RELATION BETWEEN VIRUSES FROM ACUTE GASTROENTERITIS OF CHILDREN AND NEWBORN CALVES

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Summary

The reovirus-like particles present in faeces of young children with acute gastroenteritis and the virus causing acute diarrhoea in newborn calves were found to be indistinguishable from each other in size and shape. The human and calf viruses were agglutinated by sera from convalescent calves. Some adult human sera neutralised the infectivity of the calf virus in tissue-cultures. Sera from convalescent children agglutinated both human and calf viruses and gave a positive immunofluorescent reaction with calf virus in cell-cultures. Since these viruses differ morphologically both from reoviruses and orbiviruses, the name “rotavirus” is suggested for them.

Introduction

Reovirus-like particles have been discovered in the faeces of young children with acute gastroenteritis. Reports that a similar virus causes acute diarrhoea in newborn calves in Nebraska and Berkshire, and that there are antibodies to the calf virus in some human sera prompted us to compare the morphology and antigenic properties of human and calf viruses. Since the human virus has not yet been cultured in vitro, all comparisons were not possible.

Samples of human and calf faeces and human and ovine sera were exchanged by the Compton and Birmingham laboratories. The virus-neutralisation experiments described below were carried out at Compton. The other comparative tests were done independently in both laboratories.

Materials and Methods

Antibodies.—Serum samples from adults were selected at random from specimens submitted to a hospital for routine biochemical investigation. Small amounts of sera from acutely ill and convalescent patients were available from young children admitted to hospital with acute gastroenteritis. These sera were obtained 2–3 days and >10 days, respectively, after the onset of disease. A concentrated sample of pooled γ-globulin from adults was also used.

Virus morphology.—Human and calf viruses were concentrated and purified from the faeces of several different children and calves. Human virus was prepared by differential centrifugation, and calf virus by differential centrifugation or differential centrifugation and sucrose-gradient centrifugation. The latter technique was needed to adequately clarify several calf faecal samples. Virus preparations were negatively stained with 2% potassium phoshpotungstate at pH 5–5 and examined by electron microscopy.

Immunoelectron microscopy.—For human and some calf faecal samples, 0-1 ml of serum was mixed with 0-5–1-0 ml of a virus suspension, which was prepared by resuspending the virus deposit after concentration at 180,000–300,000 g for 30 minutes in an appropriate amount of phosphate-buffered saline (pH 7-4). After standing at room temperature for 2 hours or at room temperature for 2 hours and 18 hours at 4°C, the mixture was made up to 5 ml with p.B.S. and centrifuged at 180,000–300,000 g for 30 minutes. The deposits were suspended in distilled water and negatively stained. This technique was modified for some calf samples which required further clarification—0-02 ml volumes of serial antiserum dilutions were added to equal volumes of virus suspensions prepared by differential centrifugation and sucrose-gradient centrifugation as described above. After incubation either at 37°C for 2 hours or 37°C for 2 hours and overnight at 4°C, a drop of the mixture was negatively stained as described above and examined in the electron microscope.

Virus neutralisation and immunofluorescence.—The method was similar to that described by Mebus et al. Serial dilutions of human and calf sera were mixed with equal volumes containing 10–10 T.C.D.50 of tissue-culture-adapted calf virus, held at 37°C for 1 hour, and inoculated to coverslip cultures of calf-kidney cells. Virus multiplication was determined by an indirect immunofluorescent test after incubation for 7 days. To avoid non-specific inhibitory factors most of the calf and human sera were tested at a dilution of 1/40 or above.

Human and calf sera were also tested for antibody as shown by immunofluorescence with the calf virus in calf-kidney cells.

Results

Virus morphology.—No difference in size or structure could be discerned between the human and calf viruses in either laboratory. Intact virus particles were 60 nm. to 80 nm. in diameter. They consisted of an error caused by an unsuspected fault in the microscope. (The dimensions given by Flewett et al. were incorrect, because of an error caused by an unsuspected fault in the microscope.) In electron micrographs these viruses differed in appearance from the reoviruses and also from blue-tongue virus, which does not possess the...
well-defined circular outline of the calf and human gastroenteritis viruses.

*Immu-no-electron microscopy.*—Sera from convalescent children caused agglutination of both human and calf viruses (figs. 1 and 2). Clumps of 4–40 or more particles could be seen at low magnification, while at higher magnifications strands of globulin could be seen linking the virions together. Some agglutination was detectable even in sera taken 2–4 days after the onset of gastroenteritis, but globulin strands could not be clearly identified in these preparations. Serum from convalescent calves agglutinated both complete calf-virus particles, and also those lacking the outer capsid layer.

**Virus neutralisation and immunofluorescence.**—Of sera selected at random from eighteen adults, fifteen had titres of less than 1/40, two had titres of 1/40, and one a titre of greater than 1/80 against the calf virus. Subsequent results showed that four of these sera had titres of less than 1/20. These titres are in the range normally found in cattle sera. The pooled human γ-globulin preparation had a titre of 1/320. Titres increased from less than 1/10 before infection to 1/640 4 weeks after experimental or natural infection by calf virus in calves. At 10 days inhibitory titres were just detectable—i.e., 1/10 to 1/20. Sera taken at 8–10 days from young convalescent children admitted to hospital with acute gastroenteritis did not demonstrate any neutralising activity against calf virus at a 1/10 to 1/20 dilution.

Human and calf sera possessing serum-neutralising-antibody fluoresced with calf virus in calf-kidney cell-cultures. Fluorescence was also seen with some human sera which did not neutralise the calf virus.

**Discussion**

The morphological and serological results suggest that the human and calf viruses are related, probably possessing a serologically similar internal capsid protein. We could discern no difference in size or structure between the two viruses. There was no increase in neutralising antibody to calf virus in the sera of acutely ill and convalescent children, although some sera from adults did neutralise the calf virus. This may mean little, since 10 days after infection calves had very little neutralising antibody against their own virus, and possibly neutralising antibodies develop slowly in children also. However, the observations that several sera from convalescent children reacted with the calf virus by immunofluorescence and
immunoelectron microscopy but not by serum neutralisation, and that sera from convalescent calves agglutinated the calf virus, suggest that the two viruses share a common group antigen but may not be identical. This view is reinforced by our inability to transmit the disease to calves inoculated orally with the human virus and to confer protection against subsequent challenge with the calf virus.12

The calf and human viruses differ from the orbiviruses both in morphology and antigenic properties. The calf virus is antigenically unrelated to blue-tongue viruses,13,14 and is known to be acid resistant, whereas the orbiviruses are acid labile.16 As far as we can judge from published micrographs, the calf and human viruses also differ in morphology from the orbiviruses. We propose therefore that these orbivirus-like or orbivirus-like enteritis viruses of calves and children are in a group separate from both orbiviruses and reoviruses, and we suggest that the term "rotavirus" (from the Latin -virus, a wheel) should be used for them.

Requests for reprints should be addressed to T. H. F.

REFERENCES

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TEMPERATURE AND WEATHER

CORRELATES OF MYASTHENIC FATIGUE

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Summary The myasthenic neuromuscular failure tested by nerve stimulation and recording of muscle responses is aggravated by local warming and relieved by local cooling. Small alterations of temperature can elicit quite striking changes. False-negative diagnostic tests are sometimes related to a spontaneous lowering of the muscle temperature, and temperature should be checked routinely. Clinical correlates are consistent in showing that the affected muscles are influenced by even small local temperature changes, such as occur in daily living. This suggests a number of practical recommendations which can help these patients.

Introduction

Patients with myasthenia gravis vary a great deal in the extent and severity of muscle weakness and fatigue. The amount of paresis of the affected muscles is typically aggravated by exercise (post-activation exhaustion) and improved by rest or by anticholinesterase drugs, but it has not been hitherto appreciated that small changes in muscle temperature also have a profound effect. Besides its interest for neuromuscular pathophysiology this finding suggests a number of practical points for the diagnosis and treatment of myasthenia.

In clinical neurophysiology and electromyography muscle temperature is recognised as a significant factor which can jeopardise diagnostic conclusions when not properly controlled. For example, the spontaneous fibrillation after denervation may not be recordable in a cold muscle with slightly impaired blood circulation. On the other hand, mild myotonic discharges are potentiated by local muscle cooling. Tissue temperature has a striking influence on motor and sensory nerve-conduction velocity which drops by about 3 m. per second for each fall of 1°C. Intramuscular temperatures of 28–31°C are by no means exceptional in the distal limbs of patients examined in a room at 22°C, and the nerve-conduction slowing related to such cooling ought to be differentiated from the genuine reductions recorded in polyneuropathies. The distal motor latency of the muscle electrical response to a nerve stimulus is also much increased by cooling which prolongs the neuromuscular refractory period for repetitive electrical stimulation of the motor nerve. Microphysiological data in animals would not have predicted that myasthenic neuromuscular block is dramatically aggravated by local warming and that it can be relieved by local cooling. Pharmacological studies have shown that the neuromuscular block by d-tubocurarine improves with cooling while the block by depolarising drugs such as decamethonium or succinylcholine is potentiated by cold. Although the molecular mechanisms of the block produced by a competitive inhibitor such as d-tubocurarine are entirely different from those in myasthenia gravis, the safety factor for neuromuscular transmission is reduced in both cases and adversely affected by even a small rise in the local temperature.

Patients and Methods

We have investigated thirty patients between 10 and 86 years old with an unambiguous diagnosis of myasthenia gravis. A wide spectrum of clinical severity was displayed in this series, from the mild localised myasthenia of recent onset to the generalised long-standing form of the disease. The diagnosis had been established by clinical signs of muscle weakness and fatigue. The safety factor for neuromuscular transmission is reduced in both cases and adversely affected by even a small rise in the local temperature.