introducing infection into the family; in this series the majority of index cases were in the age-group under 5 years.

Outbreaks of enteroviral infection such as this are likely to be identified with increasing frequency as a result of the development of new techniques in the virus laboratories and the appreciation that infection with these viruses may give rise to diverse clinical syndromes. The 1960 epidemic in Glasgow not only provided an opportunity to become familiar with the variable features of E.C.H.O. type 9 virus infection but also offered an occasion for fruitful co-operation between the general practitioner and the hospital with its special diagnostic facilities.

Summary

During an epidemic of E.C.H.O. 9 virus infection details of illness were recorded in 20 families comprising 80 persons. Of 43 who reported "sick," 31 were children. The majority of illnesses were mild, but one boy developed meningitis.

Specimens of faeces or throat swabs for virus isolation were obtained from patients and contacts, and sera were examined by complement-fixation (C.F.) for antibodies to E.C.H.O. 9 virus. Infection was confirmed in 19 of the 20 families. Virus was isolated from 15 of 19 faecal specimens (79%) and from 13 of 26 throat swabs (50%) from sick persons. Titres of C.F. antibody were diagnostic of infection in 12 of 15 sick examined. Virus was isolated from 5 of 14 throat swabs and one of two rectal swabs from 15 "well" persons.

The pattern of illness differed in adults and children, but headache and vomiting were common features at onset at all ages. Illness in adults sometimes suggested "influenza," but in children fever and rash were most prominent. Twenty-three out of 31 children and 1 out of 12 adults had rash, which appeared in two forms—minute discrete papules, pink or colourless, and larger maculo-papular elements, pink or red.

Some of the features of the study are compared with those of other outbreaks of E.C.H.O. 9 infection.

We thank Drs. W. J. Weetch and H. MacAnespie for allowing us to study their patients, and Dr. Constance A. C. Ross for carrying out the complement-fixation tests.

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The London Group of doctors of the Family Planning Association held a week-end conference in London from November 3 to 5 which was attended by 86 doctors working in F.P.A. clinics in all parts of Great Britain. The theme of the conference was "The Marriage Relationship," and it covered aspects of this relationship in different cultures, adolescent and teenage attitudes towards it, marital difficulties in the new towns, and the part that the F.P.A. clinic can play in the treatment of marital problems.

ISOLATION OF COXSACKIE VIRUSES FROM NORMAL CHILDREN AGED 0–5 YEARS

BY

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Since the widespread use of poliomyelitis vaccines, more attention has been given to other viruses which cause meningitis and poliomyelitis-like illnesses. Coxsackie viruses have been isolated from patients with a variety of illnesses and occasionally from apparently normal people, so that the aetiological significance of their presence is often difficult to assess. A knowledge of the incidence of these viruses in the normal population should prove useful in the interpretation of the significance of Coxsackie virus isolations from patients.

There have been numerous reports in the literature of the presence of Coxsackie group B viruses in this country but few of the group A viruses apart from type 9, which grows readily in tissue culture and has been isolated frequently. Forrester and Tobin (1951) isolated a type A1 virus from the faeces of a child suffering from muscular weakness whose cerebrospinal fluid had increased protein and a normal cell count. Type A2 virus has been reported on three occasions (Findlay and Howard, 1950; Tobin, 1953). Howard isolated an A4 strain from a case of poliomyelitis-like illness and Stuart-Harris isolated a type A10 virus from a case of herpangina (Tobin, 1953). Grist (1960) isolated type A7 viruses from 33 patients in an outbreak of poliomyelitis-like illness in Scotland in 1957. Alsop, Flewett, and Foster (1960) grew a number of type A16 viruses from patients with pyrexia and vesicular lesions in the mouth and on the hands and feet.

These scanty reports of group A viruses probably reflect a reluctance to use suckling mice for virus isolation rather than the rarity in this country of group A viruses. According to Gear (1959), infections with this group are among the most prevalent of all virological infections.

In 1957–9 the Public Health Laboratory Service undertook a survey of the incidence of poliomyelitis virus in normal children aged 0 to 5 years in England and Wales, and the opportunity was taken to use some of the specimens of faeces collected to determine the incidence of Coxsackie virus infection.

Materials and Methods

The children included in the poliomyelitis survey were taken at random from the birth registers. Specimens of faeces were collected over a one-year period beginning in June, 1958, from urban areas (Table I) within greater London north of the Thames. The random sampling method used for the selection of children and the method of preparing faecal extracts have been described by Spicer (1961). Faecal extracts from some of these specimens were prepared by Dr. C. E. D. Taylor, of the Central Public Health Laboratory, Colindale, who kindly made them available to me after their examination in tissue culture.

Mouse Inoculation.—Before inoculation, faecal extracts were stored at −10° C. or less until

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litters of mice became available. Sometimes this necessitated storage for periods of up to one year. Equal parts of three consecutive extracts were pooled and 0.03 ml. of each pool was injected intracerebrally and subcutaneously in the interscapular region of each member of a litter of at least six suckling mice. The mice used were all obtained from a single dealer and all litters were used within 24 hours of birth. Mice were examined daily for 12 days; any litters showing abnormality within 24 hours of inoculation were discarded and the test was repeated. When a pool proved positive, its three component extracts were injected individually in separate litters. In February, 1959, the isolation rate had fallen to a low level, and, to conserve litters, every third specimen collected was examined. Paralysed mice were killed and kept at −30° C. until torso suspensions could be prepared. Suspensions were made in 0.85% saline with added penicillin and streptomycin. The feet, head, tail, and viscera were removed from these mice and a 25% suspension was prepared by grinding the torsos in an M.S.E. homogenizer for two minutes at approximately 10,000 r.p.m. This was then centrifuged at 5,000 r.p.m. for one hour at +4° C. and the supernatant used for further passage and for virus typing. All positive specimens were given one or more further passage in suckling mice until a clear-cut paralysis was produced in all or most of the members of the litter inoculated. A suspension prepared from the final-passage mice was used for virus typing.

Virus Typing.—The torso suspensions from paralysed mice were first inoculated in rhesus-monkey-kidney and HeLa-cell tissue culture. Those producing a cytopathic effect were typed by a neutralization test in monkey-kidney-tissue culture using Coxsackie B1 to 5 and A9 antisera prepared in rabbits or monkeys. All suspensions were also tested against Coxsackie A1 to 19 mouse immune ascitic fluids using the complement-fixation test. The typing of all Coxsackie group A viruses was confirmed by neutralization tests in suckling mice. Double infections were demonstrated in two specimens, type B4 and A2 viruses in one and type B4 and A6 in the other. In neutralization tests in mice with these specimens paralysis was prevented only when antisera to both viruses were used.

Results

During the year specimens from 2,084 children were examined and 138 Coxsackie viruses isolated, of which 112 belonged to group A and 26 to group B. The group A viruses isolated were of types 2, 4, 5, 6, 8, 10, and 12, and the group B of types 3 and 4, which were the prevalent group B types in England at that time (Table I). It was evident that these viruses were widely disseminated in the community studied, but from the time relations and geographic locations of virus isolations of each type it was not possible to demonstrate any spread of infection; in fact, the viruses appeared almost simultaneously in a number of areas. The normal seasonal pattern, common to most enteroviruses, was followed (Table II). Considering groups A and B together, the isolation rate for the half-year July to December was 91.1 per thousand and for January to June 24.0 per thousand. The maximum isolation rates occurred in September for group A viruses with 119 per thousand, and in October for group B viruses with 30.4 per thousand.

TABLE I.—*Isolation of Coxsackie Viruses from 2,084 Specimens of Faeces Taken from Children Aged 0 to 5 Years in North London*

Coxsackie Virus	No. Isolated	Isolation Rate per 1,000 Specimens
Group A	112	53·7
Type 2	24	11·5
,, 4	18	8·6
,, 5	33	15·8
,, 6	15	7·2
,, 8	11	5·3
,, 10	9	4·3
,, 12	2	1·0
Group B	26	12·5
Type 3	1	0·5
,, 4	25	12·0
Totals	138	66·2

The area studied included Ealing, Edmonton, Enfield, Feltham, Finchley, Hayes and Harlington, Heston and Isleworth, Hornsey, Muswell Hill, Ruislip and Northwood, Southgate, Spelthorne, Tottenham, Twickenham, Uxbridge, Wembley, Willesden, Wood Green.

TABLE II.—*Isolation of Coxsackie Viruses in Each Month of the Survey*

Month	No. Specimens Examined	No. of Coxsackie Viruses Isolated (and Isolation Rate per 1,000 Specimens)		
		Group A	Group B	Both Groups
June	364	9 (24·7)	2 (5·5)	11 (30·2)
July	226	13 (57·5)	4 (17·3)	17 (75·2)
August	234	21 (89·8)	6 (25·9)	27 (115·4)
September	176	21 (119·0)	3 (11·4)	24 (136·4)
October	263	19 (72·2)	7 (30·4)	26 (98·9)
November	232	17 (73·3)	4 (17·2)	21 (90·5)
December	186	5 (26·9)	0 (0)	5 (26·9)
January	107	2 (18·7)	0 (0)	2 (18·7)
February	68	1 (14·7)	0 (0)	1 (14·7)
March	68	2 (29·4)	0 (0)	2 (29·4)
April	72	0 (0)	0 (0)	0 (0)
May	71	1 (14·1)	0 (0)	1 (14·1)
Totals	2,067	111 (53·7)	26 (12·6)	137 (66·3)

Seventeen specimens, one giving type A5, are not included as the month they were taken was not recorded.

The isolation rates in relation to the age of the children (Table III) showed for groups A and B together the lowest rate occurring in the first year of life, the highest in the second and third years, and a slight decline in the fourth and fifth years. The relation of isolation rates to sex was also examined, but no significant difference between the rates in males and females was demonstrated; isolation rates of 73 and 56 respectively were obtained.

TABLE III.—*Isolation of Coxsackie Viruses from Different Age-groups*

Age Group (Years)	No. of Specimens Examined	No. of Coxsackie Viruses Isolated (and Isolation Rate per 1,000 Specimens)		
		Group A	Group B	Both Groups
0–1	469	12 (25·6)	2 (4·3)	14 (29·9)
1–2	453	31 (68·4)	6 (13·2)	37 (81·7)
2–3	422	27 (64·0)	7 (16·6)	34 (80·6)
3–4	380	22 (57·9)	4 (10·5)	26 (68·4)
4–5	347	17 (49·0)	7 (20·2)	24 (69·2)
Totals	2,071	109 (52·6)	26 (12·6)	135 (65·1)

Thirteen specimens, two giving type 4A and one giving type 5A, are not included as the age-group from which they were taken was not recorded.

Discussion

The most striking finding was the large number of Coxsackie viruses, particularly group A strains, present in this sample of the population. This is in agreement with the findings of workers in other countries where Coxsackie group A viruses have been found to be extremely common (Huebner *et al.*, 1952; Gear, 1959). Some of the specimens of faeces had previously been examined for viruses using tissue cultures but not suckling mice, and the quarterly isolation rates of Coxsackie viruses were 23.1, 3.2, and 0 per thousand for July–September, 1958, October–December, 1958,

and January–March, 1959, respectively (Spicer, 1961). Using suckling mice, the corresponding rates for group B viruses were 20.4, 16.2, and 0 per thousand for the same quarters, results similar to those using tissue cultures. When, however, the Coxsackie A isolations using suckling mice are included, rates of 107, 76.4 and 20.6 per thousand are obtained. The increasing recognition of this group of viruses, together with their ubiquity, constitutes a strong argument for the routine diagnostic use of suckling mice.

The group A viruses isolated belonged to types 2, 4, 5, 6, 8, 10, and 12. Of these, types 5, 6, 8, and 12 have not previously been reported in this country. Types 2, 4, 5, 6, 8, and 10 have been commonly associated with herpangina and their aetiological relationship to this condition has been clearly established (Huebner *et al.*, 1951 ; Cole *et al.*, 1951 ; Beeman *et al.*, 1952a, 1952b). Herpangina, as originally described by Zahorsky (1920), appears to be surprisingly uncommon in this country, considering the frequency with which the causative agents have been found. Johnsson (1955) has also commented on the rarity of the herpangina syndrome in his series of patients from whom group A viruses were isolated in Sweden. The finding, however, of these viruses in an apparently normal section of the population serves to emphasize the necessity for caution in attributing to them aetiological significance, even when supported by serological evidence of infection.

Although more than 95% of the Coxsackie viruses isolated in this investigation were from specimens collected between June and December, 1958, occasional isolations were made during the first five months of 1959. It seems probable that the viruses persist in the community throughout the year and spread in the summer and autumn when conditions become favourable. This view is also supported by the apparently simultaneous appearance of the viruses in a number of areas, although in a metropolis such as London, where dissemination may be rapid and widespread, such evidence can carry little weight.

The incidence of Coxsackie infection in the different age-groups suggests that these viruses are very common and are encountered early in life. Beeman *et al.* (1952c) showed by serological investigations that adults had neutralizing antibodies to most of the types against which their sera were tested, whereas children had antibodies to fewer types. The protection afforded by maternal antibody is probably responsible for the low incidence of infection during the first year of life.

An analysis of the carrier rates according to sex was made because of the unexpected finding that the carrier rate of type I poliovirus was significantly higher in males than in females (Spicer, 1961). The carrier rates of Coxsackie viruses of groups A and B, however, showed no significant sex difference.

Summary

A survey was made over a one-year period beginning in June, 1958, to determine the incidence of Coxsackie virus infection in normal children aged 0 to 5 years in the greater London area.

Faecal extracts from 2,084 children were examined by the inoculation of suckling mice and 138 Coxsackie viruses isolated of which 112 belonged to group A and 26 to group B. The viruses isolated included types 2, 4, 5, 6, 8, 10, and 12 of group A and types 3 and 4 of group B. No record has been found in the literature of the previous isolation in this country of types 5, 6, 8, and 12 of group A.

The monthly isolation rates showed a seasonal prevalence with its peak in August, September, and October, but some types of viruses persisted at a low rate throughout the winter.

The carrier rate was lowest in the first year of life and highest in the second and third years. There was no significant sex difference in the carrier rates.

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ABSENCE OF INTERFERON IN LUNGS FROM FATAL CASES OF INFLUENZA

BY

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Interferon is a potent antiviral substance which is produced by virus-infected cells both *in vitro* and *in vivo* and which is thought to be important in recovery from viral infections (Isaacs and Hitchcock, 1960 ; Baron and Isaacs, 1961). We have recently developed a quantitative assay for human interferon, using primary cultures of human thyroid cells, and this has made it possible to study the production of interferon during viral infections of man. Through the co-operation of four laboratories we obtained human lung tissues from patients with fatal influenzal pneumonia. Influenza virus had been isolated from all these samples, which had then been stored at low temperatures.

To our surprise there was no detectable interferon activity in any of the specimens we have tested. This paper reports the results and discusses their possible significance.

Materials and Methods

Human lung specimens were generously supplied by Dr. J. Mulder, Leyden ; Dr. N. R. Grist, Glasgow ; Dr. J. Hambling, London ; and Miss E. Minuse, Ann

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