COXSACKIE VIRUS INFECTION IN HUNGARY

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Since the discovery by Dalldorf and Sickles (1,2) of a new group of viruses pathogenic for newborn rodents and capable of inducing human infection, numerous reports have been published in the USA concerning the incidence of this group of agents as well as its epidemiological importance (3, 4, 5). Very soon the pathogenic and epidemiologic role of this virus was discussed even in Europe. In Denmark (6), France (7), Switzerland (8), England (9), and later in Germany (10) the virus was isolated from cases appearing in various clinical forms.

During the last two years attempts have been made in our institute to isolate the virus first of all from cases which seemed clinically characteristic. The investigations included 4 cases of clinically typical poliomyelitis, 4 cases of myalgia, 8 cases of serous meningitis, and 8 cases of febrile disease of uncertain diagnosis. Further seven cases were studied in 1952, and from one of the patients with a diagnosis of nonparalytic poliomyelitis C virus was isolated. This finding offered new data on the geographical occurrence of Coxsackie viruses. It seemed of interest to give a short account of our observations.

Case history. E. M., female, aged 15 months, was admitted to the Department of Paediatrics of Szeged University Medical School on the 28th of July, 1952. Her illness had begun two days earlier with fever and on the day prior to admission she became unable to stand.

State at admission. The patient was a well developed child with a normal skeletal system. She was prostrated, unable to stand or walk. The pharyngeal mucous membrane was inflamed. Nuchal rigidity was present, Brudzinski's sign was just noticeable. Reflexes were depressed and there was loss of the right patellar reflex. The right lower extremity was atonic but the active movement of the toes was spared. In the CSF, pressure was slightly elevated; Pándy's test opalescent; fibrin negative; cell count 45, with 90 per cent lymphocytes, 10 per cent leucocytes; sugar content, 47 mg per 100 ml; protein, 44 mg per 100 ml. Blood counts were normal.

Clinical course. During the first 12 days of the illness the patient was subfebrile. In the next 5 days the temperature varied between 38 °C and 39 °C, then the patient became subfebrile again, and on the 21st and 22nd days of the disease a rise of temperature to 39 °C occurred. At the same time an impetiginous eruption appeared. After penicillin administration the temperature returned to the normal level. On the 8th day of the disease the patellar reflex returned; adynamia and prostration were also somewhat improved. Subsequently, the adynamic state and the atonicity of the right lower extremity improved and the child was discharged completely recovered.

The method of the isolation and study of the virus is summarized as follows.

The virus was isolated from faeces of which a 10 per cent emulsion in saline was made and sedimented with centrifuging at 10 000 rpm for 30 minutes; then the supernatant was collected and 2000 units of penicillin and 2 mg of streptomycin per ml were added. The material was inoculated subcutaneously into mice 1 to 3 days of age. Usually 6 to 8 animals were inoculated with each sample and the amount injected was 0,03 ml. The animals were kept under observation for two weeks and were carefully checked every day. Animals revealing characteristic symptoms of infection were sacrificed in a moribund state; from their musculature and brain a suspension was prepared and inoculated into a new litter.

The demonstration of neutralizing antibodies in the sera of the patient and of animals immunized with the virus was carried out as follows. The brain and the musculature of a number of animals infected with the virus was pooled and a 20 per cent suspension was made in broth, centrifuged, distributed in ampoules, stored in frozen state and used as a stock of virus material. The infectivity of this suspension was titrated by inoculating with a tenfold dilution several groups of animals. The *Reed-Muench* (11) method was used to calculate the 50 per cent endpoints. Various dilutions of serum were mixed with an equal volume of the tissue extract containing 200 DL_{50} per 0,03 ml of the virus, and allowed to stand at room temperature for 60 minutes Six to eight newborn mice were then inoculated subcutaneously with 0,03 ml of each of the mixtures. Thus 100 DL_{50} of virus was administered to each animal.

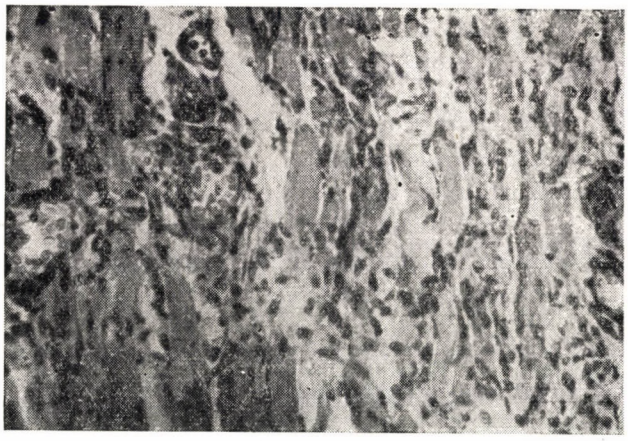

Fig. 1. Muscle tissue of a mouse infected with virus isolated from the patient

The isolation of the virus was successfully attempted for the first time on the 13th day of the disease. All of the animals inoculated with the specimen became partly or totally paralyzed and died on the 3rd day. The suspensions made from both the musculature and brain of the animals proved to be bacteriologically sterile. The suspension of the brain as well as that of the musculature inoculated into a new litter caused similar symptoms and death of the animals. The same materials filtered through a Berkefeld N candle was infective also for newborn mice. The DL_{50} value of the suspension prepared from the brain and muscle tissue obtained in the 2nd passage was 10^{-6}. Further passages are in progress.

Mice 14 days old inoculated with the virus material remained free from symptoms even after a period of 3 weeks. Histological examination of the mice dead following virus inoculation revealed findings ccharateristic of C-virus infection. Fragmentation of striated muscle fibres, sometimes their hyalinous degeneration with interstitial cellular infiltration, occurred (Fig. 1). In the brain, circumscribed destruction of nerve cells and round cell infiltration of the meninges were observed (Fig. 2).

The isolation of the virus was successfully repeated from the same specimen of faeces and from another sample obtained on the 19th day of the illness. An attempt was made to identify the type of our strain with a neutralization test using hamster immune sera corresponding to the types A_1, A_2 and B of *Dalldorf*. As these sera did offer no protection against our strain, it was concluded that the virus strain isolated from the patient does not correspond to the types mentioned above.

Fig. 2. Brain of the same animal

Serological investigations carried out with the patient's serum proved the relation between the virus findings and the disease. The results of the neutralization tests performed with the sera obtained on the 16th and 31st days of the disease are summarized in Table 1.

It is obvious that the blood serum taken on the 16th day showed only an insignificant neutralizing effect which could hardly be evaluated owing to the fact that it protected in a dilution of 1 to 2 only the half of the animals inoculated. The specific nature of the infection is supported by the fact that by the 31st day of the disease an immense increase of the virus neutralizing antibody occurred in the patient's serum, the 1 to 250 dilution of which has already offered a practically full protection.

Table I

Results of neutralization tests on sera of patient M. E. against an infective dose of 100 DL_{50}

Serumdilution	Day of bleeding after onset of disease	
	16th	31st
1 : 2	3/6	0/6
1 : 10	5/7	0,7
1 : 50	5/5	0/7
1 : 250	5/5	1/7
Control 100 DL_{50} .	6/6	6/6

Numerator = number of animals succumbed
Denominator = number of animals inoculated.

Discussion

From the faeces of a female child admitted with the diagnosis of atypical poliomyelitis to the Department of Paediatrics in Szeged a strain with properties characteristic of the Coxsackie viruses was isolated. The relation between the virus finding and the disease was demonstrated by the fact that in the course of the disease the occurrence of a specific antibody could be registered in increasing amounts in the blood serum. Attempts to identify the strain with three of the known types of C-virus remained unsuccessful. In want of proper immune sera, identification with other types could not be attempted.

The clinical picture of our case corresponded to an atypical poliomyelitis as well as to a characteristic form of C-virus infection. Although the C-virus infection was supported by both the clinical course of the illness and the early convalescence, theoretically the possibility of such an infection mixed with poliomyelitis virus cannot be excluded. Owing to the lack of facilities, demonstration of poliomyelitis virus could not be attempted.

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SUMMARY

A strain of Coxsackie virus could repeatedly be isolated from the faeces of a female child 15 months of age. The strain did not prove identical with the strains of the types A_1, A_2 and B of *Dalldorf*. The virus was neutralized even by high dilutions of the patient's blood serum. The observations have proved the occurrence of C-virus in Hungary.

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ИНФЕКЦИЯ ВИРУСОМ COXSACKIE В ВЕНГРИИ

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Р е з ю м е

Из кала 15-летней девочки, лежащей в Детской Клинике г. Сегед с диагнозом атипичного полимиелита, нам удалось больше раз выделить штамм вируса Coxsackie. Выделенный штамм не оказывался идентичным штаммам типа A_1, A_2, B Даллдорфа. Сыворотка больной нейтрализовала вирус до высоких разведений. Этот случай свидетельствует о наличии вируса C в Венгрии.