OUTBREAK OF ILLNESS ASSOCIATED WITH E.C.H.O. TYPE 7 VIRUS

THOMAS M. BELL, Ph.D., B.Sc.
Lecturer in Bacteriology, University of Aberdeen

BY

NORMAN S. CLARK, M.D., B.Sc., M.R.C.P.
M.R.C.P.Ed., D.C.H.
Senior Lecturer in Child Health, University of Aberdeen

WILLIAM CHAMBERS, M.D.
Consultant Physician, Aberdeen City Hospital

Of the 27 recognized members of the E.C.H.O. virus group only a few have so far been reported to be associated with outbreaks of disease of the central nervous system. These include E.C.H.O. type 4 (Lehan *et al.*, 1957), type 6 (Von Zeipel and Svedmyr, 1957), type 9 (Nihoul *et al.*, 1957), type 11 (Elvin-Lewis and Melnick, 1959), and type 16 (Kibrick *et al.*, 1957). In addition, the virus known as Frater (Duncan, 1960; Bell, 1962) also appears to be a member of the E.C.H.O. group and was associated with outbreaks of meningitis in Scotland in 1959. In 1961 E.C.H.O. type 7 virus infection was associated with about one-quarter of the cases of aseptic meningitis seen in the Aberdeen area. Nineteen persons were shown to have been infected during April to November, with the peak in July.

The only previous record of E.C.H.O. type 7 infection in Scotland was in association with cases of infant diarrhoea in Glasgow in 1957 (Sommerville and Carson, 1958). Cases of meningitis associated with E.C.H.O. type 7 virus have also been reported elsewhere (Melnick and Sabin, 1959), but so far occurring only sporadically.

Methods and Materials

All specimens were inoculated into one tube each of human amnion, human embryo kidney, and HeLa cells (line 4). All positive specimens yielded virus in all three cell types after the same period of incubation. Most isolates in HeLa cells, however, required to be passed before the typical cytopathic effect could be seen. All strains were identified as E.C.H.O. type 7 using antiserum supplied by the Standards Laboratory, Colindale.

Neutralization tests were carried out on both rabbit and human sera using 100 TCD₅₀ of virus mixed with equal volumes of serum dilutions and incubated for one hour at room temperature before inoculation into tubes of human amnion cells. The end-point was read after six days' incubation at 37° C. in roller-drums and the titre expressed as the reciprocal of the highest dilution inhibiting virus growth. All sera but one were tested against both the Wallace prototype strain and the local outbreak (Turiff) strain simultaneously. Media and cells were prepared and used as described previously (Bell, 1962).

Haemagglutination-inhibition tests were carried out using a 1% suspension of human group O red cells in Wassermann tubes. The method was similar to that described by Rosen (1960) for adenovirus and reovirus investigations. For titration of the haemagglutinin 0.4 ml. of virus dilution was mixed with 0.2 ml. of the cell suspension for one hour at 37° C. The titre was taken as the highest dilution giving definite haemagglutination. Haemagglutination-inhibition tests were performed by mixing 0.2 ml. of the serum dilution with 0.2 ml. of that dilution of antigen containing four haemagglutinating units and allowing the mixture to stand for one hour at room temperature. Then 0.2 ml. of red-cell suspension was added and the resulting mixture incubated for one hour at 37° C. The end-point was taken as the highest dilution giving inhibition of the haemagglutination. An antigen titration was included in each test and 0.85% saline was used as diluent throughout.

Clinical Features

Of the 19 infected individuals, 15 were admitted to hospitals in Aberdeen and four were looked after by their family doctors at home (three of them in one practice). Ten patients presented with a one-day or two-day history of severe headache, vomiting, fever, and general malaise. Three complained also of photophobia and three of muscular pains. All but one showed slight or moderate neck stiffness and Kernig's sign was doubtfully positive in the majority. Two showed a scattered faint pink macular rash and one (Case 6) an extensive petechial rash most pronounced on the limbs and buttocks but also present on the trunk. This child alone appeared to be acutely ill on admission to hospital, and her rash in conjunction with her meningitic signs suggested a diagnosis of meningococcal septicaemia. Blood culture was sterile and C.S.F. examination gave a positive virus culture but showed no rise in cells or protein; her fever settled slowly over a period of five days. In every other case the fever and symptoms settled within 48 hours, though one or two patients complained of some lassitude for several weeks. There was no known association between any of the 10 patients.

Details of the C.S.F. findings and virus studies in these 10 cases are shown in Table I.

TABLE I.—Laboratory Findings in 10 Cases of Aseptic Meningitis

Case No.	Age (Years)	C.S.F.		E.C.H.O. 7 Virus Isolation	E.C.H.O. 7 Neutralizing Antibody Titres	
		Cells	Protein (mg./100 ml.)		Wallace	Turiff
1	16	P62. L42	60	Faeces +	0 : 20	10 : 160
2	9	L52	80	Faeces +, C.S.F. —	0 : 5	0 : 80
3	9	P30. L26	25	C.S.F. +, Faeces — *	5 : 40	5 : 320
4	19	N.D.	N.D.	Faeces +, Throat +	0 : 80	0 : 640†
5	34	P10. L336	60	Faeces +, C.S.F. +	0 : 80	0 : 80
6	5	0	30	C.S.F. +	N.D. : 10	N.D. : 80
7	9	P11. L35	45	Faeces +, C.S.F. —	5 : 10	160 : 160
8	25	N.D.	N.D.	Faeces —	640 : 1,280	320 : 320
9	9	"	"	"	N.D.	320 : 320
10	18	"	"	"	0 : 0	10 : 80

N.D. = Not done. P = Polymorphs (per c.mm.). L = Lymphocytes (per c.mm.). * Faeces culture from Case 3 gave a growth of E.C.H.O. 9 virus. Antibody titres to this virus were 20 : 20.

† These sera also showed E.C.H.O. 9 antibody titres of 80 : 320.

These 10 patients presented a fairly uniform clinical picture and in all of them the serological findings were in favour of a concomitant infection with E.C.H.O. 7 virus, suggesting that the virus was in fact the cause of their illness. One other case, a girl aged 18, had a prolonged illness with encephalitic features; no virus was recovered from her stools or C.S.F., but paired sera showed antibody titres of 5:5 to the Wallace strain and 20:320 to the Turiff strain. It is thought possible that the virus may have been responsible for her illness, all other investigations having proved negative.

In three cases no opinion can be offered on the relation of the virus isolation to the associated symptoms in the absence of antibody studies. A man aged 59 was admitted to the neurosurgical unit in a comatose state nine days after

the onset of an illness diagnosed as measles. He died within 48 hours; the necropsy diagnosis was bronchopneumonia and possible encephalitis. E.C.H.O. 7 virus was isolated from his faeces. A girl aged 3 months was admitted with fever, vomiting, and continuous screaming, starting shortly after her first immunization with triple antigen. She recovered within 24 hours and her immunization course was subsequently completed without event. E.C.H.O. 7 virus was obtained from a throat swab on the day of admission. In the third case the virus was recovered from an enlarged mesenteric lymph node removed at operation on a 4-months-old girl with intussusception. The possible relationship between virus infections and intussusception has been discussed by Bell and Steyn (1962).

In the remaining five cases it is probable that the virus isolation was an incidental finding. Two of these patients had no symptoms; one was the 1-year-old son of a patient (Case 4) whose throat swab was positive on one occasion and whose stools were positive on two occasions; the other was a child convalescent from an illness associated with an E.C.H.O. 9 infection whose throat swab was positive for E.C.H.O. 7 one month after the onset of her symptoms. Serological tests were not carried out in these cases. One patient was admitted three days post-partum with left parotitis thought to be due to mumps: E.C.H.O. 7 virus was recovered from the blood, but paired sera showed a rise of antibody of only 0.10; mumps antibody was not detected. The fourth patient, a child of 17 months, was admitted to hospital in status epilepticus and subsequently developed a left hemiplegia: E.C.H.O. 7 virus was isolated from the faeces, but paired sera showed no significant antibody in either specimen. The fifth patient, a youth of 17, was admitted after having been found collapsed and semiconscious: he remembered having been seized with a severe upper-abdominal pain before his collapse and having a severe headache for 24 hours before this. He recovered rapidly and completely, and all investigations were negative apart from the isolation of E.C.H.O. 7 virus from his faeces. Serological tests showed no significant antibody in either specimen.

Laboratory Studies

E.C.H.O. 7 virus was recovered from 15 of the 19 persons thought to have been infected. Positive results were obtained from 11 out of 26 specimens of faeces, 4 out of 9 throat swabs, 3 out of 7 samples of C.S.F., and the single blood specimen and lymph node examined. In addition, E.C.H.O. type 9 virus was isolated from the faeces of one patient on two occasions.

Paired sera were obtained from nine of the persons yielding virus, and single specimens from two others. When the nine paired sera were tested for the presence of neutralizing antibody, using the Wallace prototype strain, only four gave fourfold or greater antibody rises and the titres in general were very low. These were then retested, using two of the strains isolated in the outbreak V2349 and V2509 (Turriff); all sera were tested against the latter but only three pairs using the former. Much higher antibody titres were obtained with these strains, seven of the pairs giving fourfold or greater rises.

As E.C.H.O. type 9 and mumps virus were prevalent in this area during 1961, all sera were tested for antibody to these viruses, and many were also tested for antibody to Coxsackie group B types 1 to 6, and two as yet unidentified enteroviruses also isolated during 1961—"Nicoll" and "Knox." In no case was there any sign of a concurrent

infection with mumps, "Nicoll," or the Coxsackie B viruses, and in only one was there any evidence of infection with Knox virus. E.C.H.O. type 9 antibody was found only in the two cases noted in Table I.

It will be seen from Table I that in seven of the nine persons tested for antibody to both strains of E.C.H.O. 7 virus the Wallace prototype strain elicited a much poorer response than did the Turriff local strains. In one case there was no difference and in the other the Wallace strain gave the higher titres.

Because of these results it was decided to prepare antisera in rabbits against the Wallace and Turriff strains and compare the titres obtained with these antisera titrated against the Turriff (V2509) strain and the Wallace type strain. Table II shows the results obtained with the two rabbit antisera and the Colindale reference serum when tested against the two strains.

TABLE II.—Neutralizing Antibody Titres with Wallace and Turriff Strains

Virus Strain	Serum Titres		
	Reference	Anti-Wallace	Anti-Turriff
Wallace	16,000	200	Less than 10
Turriff	16,000	265	565

The neutralization tests on monotypic rabbit antisera were therefore similar to those obtained with the convalescent human sera; antiserum prepared against the type strain neutralized both strains equally, while antiserum against the Turriff strain neutralized that strain only. Six further strains isolated during the outbreak were tested and all were neutralized by both sera to approximately the same titre as the V2509 strain. Only two strains were found which gave a sufficiently high titre of haemagglutinin to be tested by haemagglutination-inhibition tests, and the results obtained with these and the type strain are shown in Table III.

TABLE III.—Haemagglutination-inhibition Titres

Virus Strain	Wallace Serum	Turriff Serum
Wallace	40	160
V2520	40	160
V2606	80	320

Forty-eight pairs of acute and convalescent sera chosen at random from specimens sent to the virus laboratory during the same period were also tested for the presence of E.C.H.O. type 7 neutralizing antibody. Thirty-eight were negative and 10 showed antibody titres of 20 or less.

Discussion

The isolation of virus from cerebrospinal fluid in three cases and the antibody responses shown in Table I suggest that E.C.H.O. type 7 infection may have been the cause of the homogeneous clinical picture presented by the 10 cases of aseptic meningitis. The significance of the laboratory findings in the other nine individuals is uncertain, but two at least appear to have been carriers. The antibody results in the 48 random samples of serum suggest that infection with E.C.H.O. type 7 virus has not recently been common in the north-east of Scotland.

When the outbreak strains were examined they were found to show antigenic differences from the type strain, and these differences appeared to be consistent. The antibody titres demonstrated with the type strain of E.C.H.O. 7 were much lower than those demonstrated with

the outbreak strains when both human and rabbit convalescent sera were tested by neutralization tests. On the other hand, preliminary tests by the haemagglutination-inhibition technique using immune rabbit sera show no difference between the strains. Further work is in progress on this point, but it is possible that this difference in the antigenic properties of the outbreak strains may be related to their capacity to cause recognizable outbreaks of infection.

Summary

Evidence of E.C.H.O. type 7 virus infection was demonstrated in 19 individuals in the north-east region of Scotland in 1961. Ten patients showed the clinical picture of aseptic meningitis; E.C.H.O. type 7 virus was isolated from the cerebrospinal fluid in three, and in all of them antibody studies suggested concurrent infection.

Neutralizing antibody tests showed antigenic differences between the outbreak strains and the prototype strain, but no differences were apparent when the haemagglutination-inhibition tests were used.

We should like to express our thanks for permission to refer to the records of cases under the care of the following members of the staffs of Aberdeen General and Special Hospitals Groups; Professor John Craig, Professor H. W. Fullerton, Mr. H. A. F. Dudley, Dr. W. H. Galloway, Dr. Campbell Murray, and Mr. W. Martin Nichols; and to Dr. W. A. Ross, general practitioner, Laurencekirk. We are also indebted to Professor A. Macdonald for his advice and encouragement.

REFERENCES

- Bell, T. M. (1962). *Scot. med. J.*, **7**, 85.
 — and Steyn, J. H. (1962). *Brit. med. J.*, **2**, 700.
 Duncan, I. B. R. (1960). *Lancet*, **2**, 470.
 Elvin-Lewis, M., and Melnick, J. L. (1959). *Proc. Soc. exp. Biol. (N.Y.)*, **102**, 647.
 Kibrick, S., Melendez, L., and Enders, J. F. (1957). *Ann. N.Y. Acad. Sci.*, **67**, 311.
 Lehan, P. H., Chick, E. W., Doto, I. L., Chin, T. D., Heeren, R. H., and Furcolow, M. L. (1957). *Amer. J. Hyg.*, **66**, 63.
 Melnick, J. L., and Sabin, E. A. (1959). In *Viral and Rickettsial Infections of Man*, edited by T. M. Rivers and F. L. Horsfall, 3rd ed., p. 547. Pitman, London.
 Nihoul, E., Quersin-Thiry, L., and Weynants, A. (1957). *Amer. J. Hyg.*, **66**, 102.
 Rosen, L. (1960). *Ibid.*, **71**, 120, 242.
 Sommerville, R. G., and Carson, H. G. (1958). *Lancet*, **2**, 1347.
 Von Zeipel, G., and Svedmyr, A. (1957). *Arch. ges. Virusforsch.*, **7**, 355.

VITAMIN A IN ACNE VULGARIS

REPORT BY THE SOUTH-EAST SCOTLAND FACULTY OF THE COLLEGE OF GENERAL PRACTITIONERS*

"When a patient runs an imminent and certain risk, it is justifiable, or at least excusable, to use every remedy, as in such a case we cannot make bad worse. Still, even in such cases, our therapeutic action must be defensible in theory and by an appeal to analogy."—TROSSEAU on Experiments on Patients.

According to Unna (1894) the basic histological picture in early acne is an even hyperkeratosis of the epidermis which spreads to the openings of the pilosebaceous follicles, with the formation of comedones. Since the early histological picture resembles that found in vitamin-A deficiency, it would appear that there is some evidence to support the theory that vitamin A might be of value in the treatment of acne vulgaris.

This underlying rationale of the use of vitamin A in acne explains the many dermatological papers on the subject. One of the first reports was that by Straumfjord (1943), who treated 100 patients with 100,000 I.U. daily for periods of six months or more. Seventy-nine showed considerable improvement and only three were unimproved. Straumfjord made the point that in many cases a definite aggravation occurred early in treatment before benefit ensued. Ten years later Fell and Mellanby (1953) gave experimental support to the theory by showing that explants of embryonic chick ectoderm grown in media containing excess of vitamin A underwent suppressed keratinization as compared with control explants grown in normal media. While the control explants formed keratinizing squamous epithelium, those with excess vitamin A underwent a differentiation into mucus-secreting, often ciliated, epithelium similar to that of normal nasal mucosa.

The largest series in which vitamin A alone has been used as therapy was described by Germeraad, Vashinder, Verbeek, and Van Der Sijde (1955) in Holland. They treated 133 cases, using 100,000 I.U. thrice daily for periods of six to nine months, and thought that the majority of these cases had been improved by the treatment. They estimated the vitamin content of the plasma at intervals and found that the best results were obtained when that level rose above 300 I.U./100 ml. They also found that

oral administration was as satisfactory as parenteral and that doses of 50,000 I.U. thrice daily were sufficient to achieve this level.

The above authors and others have agreed that acne which was characterized by prominent follicular plugging in addition to the formation of comedones responded best to treatment with vitamin A, whereas the results were disappointing in the pustular and cystic types. Most trials suffered from lack of proper control cases. The place of vitamin A in therapy therefore did not appear to us to be established on firm grounds, especially as further workers—Lynch and Cook (1947) and Mitchell and Butterworth (1951)—found their results disappointing. It seemed that with a long-term and fluctuating condition such as acne there was a good case for a double-blind therapeutic trial, using an objective method of assessment to judge results. Furthermore, we wished to study the problems such an objective method might raise in general practice.

Logan (1960), analysing the records of members of the College of General Practitioners, indicated that 4.1 per thousand of the population sought advice for this condition annually and that most of these were in age-group 12–24 years, which comprised 18% of the population. A group of general practitioners would therefore be likely to see sufficient numbers for statistical purposes, and the criteria of the disease would be relatively easy to define—a very important point in any group research. Control of therapy during the trial would be simple in general practice, and the fact that there were no known cures for the condition meant that ethical objection to a double-blind trial using dummy tablets would be absent. Having a large number of observers to assess the clinical picture, however, presented difficulties; therefore it seemed wise to establish a precise method of final assessment of results, uniform for all cases in the trial. Colour photography judged by a panel of three was the method selected.

Method

The trial consisted of a comparison of the effects of tablets of vitamin A, each containing 50,000 I.U., with dummy tablets containing lactose 10 mg., starch 110 mg.,

*Recorders: Dr. J. A. D. Anderson and Dr. I. H. Stokoe.