strains of Frater virus were isolated were each injected into a family of suckling mice intra-peritoneally in a dose of 0.03 ml. per mouse. All suckling mice were observed for at least 2 weeks.

b) **Chick embryos.** 0.1 ml. of 10th pass seed of Frater virus strain 1 was inoculated on to the chorio-allantoic membranes of 3 14-day hen’s eggs, intra-amniotically to 3 12-day eggs, intra-allantoically to 3 12-day eggs, and into the yolk sac of 3 7-day eggs. The eggs inoculated by the yolk sac route were observed until just short of hatching and the others were killed and examined at 3 and 4 days.

**Haemagglutination.** 7 strains of Frater virus including strains 1 and 2 were grown in tubes of the same batch of human amnion tissue cultures. A control preparation was made by harvesting uninoculated tubes of this batch containing the same maintenance medium in the same way as the virus-infected cultures. The 7 viruses and the control fluid were tested for haemagglutination by mixing 0.3 ml. volumes of tissue culture fluids and red cell suspensions in plastic plates. Each was tested against a 1% saline suspension of human group 0 red cells and against a 0.5% suspension of fowl red cells of a type known to be strongly agglutinable by vaccinia virus. All these tests were done in replicate at 4°C, 23°C, and 37°C.

**Preparation of tissue cultures.** All the types of tissue used were grown in 4 x ½ inch test tubes in stationary racks. Human thyroid, human amnion, rhesus monkey kidney and guinea pig kidney cells were obtained by trypsinization and used to prepare first generation cultures. Propagation medium was Hanks’ solution + 10% human serum for thyroid, Hanks’ + 20% human serum for amnion, Hanks’ + 10% human and 10% calf serum for monkey kidney, and Hanks’ + 10% calf serum for guinea pig kidney. Details of the methods of growing thyroid (3) and amnion (4) are given elsewhere. All 4 tissues were maintained in Earle’s solution + 5% calf serum. HeLa cell tube cultures were propagated and maintained in Hanks’ solution + 4% calf serum from stock cultures grown in 340 ml. flat bottles in Hanks’ solution + 20% human serum. All these media contained also 0.5% lactalbumin hydrolysate and antibiotics.

17*