

Prevalence of cytomegalovirus in France*

BY M. JEDDI, O. G. GAUDIN AND R. SOHIER

*Section de Virologie du Laboratoire National de la Santé Publique,
8, Avenue Rockefeller, Lyon 8e, France*

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SUMMARY

Between October 1968 and February 1970, 30 strains of cytomegalovirus were isolated from the urine of children admitted to hospitals in Lyon. Three groups of children up to the age of 14 years have been investigated.

The first group consisted of 304 newborns and infants up to the age of 1 year; cytomegalovirus was grown from five of these (1.6%). Among these five children, two had cerebral disorders. None of them had ever shown any sign of typical CMV infection.

The second group comprised 102 children between the ages of 1 and 14 years, from a special service for neurological and mental diseases. Cytomegalovirus was grown from 19 (18.6%).

The third group was 27 children also between 1 and 14 years of age, admitted to hospital for miscellaneous diseases excluding cerebral disorders; cytomegalovirus was grown from six (22.2%).

It appears that cytomegalovirus has a very low incidence in neonatal disease. The virus spreads at a higher rate in children 1–14 years old. No difference has so far been shown in the excretion rates of two groups of children, one with cerebral disorders and one with other diseases, but the number of children in the last group is too small to allow definite conclusions to be drawn.

INTRODUCTION

Several studies carried out in different countries have shown that cytomegalovirus (CMV) may be involved in pathological processes in new-born as well as in older children. Among the older children the virus has been incriminated as the origin of mental defects.

In a previous study (Jeddi, Gaudin, Terraillon & Sohier, 1969) we have investigated the immunity to CMV of a sample of the French population by means of the complement fixation test. We found that the percentage of positive sera increased with age at roughly the same rate in France as in other countries. At the age of 20, 50% of the population have acquired CMV antibodies.

The present paper reports a study set up in Lyon on different groups of patients to establish the frequency of infection during childhood.

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MATERIALS AND METHODS

Collection of urine

Urines were collected from 433 children for first isolation. In addition, eight samples were collected for re-isolation of the virus from children who had been shown to be excreting. Samples were collected in sterile containers, refrigerated and rapidly transported to the laboratory, where cultures were put up within 3–4 hr. after collection.

Tissue culture

The cell line W.I. 38 was grown in plastic bottles.* The growth medium was Eagle's medium† to which 10% calf serum was added. For maintenance, medium 199‡ was supplemented with 2% foetal bovine serum.§

Virus isolation

Each sample of urine was treated with antibiotics, the following amounts being added to 3 ml. of urine: penicillin, 10,000 units; streptomycin, 10 mg.; kanamycin, 5 mg.; Negram (naladixic acid), 2 mg.; nystatin, 5000 units. The samples were then kept at 4° C. for 30 min., after which 0.3 ml. of each urine was allowed to adsorb on a cell sheet of 25 cm.². After incubation at 37° C. for 3 hr. the medium was changed. The cultures were observed for cytopathic effect (CPE) for a period of 2 months, during which the medium was changed twice weekly. At the end of the observation period about 100 TCID₅₀ of echovirus type 11 was inoculated on the cells which showed no CPE, to test the susceptibility of the cells at the end of the experiment and also to detect any possible interfering agent. The strains of virus were characterized by the appearance of the degeneration and cytology after staining with Erythrosin Orange G Toluidin Blue.

RESULTS

Isolation of cytomegalovirus

Group 1; 304 newborns and infants. All these patients, under 1 year of age, had been admitted to a paediatric clinic for miscellaneous syndromes excluding typical CMV inclusion disease at birth. Five strains were isolated (Table 1). The clinical histories of the five positive children may be summarized as follows: (1) 1 year old: lower respiratory infection; (2) 1 year old: neonatal cerebral defect; (3) 1 year old: neonatal cerebral defect; (4) 10 months old: mild gastroenteritis; (5) 7 months old: vomiting at the time of isolation of CMV; hepatitis occurred 3 months later.

Groups 2 and 3; children from 1 to 14 years of age. Group 2 consists of 102 children admitted for long-term stay in a service for neurological and mental diseases, and group 3 consists of 27 children admitted for miscellaneous diseases, excluding cerebral disorder. The results are shown in Table 1. It is seen that the percentage of

* Falcon Plastics (Becton Dickinson).

† Dried MEM medium from Wellcome Laboratories, London.

‡ Ten times concentrated, from B.D. Merieux, Lyon.

§ From SORGA laboratories, Paris.

positives is in each case much higher than in group 1; it is roughly the same in these two groups, but the small number of children examined in group 3 prevents any definite conclusions.

Table 1. *Results of isolation of cytomegalovirus in 3 groups of children*

	Total no. of children investigated	No. of children excreting cytomegalovirus	Percentage
Newborns and infants (1 year)	304	5	1.6
Children 1-14 years with cerebral disorders	102	19	18.6
Children 1-14 years without cerebral disorders	27	6	22.2
Total	433	30	

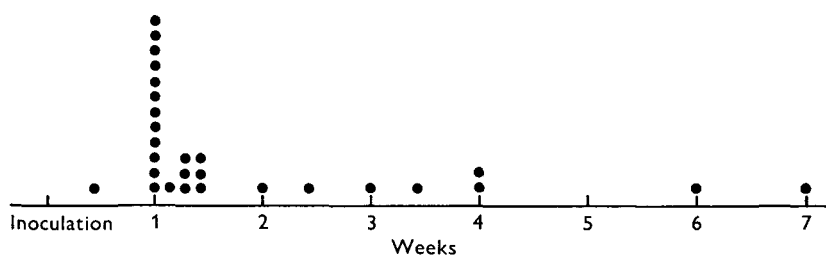


Fig. 1. Rate of appearance of cytopathic changes in W.I. 38 cell cultures at first isolation of cytomegalovirus from urine.

Speed of appearance of cytopathic effect in tissue culture

Fig. 1 shows the time of first appearance of CPE on W.I. 38 cells. It is seen that most of the viruses can be detected in 7-10 days after inoculation, though a few may show a delay of several weeks.

Duration of excretion of cytomegalovirus

In eight cases it was possible to get a second specimen of urine from which the virus was isolated again. In three of these eight cases a third specimen was obtained; these three were positive at 101, 116 and 182 days after the first evidence of viral infection.

DISCUSSION

The incidence of cytomegalovirus infection in neonatal disease does not appear very important in spite of the percentage of infants excreting the virus. This confirms the findings of other authors in Europe and North America (Cherry, Soriano, Jahn & Wis, 1968; Feldman, 1969; Krech, Jung, Bärlocher & Sege, 1968; Sanders & Cramblett, 1968; Starr & Gold, 1968; Stern, 1968), although a higher incidence of CMV has been found in Japan by Chiba, Osaki, Hanazono & Nakao (1968).

No clear evidence of disease related to CMV has been found in the course of our

study, with the possible exception of the two cases of cerebral defect among the five positive infants in the first group, and also the case of hepatitis which developed 3 months after first isolation of the virus. It should be noted that in this last case no typical inclusions have been shown by histological examination of a liver biopsy. Some previous reports have mentioned the possibility of cerebral lesions (Crome & France, 1959; De Fouquet, 1956; Marie *et al.* 1957; Nezelof, Gaquière & Brousse, 1961), and more recently Hanshaw (1966) and Stern, Elek, Booth & Fleck (1969) have drawn attention to a possible connexion of CMV with mental defects. We are not yet able to confirm this connexion, and in the two groups of children between 1 and 14 years of age which we investigated the excretion rates for CMV appear to be roughly the same.

Although our control group is as yet too small to provide quite reliable figures, we wonder whether the conditions of life of mentally retarded children in specialized communities may not be responsible for the high prevalence of CMV among them, since the wide spread of viruses in hospital wards is a well-known phenomenon. A similar percentage of excretors has been found in a population of poor economic status by Li & Hanshaw (1967).

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