If, on the other hand, we deal only with those washings which produced colds in volunteers and consider how many viruses we could cultivate from them, we obtain the figure of 6/10, or 60%. This may be too high a value, as they do not represent a fair sample: some of the washings were tested in volunteers because we had already obtained a cultivable virus from them.

We may perhaps expect the true figure to be between 25 and 50%, though it is likely that in some places and at some times cultivable or non-cultivable viruses will tend to prevail.

Summary

Cytopathic viruses have been isolated from 25 nasal washings taken from subjects suffering from colds.

Nine of these viruses apparently caused colds in human volunteers. Five washings from which cytopathic viruses were not isolated were shown to cause colds in volunteers.

Washings were collected from a patient during four colds which occurred in a three-year period. The washings were shown to contain three distinct agents, two of which were cytopathic.

There is preliminary evidence suggesting that different agents cause colds with slightly different clinical patterns, and induce the production of specific neutralizing antibodies.

We are indebted to the volunteers for their willing and conscientious co-operation, and to Miss J. Bullock for help with the clinical observations. We thank Dr. C. H. Andrewes for help in preparing the manuscript; Dr. P. K. Hopper, Dr. H. G. Pereira, Dr. R. E. Hope Simpson, Dr. D. Hobson, and Dr. E. J. C. Kendall, for supplying clinical specimens; and Dr. H. E. M. Kay, Mr. J. R. Reynolds, and the M.R.C. poliomyelitis control laboratories, for supplying tissues or cultures. We also thank Mr. J. May for preparing the cultures, and Mr. M. J. Young for the photomicrograph.

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At the outset of the year 1959 to 1960 the Institute of Cancer Research, Royal Cancer Hospital, was concerned about its finances as there was then no guarantee that the grants it had received from the United States Public Health Service would be renewed. A generous new grant of £57,589 was, however, made by the U.S.P.H.S., and the Institute's finances were further assisted by an increase in the block grant made by the Medical Research Council (£212,800) and that made by the British Empire Cancer Campaign (£66,803). Throughout the year work proceeded on the extension to the Chester Beatty Research Institute in Fulham Road—a major undertaking involving the expenditure of £238,000 from the Trust Fund administered by the Board of Governors of the Royal Marsden Hospital in aid of the Institute, and £40,000 from the Wellcome Foundation. *(Report for the Year 1959–60.* Institute of Cancer Research, Royal Cancer Hospital.)

INOCULATION OF HUMAN VOLUNTEERS WITH E.C.H.O. VIRUS TYPE 20

BY

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E.C.H.O. virus type 20 was first isolated in the U.S.A. from children and infants living in a residential home. The virus was recovered by inoculating tissue cultures of monkey-kidney cells with extracts of faeces and throat swabs. Many of the properties of the virus have been described (Rosen, *et al.*, 1958). Some of the children infected with the virus were well, others had minor illnesses resembling common colds, and some were admitted to hospital with a moderately severe febrile illness manifesting pharyngitis, bronchitis, diarrhoea, and conjunctivitis. It was not possible to decide whether these illnesses were due to the virus infection. Accordingly we have inoculated volunteers living in strict isolation to determine, firstly, whether adults can be infected by intranasal inoculation of the virus, and, secondly, whether such infections produce an illness, and if so of what clinical type.

Materials and Methods

Forty-three volunteers of both sexes, aged 18–45 years, were used in these experiments. They were isolated, usually in pairs, as described elsewhere (Andrewes, 1949). At the same time 33 volunteers were given culture fluid without virus. Volunteers were allocated at random to the experimental groups, and the clinical observer was unaware of the nature of the inoculum until he had completed the records. Volunteers were considered to be "ill" when the symptoms and physical signs developed after inoculation seemed to differ significantly from the state observed during the four-day quarantine period. Blood was collected before inoculation. Throat swabs and faecal specimens were collected on the first, third, and fifth days after inoculation, and a second specimen of serum and a fourth faecal specimen on the fourteenth day, as in the trials of E.C.H.O. 11 virus (Buckland *et al.*, 1959).

Tissue Cultures.—Human-embryo-lung cells were cultured as explants or after trypsinization, and monkey-kidney cells after trypsinization. All cultures were rolled at 36° C. and the medium at the time of inoculation contained 2% calf serum, 0.25% lactalbumin hydrolysate in Hanks's saline containing 0.1% sodium bicarbonate and antibiotics.

Virus Strains.—Three throat swabs from each of two patients in the U.S.A. were immersed in medium, and the fluid was tested in Bethesda for the presence of virus. The fluids were then transported in the frozen state to Salisbury and tested again. Separate pools were made of the fluid from each patient. These pools were diluted in Hanks's saline and 1 ml. was administered as nasal

drops to volunteers. The prototype strain of virus, which had been passed five or more times in monkey-kidney cells, was used for serum neutralization tests.

Virus isolations were performed in explant or trypsinized cultures or human-embryo-lung cells. Specimens were concluded to be negative after three attempts at isolation using a total of 0.6 ml. of inoculum and six tube cultures. Cultures were observed for cytopathic effects for 10 days. When the faeces or throat swabs of a volunteer yielded a virus, at least one isolate from each site was identified by a neutralization test with a specific antiserum prepared in rabbits.

Serum neutralization tests were performed by mixing serial fourfold dilutions of inactivated serum with 10–100 TCD_{50} of virus. These mixtures were held overnight at 4° C. or at room temperature for one hour because thermal inactivation of the virus was rapid at room temperature and 37° C. (see Lehman-Grube and Syverton, 1959). Each mixture was tested by inoculation into two or more cultures.

Infection of Volunteers with Swabs and Cultures

The pooled swab fluids did not contain much virus and were small in volume. As can be seen from Table I, the fluids were administered to eight volunteers. A mild illness was observed in four of the five volunteers from whom virus was recovered and in one of the three from whom it was not recovered. (However, the main feature of this one volunteer's illness was a pyrexia of 100° F. (37.8° C.), which occurred six hours after inoculation and which may not have been due to virus infection.) One illness in a virus-infected volunteer (Sun.) showed the clinical features of a mild common cold. Nasal washings were taken near the onset of this illness; these were inoculated into six further volunteers. Virus was recovered from all of these; moreover, five of them became ill.

Virus from a throat swab (collected from Sun. at the same time as the nasal washings) was passed serially in cultures of human embryo lung, human embryo kidney, and monkey kidney. The identity of the virus was checked in each case after three passages, and an attempt was made to detect contaminating viruses by adding the fluid mixed with monkey or rabbit immune serum to tissue cultures, which were then kept for two or three weeks and observed for cytopathic effects. No contaminating virus was detected. Dilutions of the pools of infected culture fluids were then administered to volunteers by the same techniques as those used for administering nasal washings. Fourteen out of 15 volunteers became infected, and 11 of these became ill. One of the illnesses which followed inoculation of material passed in human-embryo-kidney cells showed the features of a common cold in addition to a febrile illness.

TABLE I.—*Summary of Results of Infecting Volunteers with E.C.H.O. 20 Virus*

Source of Virus	Tissue-culture Passages	Dilution of Virus Inoculated	Dose Inoculated*	No. of Volunteers: Inoculated	No. of Volunteers: Infected †	No. of Volunteers: Ill	Virus Isolated from: Throat Swab	Virus Isolated from: Faeces	No. of Volunteers with Antibody Rise
Patient C	Nil	1/2	30	4	3	2‡	2	3	3
,, Str.	,,	1/2	20	4	2	2	1	1	0
,, ,,	,,	1/2	20+ antiserum	4	0	0	0	0	0
Volunteer Sun. . .	,,	1/4	300	6	6	5	6	6	5
Volunteer Sun.	3 in H.E.L.§	1/2	300,000	8	7	7	7	7	3/6
,,	3 in H.E.L.	1/2	300,000+ antiserum	8	5	3	1	5	4/7
,,	3 in H.E.K.	1/100	45,000	3	3	3	3	3	3
,,	3 in M.K.	1/300	56,000	4	4	1	4	4	1/2
Patient C	3 in M.K.	1/10	300,000	2	2	2	2	2	1/1

* Tissue-culture infectious doses determined in cultures of human-embryo-lung cells.
† Number of volunteers from whom virus was recovered on one or more occasions.
‡ In addition, one volunteer was ill but not infected.
§ Human embryo lung, human embryo kidney, and monkey kidney.

Neutralization Experiments

Each of two pools of virus prepared in human embryo lung was mixed with an equal volume of undiluted hyperimmune monkey or rabbit antiserum and held overnight at 4° C. The mixture was diluted immediately before inoculation to give the same final concentration of virus pool as was administered in the parallel tests without serum (shown in the previous line of Table I). The virus was not completely neutralized in one pool, as judged by tests in tissue cultures, and some volunteers did become infected after receiving each of the pools. Two illnesses occurred in the five volunteers infected with the virus and one in a volunteer from whom a virus was not recovered. In contrast to this result, no illnesses or virus infections were observed in four volunteers who received the throat swab material from patient Str. after similar treatment with the monkey immune serum which had been used with the tissue-culture-passed virus.

Recovery of Virus

The data in Table I on the recovery of virus show some points of interest. Firstly, virus was recovered both from throat swabs and from faecal suspensions. Thirty-seven volunteers sent back by post specimens of faeces taken about two weeks after the day of infection, and 18 of these contained virus. The general pattern of virus excretion is thus like that found in similar experiments with E.C.H.O. 11 virus (see Buckland *et al.*, 1959). However, the virus was usually found in the throat on the day after inoculation of a large dose, but generally not until later after inoculation of a small dose.

Where practicable the amount of virus in the throat swab and faecal specimens was determined by titration. Fig. 1 summarizes these data. It can be seen that there are no gross differences in the levels of virus reached after successful infection by the various inocula used. Furthermore, the general pattern of excretion is similar to that observed in earlier experiments with E.C.H.O. 11 virus in that the titres rise earlier in the pharynx than in the faeces, while later on more virus is found in the faeces than in the throat swabs. However, there was no significant difference in the amount of virus excreted by volunteers who were infected but remained well and those who were infected and became ill. The pattern of excretion is rather different from that found with the Coe virus, in which virus was usually more readily recovered from the throat than from the faeces (Parsons *et al.*, 1960).

Paired sera from most volunteers were titrated for antibodies though none or only single samples were available from a few volunteers. No neutralization (titre <4) was found in the first specimens of serum from 24 out of 34 volunteers. Only four volunteers failed

to become infected after being given virus, and two of these had the highest levels of neutralizing antibody of all. No antibody was detected in the first specimens from the other two volunteers who were not infected, but they received only a small dose of virus. Fourfold or greater rises in antibody titre were found in 20 of 25 pairs of sera of the volunteers from whom virus was recovered. No rises occurred in volunteers from whom virus was not recovered. Small batches of human-embryo-lung and human-thyroid culture had perforce to be used in some of the neutralization experiments: some inconsistent results were found, probably due to the differing sensitivity of the tissues to virus. Twenty-seven pairs of sera were then tested at the National Institute of Health, and some of them again at Salisbury, using monkey-kidney cultures throughout, and except in three instances, where antibody rises were found at the former but not at the latter laboratory, the results were in agreement. In the three instances mentioned the results have been regarded as positive.

Illnesses and Their Relation to Virus Infection

Virus was recovered from 32 of the 43 volunteers who were inoculated, and 24 of these were thought by the clinical observer to have a definite illness. One of the 11 volunteers who were not infected with the virus became ill. The type of illness observed was in general not that of a typical common cold, but two volunteers showed typical coryza with some additional symptoms as described below. The incubation period varied from one to five days (average 2.5 days) and then followed generalized influenza-like symptoms and some respiratory symptoms. As can be seen from Table II the main constitutional symptoms were malaise, headache, and aching limbs, and it is of interest that 14 volunteers were febrile (oral temperature greater than 99.2° F.: 37.3° C.), the maximum oral temperatures ranging up to 102° F. (38.9° C.) (average 100.3° F.: 37.9° C.). The commonest respiratory-tract symptom was sore throat, noted in 17 cases, without observable changes except redness of the fauces in five cases. There was, however, tender enlargement of the cervical lymph nodes in six volunteers. Alimentary-tract symptoms were not prominent but occurred in eight subjects. Fig. 2

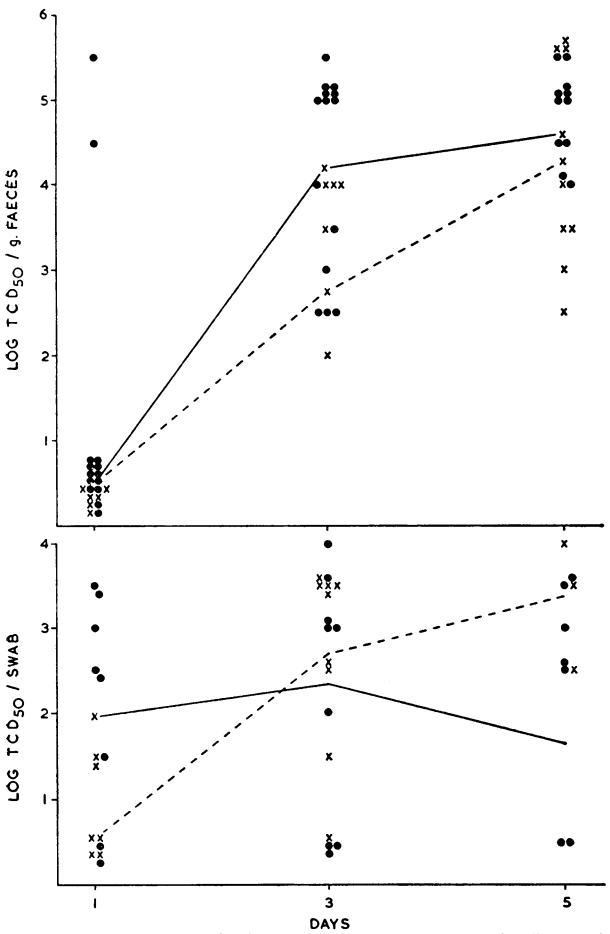

FIG. 1.—Titre of virus in faeces (top) and throat swabs (bottom) of volunteers infected with E.C.H.O. 20 virus. Abscissae indicate the number of days after inoculation. × shows the titre of a specimen from a volunteer infected with throat swab or nasal washing, and × - - - × indicates the mean titre for this group. ● and ×——× are corresponding data for volunteers infected with tissue-culture fluids.

TABLE II.—*Frequency of Various Symptoms in Volunteers*

	No. of Volunteers with Indicated Symptoms		
	Inoculated and Infected* (32)	Not Inoculated (33)	Inoculated but Not Infected† (11)
Malaise	20	0	2
Headache	15	1	3
Aching limbs	12	0	0
Backache	5	0	0
Chill	7	0	1
Pyrexia	14	0	1
Cervical adenitis	6	0	1
Nasal obstruction	5	3	1
More than 4 handkerchiefs a day‡	4	1	1
Coryza	2	0	2
Sore throat	17	2	2
Cough	1	0	0
Abdominal distension	3	0	0
Diarrhoea	1	0	0
Constipation	1	0	0
Vomiting	2	0	0
Nausea	3	0	0

* Virus recovered from throat or faeces or both.
† Includes those given neutralized virus.
‡ Two volunteers used more than 4 handkerchiefs a day before inoculation but no increase in nasal discharge occurred thereafter.

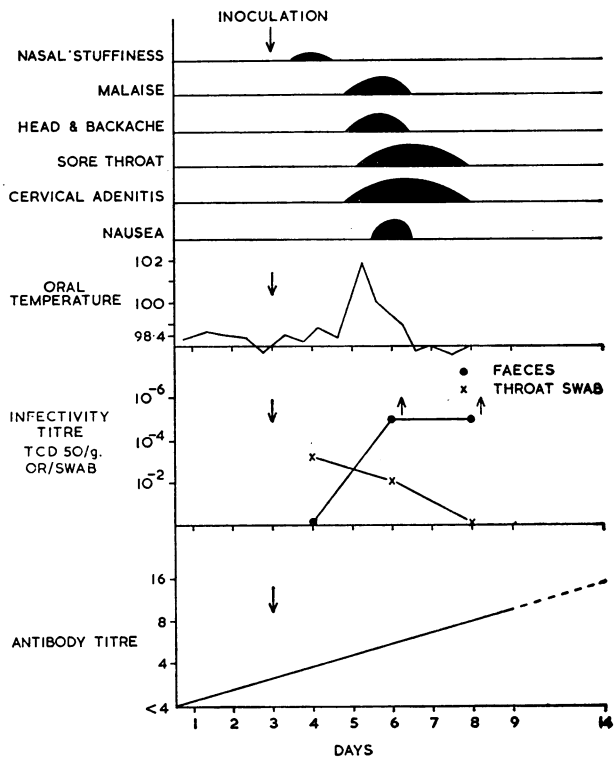

FIG. 2.—Diagrammatic summary of the course of events in a volunteer infected on day 3 with 300,000 TCD_{50} of E.C.H.O. 20 virus passed three times in human embryo lung cultures.

correlates in a graphical form the clinical and laboratory observations on one volunteer who showed a typical response of rather more than average severity.

Thirty-three volunteers were inoculated with Hanks's saline or medium from uninfected cultures of human-embryo-kidney cells. One of these developed a mild cold and six developed mild symptoms, mainly respiratory, at some time after their inoculation; but only two or three of these volunteers would have been regarded as experiencing mild illnesses by the criteria used in evaluating the clinical data on the volunteers receiving E.C.H.O. 20 virus.

However, the volunteers who were inoculated with virus and from whom virus was not recovered had more symptoms than the uninoculated controls and fewer than the virus-infected volunteers. This might have been due either to infection by another virus, which would not multiply in the cultures used, or to infection by E.C.H.O. 20 virus which we failed to detect. We think the latter is the most probable explanation, since most of the illnesses occurred in the experiments in which large doses of partly neutralized virus were given; while, on the other hand, no illnesses occurred in the volunteers inoculated with the mixtures of low-titre throat-washing virus and immune serum.

Discussion

The following conclusions can be provisionally drawn. When material containing E.C.H.O. 20 virus from throat swabs, nasal washings, or tissue culture was given to volunteers, illness as a rule followed and virus multiplication could generally be demonstrated in those that were ill. The illnesses were mainly undifferentiated minor febrile disease with sore throat; coryza occurred rarely.

These results support the view that the illnesses suffered by some of the children in the U.S.A. from whom the viruses were isolated (see Cramblett *et al.*, 1958) were probably due to E.C.H.O. 20 virus. The general results of these studies may be compared with those obtained using Coe virus and E.C.H.O. 11 in similar experiments. E.C.H.O. 20 produced illness rather less often than did these viruses and the clinical picture was different. In Coe virus infections general symptoms with fever were quite common, but coryza occurred in every case. In E.C.H.O. 11 virus infections coryza was not seen, but sore throat occurred and abdominal symptoms were quite common.

Summary

Forty-three human volunteers living in isolation were inoculated with E.C.H.O. 20 virus as nasal washings or tissue-culture fluids. Twenty-seven volunteers became ill. The main symptoms and signs observed were headache, malaise, aching limbs, sore throat, and fever. Two volunteers showed a syndrome resembling the common cold and eight had abdominal symptoms. Virus was readily found in the throat and faeces of most volunteers, and antibody responses fourfold or greater occurred in 20 out of 25 volunteers from whom virus was recovered.

We thank Miss J. B. Macdonald for help with the clinical observations and Miss P. K. Pearce for technical assistance. The experiments would have been impossible without the willing help of the volunteers, to whom we are grateful.

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ACUTE DIVERTICULITIS

A REVIEW OF EMERGENCY ADMISSIONS

BY

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This paper is based on 138 cases of diverticulitis admitted as emergencies to a general hospital for acute cases over a recent two-year period. The diagnosis was confirmed in every case by operation, barium enema, sigmoidoscopy, or necropsy—often by a combination of these methods. "Diverticulitis" is a general term including a range of conditions that are defined below.

Incidence

In a series of 116 consecutive necropsies in patients over the age of 40 the colon was examined specifically for diverticulitis. This gives the absolute incidence of the condition. Of the 116, 14 (12%) showed the presence of diverticulitis. This incidence compares with that of other series (Edwards, 1954; Aird, 1957), but is greater than that of Wakeley (1936). In men, 6 out of 68 necropsies showed diverticulitis in the colon (9%), and in women 8 out of 48 (17%). The absolute incidence rose with age: in the 60–69 decade 3 out of 26 showed diverticulitis (12%), in the 70–79 decade 3 out of 16 (19%), in the 80–89 decade 4 out of 16 (25%), and in the over-90's 3 out of 3 (100%).

The relative age incidence of the 138 clinical cases is shown in Fig. 1. It can be seen that the highest number of cases is in the 70–79 age-group. None occurred below the age of 40.

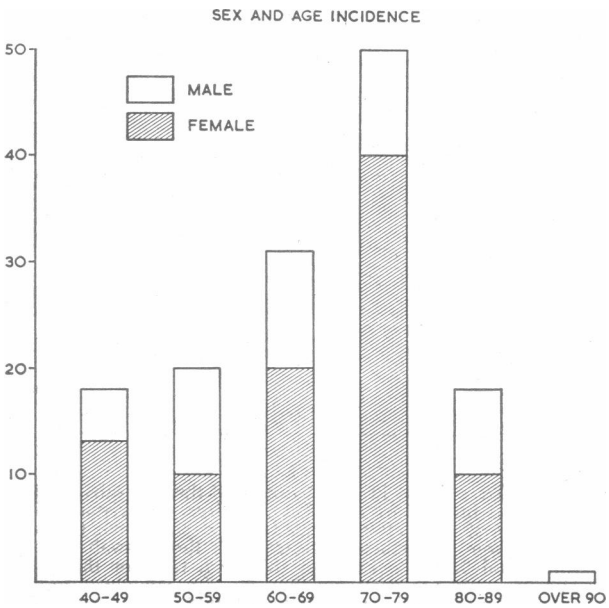

FIG. 1.—Age and sex incidence in 138 cases of acute diverticulitis.