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Type A7 Coxsackie (type 4 poliomyelitis) virus infection in Scotland

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INTRODUCTION

In 1952, Russian workers isolated strains of what they considered to be a fourth type of poliovirus from cases of clinically typical paralytic poliomyelitis (Chumakov, Voroshilova, Zhevandrova, Mironova, Itzelis & Robinson, 1956). These viruses were later shown to belong to the already-known Coxsackie type A 7 (Johnsson & Lundmark, 1957; Habel & Loomis, 1957). Additional strains were isolated from paralysed patients in a second outbreak in the U.S.S.R. (Voroshilova & Chumakov, 1959), from single cases of paralytic disease in the U.S.A. (Ranzenhofer, Dizon, Lipton & Steigman, 1958) and Scotland (Grist, Larminie, MacGregor, McIntosh, McLaughlin & Sommerville, 1960), and from three of nine cases of paralytic Coxsackie infections reported from Switzerland by Rentsch (1961, and personal communication).

In 1959, Coxsackie A 7 and Frater virus (Duncan, 1960) were jointly responsible for a considerable outbreak of aseptic meningitis in Scotland, a general description of which has been published (Combined Scottish Study, 1961). Although poliovirus was almost completely absent from the community, there were a number of paralytic illnesses. Coxsackie A 7 virus was the infecting agent most frequently associated with paralysis. Details of the Frater virus outbreak were recorded by Duncan (1961). The present paper describes investigations of the 1959 outbreak of Coxsackie A 7 virus infection in Scotland together with additional studies of its prevalence in recent years.

MATERIALS AND METHODS

Population studied

Specimens of stool and blood were received from cases of paralytic poliomyelitis, aseptic meningitis, and similar conditions admitted in 1959 to hospitals in the Western and Eastern Regional Hospital Board areas of Scotland. These areas include the large populations of the Clyde Valley and Dundee. Residues of sera stored at $-40°$ C. were available from similar patients investigated during the three previous years. Serological tests were also performed on the same blood donor sera and gamma globulins tested for Frater virus antibodies by Duncan (1961).

Laboratory methods

A preliminary account of the methods has been reported (Grist, 1960*a*) and a detailed description will be given elsewhere. In brief, isolation of Coxsackie A 7 virus was accomplished by inoculating suckling mice intraperitoneally each with 0·03 ml. of 20 % extract of stool clarified by centrifugation and containing antibiotics. Animals were observed for 14 days; those which became paralysed were killed by ether anaesthesia and examined histologically and virologically. Approximately 20 % extracts of carcasses of paralysed mice were clarified by centrifugation after storage in the frozen state for several days or weeks. These clarified extracts were tested for the presence of haemagglutinins against vaccinia-agglutinable fowl red cells. If these were present, haemagglutination-inhibition tests were set up with fowl antiserum to the local strain 1034/59 which has been shown to belong to type A 7 by serological cross-reactions with Dalldorf's prototype and the Russian ABIV strains (Grist, 1960*a*). Confirmatory tests were made by complement fixation against ascitic fluid from mice immunized with prototype A 7 virus and by neutralization tests in suckling mice with fowl antiserum to strain 1034/59. All the strains of Coxsackie A 7 virus produced myositis of typical group A Coxsackie character in suckling mice.

Tests for neutralizing antibody in sera were carried out in monkey kidney tissue culture using the ABIV strain of virus adapted by Habel & Loomis (1957). Serum dilutions, previously inactivated at 56° C. for 30 min., were mixed with equal volumes of virus (100 T.C.D.$_{50}$ per 0·1 ml.). After 1 hr. at room temperature, each mixture was inoculated in 0·2 ml. volumes into two tissue culture tubes which were incubated at 37° C. The antibody titres were taken as the reciprocals of the highest dilutions protecting the cultures against degeneration caused by virus. Dilutions are expressed as final dilutions of serum in serum-virus mixtures. Poliovirus antibodies were measured by a similar method using human amnion tissue cultures. For haemagglutination-inhibition (H.I.) and complement-fixation (C.F.) tests the antigen consisted of 20 % saline suspensions of skinned, decapitated, eviscerated carcasses of suckling mice paralysed by strain 1034/59 of Coxsackie A 7 virus. These suspensions were stored frozen at −40° C. for some weeks or months, thawed and clarified by centrifugation before use. Their haemagglutinin content was titrated by mixing serial twofold dilutions of antigen in saline with equal volumes (0·3 ml.) of 0·5 % suspension of agglutinable fowl cells: the end-point was determined by observing the pattern of settled cells. For H.I. tests, four 50 % doses of haemagglutinin in 0·3 ml. volumes were added to equal volumes of serial twofold dilutions of serum. Tests were left for 30 min. at room temperature; 0·3 ml. volumes of 0·5 % fowl cells were then added and the tests read after settling for 1 hr. Serum titres were taken as the highest initial serum dilution showing complete or almost complete ('one-plus') inhibition of haemagglutination.

Complement-fixation tests were carried out by the standard small-volume cold-overnight method of this laboratory which was described by Ross (1961).

Standard antisera were obtained from a cockerel immunized with strain 1034/59, and from mice immunized with Dalldorf's prototype A 7 strain (Grist, 1960*a*).

RESULTS

The 1959 outbreak

The clinical and epidemiological features of thirty-four cases of Coxsackie A 7 infection have been described in detail (Combined Scottish Study, 1961). Coxsackie A 7 strains were also isolated in 1959 from three additional patients in hospitals not included in that survey, and the following account is thus based on thirty-seven cases. The outbreak started with one case whose illness began in April, continued with one in May, reached a peak of fifteen cases in June, diminishing to eleven in July, eight in August, and one in September. With the exception of a male aged 21 years, all the cases were children, twenty-three aged 3 years or less. Males (twenty-four cases) predominated. Most of the illnesses were characterized by fever, vomiting, and signs of meningeal irritation. Paralysis affected seven children, in one case fatal. There was a suggestion that illness was more severe in the younger patients (Table 1).

Table 1. *Age and severity of illness*

	Age (years)					
Type of illness	< 1	−2	−4	−6	−21	All ages
Fatal	1	0	0	0	0	1
Paralytic	2	1	2	1	0	6
Aseptic meningitis	2	9	10	4	4	29
Fever and headache	0	0	0	0	1	1

Table 2. *Isolations of Coxsackie* A 7 *virus during June–July* 1959

	Coxsackie A 7 virus in stool		
Category of illness	Present	Absent	Total
Paralytic poliomyelitis	5	1	6
Non-bacterial meningitis	18	39*	57
Other diseases	1	59	60
Total	24	99	123

* Nineteen other enteroviruses isolated.

Coxsackie A 7 *infection and paralysis*

Paralysis affected the face in one case, one upper limb in three cases (one fatal), one lower limb in two cases, and both the right hand and right leg in one case. Recovery from paralysis was generally more rapid than would be expected in typical poliovirus infection, but residual paralysis persisted in at least two cases (Combined Scottish Study, 1961).

Altogether thirteen cases of clinical paralytic poliomyelitis were investigated during 1959. Coxsackie A 7 virus was isolated from the majority (7) of these, Frater virus from one case and type 1 poliovirus from one case. Four of the Coxsackie A 7 and the single Frater virus paralytic infections were in children who had received two or more injections of poliovaccine. The fatal Coxsackie infection

developed a fortnight after injection of the first dose of poliovaccine. The single case of poliovirus infection was unvaccinated.

In order to evaluate the association of paralysis with Coxsackie A 7 virus infection, every available extract of stools received at the laboratory during the peak epidemic months, June and July, was inoculated into suckling mice. The results are summarized in Table 2. It should be noted that of the sixty cases of 'other diseases', twenty-seven of the patients were infants or children in the under-8-year age-group in which most of the Coxsackie A 7 infections were found. There was strong association between Coxsackie A 7 infection and the clinical diagnosis of paralytic poliomyelitis and, to a lesser degree, non-bacterial meningitis. Only one strain of Coxsackie A 7 virus was isolated from the 'other diseases' group, from a 13-year-old girl with fever and headache suggestive of sinusitis (Combined Scottish Study, 1961).

Table 3. *Neutralizing antibody titres to poliovirus in sera of paralytic cases of Coxsackie* A 7 *infection*

	Poliovirus antibody titres						
	First sera			Second sera			
Case	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Poliovaccination history
1	—*	—	—	< 16	< 16	< 16	0
2	8	—	8	8	< 8	8	+
6	—	—	—	< 8	< 8	< 8	?
7	—	—	—	< 8	< 8	< 8	0
15	64	512	64	64	512	64	+

* — = Not tested.

No evidence of poliovirus infection was found by inoculation of human amnion and thyroid tissue cultures with extracts of faeces from these Coxsackie A 7 cases. In addition, neutralization tests for poliovirus antibodies were performed with paired sera which were available from five paralytic cases (Table 3). Poliovirus antibodies were not detected in three, of whom two certainly and one possibly had not been vaccinated against poliovirus infection. Low titres of antibody to Types 1 and 3 were found in case 2 who had received two doses of vaccine; higher titres to all three types of poliovirus were found in case 15 of Table 3 who had received a third injection of poliovaccine 4 weeks previously.

Serological studies of 1959 *cases*

Neutralization tests for Coxsackie A 7 antibodies were performed with available paired sera from eighty-seven patients with aseptic meningitis, paralytic or encephalitic illnesses. The group included twenty patients from whom Coxsackie A 7 virus had been isolated, from eight of whom third sera were collected a year or more after illness. Table 4 summarizes the serological tests of these twenty cases, including the results of H.I. and C.F. tests with Coxsackie A 7 antigens. Neutraliza-

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Table 4. *Serological tests for Coxsackie* A 7 *antibodies in sera of patients from whom Coxsackie* A 7 *virus was isolated*

Case	Age (years)	Stage of illness when serum taken	Antibody titres N.*	H.I.*	C.F.*
1†	< 1	3D	32	—	—
		15D	32	—	—
		15M	16	< 10	—
2†	< 1	2D	64	320	—
		16D	64	—	—
3	1	4D	16	160	—
		14D	32	—	—
4	1	8D	64	320	< 12
		16D	64	320	96
5	2	3D	16	80	—
		14D	64	160	—
6†	2	5D	64	160	—
		16D	64	—	—
		14M	64	80	—
7†	3	1D	64	640	192
		11D	64	160	—
		15M	64	40	32
8	3	5D	32	320	—
		15D	32	—	—
9	3	3D	64	—	—
		14D	64	640	< 10
		12M	64	640	< 10
10	4	4D	8	—	—
		19D	32	640	—
11	4	5D	128	160	—
		15D	64	—	—
12	4	6D	64	320	—
		14D	64	—	—
13	4	2D	128	640	—
		13D	128	320	—
14	5	2D	64	160	—
		15D	64	160	—
		15M	64	80	—
15†	5	4D	64	160	10
		13D	64	—	—
		16M	64	40	< 10
16	6	4D	64	320	—
		16D	64	320	48
		13M	64	320	< 12
17	6	5D	128	320	< 10
		16D	128	320	10
		14M	128	320	< 10
18	13	2D	32	320	—
		13D	32	320	—
19	13	4D	8	< 10	< 10
		14D	128	160	10
20	21	4D	32	—	—
		13D	32	—	—

* N. = neutralization; H.I. = haemagglutination inhibition; C.F. = complement fixation.
† = paralytic case. *D* = *day of illness*; *M* = *months after onset of illness*.

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tion titres ranged from 32 to 128 in second sera, with fourfold or greater rising titres in three cases (5, 10, 19). Haemagglutination-inhibiting antibodies ranged from 160 to 640 in second sera with significantly rising titres in only one (case 19). Observations on C.F. reactions were less extensive, but showed lower titres than the other tests with significantly rising titres in one (case 4). Tests of the third sera showed that neutralizing and H.I. antibody titres were maintained for a year or more after infection, whereas C.F. titres declined to low or undetectable levels.

Evidence of Coxsackie A 7 *infection in other years*

During the first two years of the continuing study of poliomyelitis-like diseases in this laboratory, tissue-culture methods of virus isolation were supplemented by inoculation of suckling mice, particularly in cases of paralytic disease and encephalitis where no causal agent had otherwise been identified. As described elsewhere (Grist *et al.* 1960), Coxsackie A 7 virus was isolated in this way from a child with paralytic illness in 1956, in whose convalescent-phase serum poliovirus antibodies were not detected. Since 1959, suckling mice have been inoculated with stool extracts from cases of aseptic meningitis, paralytic poliomyelitis, and encephalitis from which no virus was isolated in tissue culture. One strain of Coxsackie A 7 virus has been isolated in this period, from a male aged 11 years with aseptic meningitis in 1961.

Table 5. *Distribution of Coxsackie* A 7 *neutralizing antibody titres*

Group	Titres						Totals
	<8	8	16	32	64	128	
1956–57 (cases)	12	4	2	0	0	0	18
1958 (cases)	28	3	4	0	1	1	37
1959 (cases: Coxsackie A 7 virus isolated)	0	0	0	6	12	3	21
1959 (cases: Coxsackie A 7 virus not isolated)	37	10	11	6	2	0	66
Blood donor sera (1958)	63	30	20	1	2	0	116
Blood donor sera (1960)	39	12	41	6	2	0	100

Neutralization tests for Coxsackie A 7 antibodies were performed on sera from a number of aetiologically undiagnosed cases of paralytic and non-paralytic poliomyelitis-like disease in 1956–57–58. The results are shown in Table 5, together with those of tests of the 1959 cases and also of adult blood-donor sera collected in 1958 and 1960. The illness cases have been tabulated by the titres of their second sera. With the exception of those previously mentioned from whom Coxsackie A 7 virus was isolated in 1959, none showed significantly rising titres. In 1958 two cases showed titres which, as judged by the findings in 1959 cases, suggested Coxsackie A 7 infection. Both of these cases were paralytic. In one, a male aged 36, no other investigation had been carried out except for a technically unsatisfactory test of stool in monkey kidney tissue culture, and it is thus impossible to exclude infection by poliovirus which was prevalent at that time (Duncan & Sommerville, 1960). The other, a male aged 4 years, had paralysis of the right biceps, triceps and deltoid,

with partial recovery and residual weakness of the deltoid. No poliovirus was isolated from his stool in monkey-kidney tissue culture. Neutralizing antibody titres of his paired sera were as follows: Type 1 poliovirus, 32 and 64; Type 2 poliovirus, 32 and 32; Type 3 poliovirus, 16 and 32; Coxsackie A 7 virus, 128 and 128; Frater virus, < 8 in the second serum. The poliovaccination status of this child was unknown, but these titres are lower than would be expected in poliovirus infection. In 1959, two patients with aseptic meningitis, from whom Coxsackie A 7 virus was not isolated, showed neutralizing antibody titres of 32 in the first and 64 in the second sera, suggestive of recent Coxsackie A 7 infection. In one, a male aged 6 years, Frater virus was isolated in tissue cultures, and serological tests showed rising titres of antibody to Frater virus. In the other, a male aged 10 years, all tests were negative; no virus was isolated in tissue cultures or suckling mice, and no Frater antibodies were detected.

Serological surveys for Coxsackie A 7 *antibodies*

The results of testing healthy blood donor sera collected in 1958 and 1960 are shown in Table 5. In 1958, antibody was found in 46 % of sera as compared with 61 % in 1960, the year after the outbreak. The second year also shows a larger proportion of sera with higher titres. The distribution of titres in 1960 is bimodal with a main peak at '16', suggesting a mixed population of individuals, the majority with titres similar to the 1958 group, and a minority with a modal titre of 16 which might represent residual antibody from infection in 1959.

Six of the γ-globulin samples investigated by Duncan (1961) were tested for neutralizing antibodies. The following titres were obtained in samples from different years: 1951, 16; 1953, 64; 1956, 32; 1957, 16; 1958, 16; 1959, 16. The globulins had been prepared from blood collected in the east of Scotland; the 1956 and 1958 pools were derived mainly from men of the armed forces. These findings provide further evidence of previous infection of the population of Scotland by Coxsackie A 7 virus. The fourfold higher titre of antibody in 1953 as compared with 1951 might suggest a wave of Coxsackie A 7 infection at some time between collection of the sera from which these globulins were prepared, but it is impossible to be certain of the significance of this finding in the absence of virological studies at that time.

DISCUSSION

The poliomyelitis-like properties of Coxsackie A 7 viruses were recently reviewed by Voroshilova & Chumakov (1959), who studied strains isolated by inoculation of monkeys from paralytic and fatal human infections. These viruses resemble poliovirus in their ability to cause paralysis and neuronal damage in monkeys, and are like Type 2 poliovirus strains in causing paralytic disease of the central nervous system of adult cotton rats. Unlike Type 2 poliovirus they are non-pathogenic for adult mice, but cause paralysis and myositis of a typical Coxsackie A character when inoculated into suckling mice or cotton rats. Antigenically, they show no relationship to poliovirus Types 1–3.

The present paper provides additional evidence that Coxsackie A 7 viruses can

cause in man the clinical syndrome of paralytic poliomyelitis, that they can cause paralysis of persons immunized against poliovirus Types 1–3, and that they can cause outbreaks of paralytic and non-paralytic illness resembling classical poliomyelitis in polio-vaccinated communities. The evidence may be considered under several headings, of which the epidemiological is the most significant since there was no opportunity to isolate virus from the nervous system of a paralysed patient. Thus, during the height of the outbreak, Coxsackie A 7 virus was isolated from 83 % of patients with paralysis, from 32 % with non-paralytic non-bacterial meningitis, and from less than 2 % of those with other diseases in the same area at the same time (Table 2). Coxsackie A 7 virus was isolated from the majority of all paralytic cases investigated during the year 1959, whereas poliovirus was isolated from only one such case during the year and was at such a low level of prevalence as to be almost undetected in surveys of sick persons in the virus laboratories of Scotland (Grist, 1960*b*).

The virological evidence of Coxsackie A 7 virus infection of paralysed patients rests primarily on isolation of the virus from faecal specimens—the standard diagnostic method for poliovirus and other enteroviruses. The serological reactions resembled those found in poliovirus infection since the majority of patients showed peak antibody titres in the first sera examined. Only in four non-paralytic cases were rising antibody titres demonstrated. Neutralizing antibody titres of 64 and over showed strong correlation with presence of virus in the faeces (Table 5). The virological evidence against poliovirus infection of these patients depends on failure to isolate poliovirus from stools by established tissue-culture techniques and the absence of detectable poliovirus neutralizing antibody from the convalescent-phase sera of three unvaccinated paralytic cases; antibody in the sera of two other paralytic cases was probably attributable to previous immunization (Table 3). The stools of one paralytic and one fatal case were additionally tested by intracerebral inoculation of monkeys, which became paralysed and showed virological evidence of Coxsackie A 7 but not of poliovirus infection (Grist & Roberts, 1962).

The serological surveys and investigations of patients in other years show that Coxsackie A 7 virus was not new to the community in 1959. Although neutralizing antibody titres were found to persist unchanged for a year or more after Coxsackie A 7 infection, the distribution of titres in various collections of sera showed that levels of 64 and over were unusual except in cases of recent infection (Table 5). On this basis, titres suggestive of recent infection were found in a few sera collected in 1958, the year before the outbreak, including sera from a paralysed child with little virological evidence of poliovirus infection. Serological examination of healthy adult blood donors showed a considerable lack of immunity in the population before the 1959 outbreak and an increase thereafter. If this increase represented the increment of immunity resulting from the 1959 outbreak, infection of adults must have been considerably more frequent than was recognized. Since there was only one young adult among the cases detected, the majority of such hypothetical adult infections must have been of minor or symptomless character, whereas among the 37 proven cases illness was more severe in younger children.

No data are available to indicate the total amount of infection, as distinct from illness, among children in 1959, but it is notable that of the recognized symptomatic infections more than half were under 5 years old. This age-distribution resembles that of poliovirus infections studied in this laboratory in recent years (Grist, Larminie, MacGregor, McIntosh, McLaughlin & Sommerville, 1958), and differs markedly from that of the 1959 epidemic of Frater virus—a relative newcomer to the community whose main impact was upon schoolchildren aged from 5 to 14 years, with a fifth of the cases in adults (Duncan, 1961).

Although several cases of synchronous infection with more than one virus were encountered in 1959, it is interesting that no case showed dual infection with Coxsackie A 7 and Frater virus, the two most prevalent enteroviruses of the season (Combined Scottish Study, 1961). This suggests the possibility of interference between these two viruses, which might also explain the rapid collapse of the Coxsackie A 7 outbreak as the Frater epidemic began. Interference might also be postulated to explain the outbreak of Coxsackie A 7 infection in a year of unusual poliovirus quiescence, but both type 1 poliovirus and Coxsackie A 7 virus were active in the Karaganda outbreak of 1952 (Voroshilova & Chumakov, 1959) and the Glasgow case of paralytic Coxsackie A 7 infection in 1956 occurred during a mixed outbreak of Types 2 and 3 poliovirus infection (Grist *et al.* 1960).

Coxsackie A 7 infection may have gone unrecognized in the past because of lack of clinical distinction from classical poliovirus infection and failure to utilize the inconvenient diagnostic procedure of suckling mouse inoculation. Until recently the virus may well have been endemic like poliovirus and other enteroviruses, infecting mainly children and causing few cases of paralysis and no sizeable outbreaks. Continued observations are required to detect whether Coxsackie A 7 virus will become important as a cause of outbreaks of paralytic disease in polio-vaccinated as well as unimmunized sections of the population.

Haemagglutination by Coxsackie A 7 virus will be described in detail elsewhere (Grist, to be published). The haemagglutinin appears to be a protein or lipo-protein, distinct from the infectious particle, serologically type-specific, and not associated with Coxsackie group A viruses other than A 7. Testing for haemagglutininin proved a convenient technique for rapid identification of Type A 7 viruses isolated in suckling mice. The results of haemagglutination-inhibition tests for antibody correlated reasonably with the results of neutralization tests, and preliminary observations suggest that this technique provides a simple and satisfactory method of measuring antibodies.

SUMMARY

Coxsackie A 7 virus was isolated from thirty-seven patients during an outbreak in Scotland in 1959. Seven cases were paralytic, one of them fatal. Evidence is presented that Coxsackie A 7 virus caused these paralytic illnesses. The virus was also isolated from a paralytic case in 1956 and from a non-paralytic case in 1961. Serological surveys suggest that it has been active in the community for some years. Specific haemagglutination by Coxsackie A 7 virus was useful for rapid identification of viruses and for measurement of serum antibodies.

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