

Virus Excretion and Bacteriological Studies in Sporadic Infantile Enteritis

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In the aetiology of infantile enteritis a significant role is ascribed to some agents other than the intestinal bacteria known for long to be pathogenic (*Salmonella*, *Shigella*, enteropathogenic *E. coli*). A considerable number of reports have discussed the eventual pathogenic role played by other bacteria (*Klebsiella*, *Proteus*, *Pseudomonas*, *Staphylococcus*), the excretion of virus, and the aetiological connexions in such cases [1, 2, 4, 7-18, 20, 22-24, 27]. These complex bacteriological and virological studies made in widely differing patient materials yielded divergent results and often failed to determine the agent responsible for the disease.

In the present study it has been attempted to investigate whether the results of simultaneous bacteriological and virus isolations from patients with enteritis and from control subjects with no enteral disease would be of aid in clarifying the aetiology of sporadic infantile enterocolitis.

MATERIALS AND METHODS

Groups. The patients subjected to study were grouped on the basis of the following points of view. The so-called enteritis group

comprised infants and small children with symptoms of acute enteritis. Patients exhibiting dysenteric or dysentery-like symptoms were excluded from this group. During the study period there was no epidemic diarrhoea at the Department of Paediatrics, and in the City Nursery there were exclusively babies with no diarrhoea. Thus, the cases studied were all sporadic. The test period lasted from March, 1961, till July, 1962; most samples were sent in during the summer months. The control group was set up of infants and small children treated at the Department for diseases other than enteric.

MATERIAL

A total of 270 faecal samples taken from 154 infants and young children were examined.

Virological studies. The about 10 per cent faecal suspensions prepared for isolation in the usual way were stored at -20°C until tested. Trypsinized monkey kidney cell suspension was obtained from the State Institute of Public Health, Budapest, in the chilled state. With every faecal sample two primary monkey kidney cell cultures were inoculated and kept under observation for 12 days. The isolated cytopathogenic viruses were typed at the Virus Department of the State Institute of Public Health, Budapest.

Bacteriological studies. Faecal samples were inoculated by the usual method on Endo, D. C., blood agar, and 6 per cent

NaCl agar media. *E. coli* cultures were tested for agglutination first with polyvalent *E. coli* OB sera, then, in the case of positivity, with the corresponding components. The polyvalent *E. coli* sera had the following composition:

OB I: 0111 : B4, 055 : B5, 026 : B6
 OB II: 086a : B7, 0127a : B8,
 0112a, c : B11
 OB III: 0112a, b : B13, 0119 : B14,
 0125 : B15, 0124 : B17
 OB IV: 086a, b : B9, 0127a, b : B10,
 0128 : B12, 0126 : B16

The sera were diluted 1 in 10 or 1 in 20, for use in the slide agglutination test.

RESULTS

Table I shows the age incidence in the enteritis and control groups. It is seen that in both groups the largest number of faecal samples tested came from the age group 0 to 6 months.

TABLE I
Age incidence

Age groups, months	Cases of enteritis	Control cases (no enteritis)
0 to 3	48	18
3 to 6	27	17
6 to 12	9	13
12 to 24	8	9
over 24	3	2
Total:	95	59

Table II shows the results of the virus isolation studies. In 6.5 per cent of the cases cytopathogenic viruses representing all three enterovirus (poliovirus, Coxsackie and ECHO) groups and the adenovirus group could be isolated.

TABLE II
Virus isolation results

Type of isolated virus	Number of isolated strains		Total
	enteritis group	control group	
Poliovirus 1		1	1
Poliovirus 3	1	1	2
Coxsackie A9		1	1
Coxsackie B4	1		1
ECHO 14	2		2
Adenovirus 4		1	1
Untyped		4	4
Total:	4	8	12

As the data in *Table III* indicate, enteropathogenic *E. coli* strains could be isolated by repeated tests in 54 per cent of the 37 cases of "acute mild enteritis". The corresponding value for the 58 cases of "acute severe enteritis" was 67 per cent. The number of successful isolations significantly increased in repeated tests, as compared with the result of single tests. In the control group the percentage of positivity was 17 per cent whether on single or repeated isolations.

TABLE III
Enteropathogenic *E. coli* strains in the various conditions by one test and by repeated testing

Clinical classification	Number of cases	<i>E. coli</i> isolated by one test number	<i>E. coli</i> isolated by repeated testing number
Acute, mild	37	5	20
Acute, severe	58	17	39
Control (no diarrhoea)	59	3	10
Total	154	25	69

The data in Table IV indicate the incidence of enteropathogenic *E. coli* excretors according to age groups.

The number of excretors under 1 year of age was considerable in both groups. It is conspicuous, however, that the incidence of enteropathogenic *E. coli* in the group 0 to 3

TABLE IV
Enteropathogenic *E. coli* strains excreted in the different age groups (out of 69 excretors)

Age groups months	with enteritis		without enteritis number of cases	excretors number
	number of cases	excretors number		
0 to 3	48	41	18	5
3 to 6	27	12	17	1
6 to 12	9	5	13	2
12 to 24	8	1	9	2
Over 24	3	—	2	—
Total	95	59	59	10

months with diarrhoea was 86 per cent, and in the 3 to 6 months and 6 to 12 months age groups, 50 per cent, while the infants having no diarrhoea showed merely one third of these incidences.

TABLE V
Isolated *E. coli* strains

Type	Number of strains
0111 : B4	43
055 : B5	31
026 : B6	3
086 : B7	9
0127 : B8	1
0119 : B14	1
Total	88

Among the isolated *E. coli* strains the 0111 : B4 strain dominated in the period of testing (Table V).

The same is indicated by the data in Table VI, according to which the 0111 : B4 strain occurred alone in 32 cases, and together with the serotype 055 : B5 in 8 cases. Other enteropathogenic *E. coli* strains occurred together in further 8 cases.

TABLE VI
Number of *E. coli* excretors and serotype of isolated strains

Type	Number of excretors
0111 : B4	32
055 : B5	16
026 : B6	2
086 : B7	2
0127 : B8	1
0111 : B4 + 055 : B5	8
055 : B5 + 086 : B7	3
055 : B5 + 026 : B6	1
086 : B7 + 0119 : B14	1
0111 : B4 + 055 : B5 + 086 : B7	3
Total	69

DISCUSSION

The results of our investigations and the data in the literature supplied only partially satisfactory answers to the question raised in the introduction. The incidence of virus excretion was low (6.5 per cent) in both groups tested. It is to be noted, however, that most of the subjects under 2 years of age were tested soon after Sabin vaccination. This is reflected also by

the fact that the isolated poliovirus strains 1 and 3 proved to be marker $\text{ret}/40^-$ ones thus presumably vaccine strains. If we exclude these 3 vaccine strains from the few strains isolated and take into account the enterovirus excretion data obtained by DÖMÖK et al. in almost the same period [5, 6], according to which in 1960 Coxsackie B4 and ECHO 14 were most often excreted by subjects under 2 years of age, it may be stated that our material, in which these two viruses represented 3 strains, reflects the actual virus excretion pattern characteristic of the age groups tested. It is also decisive that, according to the estimate of DÖMÖK et al. [6] in that period in Hungary the symptom-free enterovirus excretors numbered about 40,000.

It would thus seem that the role played by the isolated viruses in the given enteral conditions should be accepted with certain restrictions, even if the incidence of positive isolations was higher. This view is supported by the following data from the literature. In 1961, REITANO and DARDANONI [20] drew the same conclusion from the results obtained in a similar material; in their group with diarrhoea the incidence of enterovirus excretion was 26 per cent, against 31 per cent in the control group. The incidence of ECHO types was 15 per cent in both groups. In 1959 BERGAMINI and ANDREONI [1] succeeded in isolating enterovirus or adenovirus in 10 per cent and intestinal bacteria in 21 per cent of their cases with diarrhoea; in 68 per cent they found no

pathogenic micro-organisms. They attributed no aetiological role to the isolated viruses. GIOVANARDI et al. [9] isolated cytopathogenic virus in 10 per cent of 160 patients with sporadic infantile gastroenteritis, while in 83 per cent the pathogen could not be identified. Their conclusions were similar to those mentioned above. McLEAN et al. [15] found enterovirus in a small percentage of their cases of epidemic gastroenteritis; they did not consider this finding to bring them nearer to the solution of the problem. SCHÖNFELD and RAMON [23] isolated enterovirus in 15 per cent of faecal samples from both gastroenteritis and respiratory cases. SAUTHOFF [22], studying infantile abacterial enteritis could isolate merely a few enteroviruses and emphasized that the success of isolation depended always on the actual degree of virus excretion, which may vary from country to country and from year to year, in dependence on a number of conditions, and this is why his results differed from those obtained in 1956 by RAMOS-ALVAREZ and SABIN [18]. The last-named authors isolated namely various types of enterovirus and adenovirus in 50 per cent of cases of diarrhoea and claimed an important role to the ECHO group, which they found to occur six times more often in patients with diarrhoea than in healthy subjects. It is, however, difficult to form an opinion concerning the role of ECHO viruses in view of the fact that the 42 isolated strains belonged to 13 different serotypes. Other authors have also attributed a role to the

ECHO viruses in enteral conditions. EICHENWALD et al. [7], for instance, consider ECHO type 14 to be pathogenic. BERGAMINI and BONETTI [2], KLEIN et al. [12] found that ECHO 11, LÉPINE et al. [14] and SOMMERVILLE [24] that ECHO 14 occurred frequently in cases of infantile epidemic diarrhoea, whereas others, for example FELICI et al. [8], and KOLTAY et al. [13], found Coxsackie B3 in a considerable number of patients.

On the basis of our own results and the above data we tend to support the view that the excretion of enterovirus, apart from the high incidence of certain types in circumscribed outbreaks, supplies information not as to the aetiological factors, but to the measure and quality of virus excretion characteristic of the given conditions. It should also be emphasized that in such studies the use of representative control groups is essential.

Some authors have supplemented the virus excretion studies with antibody response tests [7, 13, 18]. Evaluation of such data is, however, rendered difficult by the fact that in general we have no sufficient information as regards the responses of symptom-free excretors, and, further, that a positive immune response does not yet decide whether it is an immune response to the aetiological factor or merely to an abortive viral infection occurring independently of the diarrhoea. We have therefore omitted to carry out such tests.

The opinions in the literature are unequivocal as regards the aetiological role played by the different entero-

pathogenic bacteria (*Salmonella*, *Shigella*, *E. coli*) in infantile enteritis and enterocolitis. In our present studies it could once again be confirmed that the enteropathogenic *E. coli* strains played a leading role in these sporadic cases of enterocolitis. Owing to the small number of the isolated strains, nothing definite can be stated concerning the role of other bacteria (*Klebsiella*, *Proteus*, *Pseudomonas*, *Staphylococcus*). (Results of previous studies in this field have been published by one of us [27]). In the present examinations no *Salmonella* and *Shigella* strains were isolated; this is only natural since cases of dysentery or dysentery-like conditions were excluded from the material.

Our results clearly mirror the importance of repeated bacteriological tests in sporadic cases of infantile enteritis. This is quite obvious from the data in Table III. The significance of serial tests is increased by the fact that in the group designated "acute severe", requiring continuous drop infusion, one test revealed the presence of the pathogen in 17 (29 per cent), whereas repeated tests in 39 (67 per cent) of the 58 cases.

The results obtained by one single examination closely agree with the 30 per cent value reported in 1952 by RAUSS et al. [19] for enteropathogenic *E. coli* excretors.

The 17 per cent incidence of enteropathogenic *E. coli* in the control group is not conspicuous. Repeated tests in normal groups often yielded similar values, as reported in the literature. In Hungary, RUDNAI et al. [21] found

about 25 per cent symptom-free excretors among 251 healthy children aged 1 to 2 years.

In spite of the repeated bacteriological and virological tests the aetiology remained unclear in 33 per cent of even the "acute severe" group. This again shows that every effort should be made to develop diagnostic methods more sensitive than those in common use. This was our reason for including into the polyvalent *E. coli* sera diagnostic ones for the infrequently occurring enteropathogenic *E. coli* strains. The number of clarified cases may be increased also by the demonstration of new pathogenic *E. coli* types [28]. A number of data are indicating that the immunofluorescence method may be used with success for the aetiological clarification of sporadic or epidemic cases of infantile enteritis. In this respect we only refer to the papers by THOMASON et al. [3, 25, 26] and our own experience that by means of the method about double the number of *E. coli* excretors can be demonstrated as by other ones [29]. On the other hand, the number of isolated viruses may also be increased by an expansion of the methods.

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SUMMARY

(i) Two hundred and seventy faecal samples obtained from 154 infants and children under 2 years of age were tested bacteriologically and virologically. Of the patients, 95 suffered from non-dysenteric enterocolitis and 59 were controls with no diarrhoea.

(ii) In 62 per cent of the enteritis cases enteropathogenic *E. coli* could be isolated. In the acute, severe cases the incidence of *E. coli* excretion was 76 per cent.

(iii) The importance of repeated testing is emphasized. While by one attempt at isolation, enteropathogenic *E. coli* strains could be isolated in about 30 per cent of the cases, by repeated testing this succeeded in 60 per cent. Thus, one screening may yield a false result concerning the incidence of enteropathogenic *E. coli* in cases of diarrhoea.

(iv) Repeated testing showed the presence of enteropathogenic *E. coli* in 17 per cent of the control patients with no diarrhoea.

(v) Cytopathogenic viruses including poliovirus, Coxsackie, ECHO and adenovirus, could be isolated in 12 cases, 4 in the diarrhoeal and 8 in the control group. The small number of the viruses isolated, the lack of uniformity, and the similarity of incidence among the population speak against a viral aetiology of the enteritis cases with a negative bacteriology. Pertinent data in the literature are quoted to support this view.

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