SOME FURTHER VIRUS ISOLATIONS FROM COMMON COLDS

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We have recently reported the successful cultivation of some viruses from nasal washings collected from three patients suffering from common colds (Tyrrell *et al.*, 1960). The viruses grew and produced a cytopathic effect in cultures of human-embryo-kidney cells when these were maintained at 33° C. rather than 36° C., and in a medium with a lower bicarbonate concentration and therefore a lower *p*H than is usually employed (Tyrrell and Parsons, 1960). These findings have been confirmed by Hobson and Schild (1960). We also presented evidence that the viruses we collected were able to cause colds after two to eight passages in tissue culture and reinoculation to man.

We have now expanded this work by testing many more specimens in tissue cultures and in volunteers, and the results are reported here.

Materials and Methods

Tissue Cultures.—Human-embryo-kidney cultures and secondary rhesus-monkey-kidney cultures were used, and were prepared as described elsewhere (Tyrrell *et al.*, 1960). The maintenance medium used consisted of 2% filtered unheated calf serum and 0.25% lactalbumin hydrolysate (Nutritional Biochemicals Corporation, Cleveland, Ohio) and Hanks's saline containing 0.03% of sodium bicarbonate with 100 μg. of streptomycin, 100 units of penicillin, and 20 units of mycostatin per ml. All cultures were maintained in a roller drum at 33° C.

Virus Isolation.—Nasal washings taken with broth saline or Hanks's saline, or fluids from swab specimens, were thawed and 0.3 ml. was inoculated into each of three or four culture tubes. The medium was changed after one day and again after about five days of incubation. If a cytopathic effect occurred the fluid phase of the culture was removed and stored at −60° C., usually at the stage at which focal lesions were present or were becoming confluent. Passages were often unsuccessful if the fluids were harvested from tubes showing advanced degeneration. Further passages, using 0.2 ml. of culture fluid per tube, were made. At first we passed fluids from both positive and negative cultures. Later, because passages from negative cultures were uniformly negative, and because of a shortage of tissue cultures, only positive cultures were passed.

Storage of Viruses.—All specimens were stored in the laboratory at about −60° C. in the absence of CO_2. Some specimens from Cirencester and Epsom were stored at −20° or −40° C. for a few days or weeks before reaching the laboratory.

Sources of Materials.—The washings were mainly collected from patients in the acute phase of typical common colds of varying degrees of severity. Two main collections had been made—at the National Institute for Medical Research at Mill Hill and at Salisbury. The first, in 1951, had been moved about from one laboratory to another, and was, of course, nine years old when tested. The second had been made between 1955 and 1960, mainly from cases of colds in laboratory staff: there were, however, practically no clinical details available about these illnesses. Dr. D. Hobson kindly sent us some selected specimens which he had collected from medical and office staff in Sheffield early in 1960, including some specimens from which he had isolated viruses. The remaining two groups were collected by general practitioners. Dr. R. E. Hope Simpson supplied nose and throat swabs and nasal washings from adults and children in Cirencester who suffered from colds in February and March, 1960, and Dr. E. J. C. Kendall supplied similar specimens from boys at a residential school in Epsom in May and June, 1960.

Isolation of Fresh Strains

The specimens tested have yielded a number of cytopathic viruses: the results are summarized in Table I. The cytopathic effects observed were in all cases indistinguishable from those described earlier (Tyrrell and Parsons, 1960). However, the extent varied from one or two foci in one tube, appearing after 10 to 14 days' incubation and regressing a few days later, to a marked cytopathic effect starting three or four days after inoculation and destroying the whole culture within a week. Human-embryo-kidney cultures often contain groups of rounded cells, and by the end of two weeks' incubation some cells at the edge of the sheet in uninoculated cultures may undergo granular degeneration and break up. It has therefore been found best to use a final magnification of ×30–70 and to search the whole cell sheet at least every second day for groups of cells in an early stage of virus degeneration (Fig. 1). These foci tend to occur near the edge of the cell sheet (Parsons and Tyrrell, 1961) and may be recognized even if the focus contains only a dozen or so cells. These virus-infected cells are refractile, angular rather than rounded in outline, and from the edge of the cell project threads and globules of cytoplasm. In later stages they become completely rounded or converted into masses of granules.

TABLE I.—*Isolation of Viruses from Washings and Swabs Collected from Patients with Colds*

Year of Collection	Place	No. Tested		No. of Cytopathic Viruses Isolated in Human-Kidney-Cell Cultures
		Swabs	Washings	
1951 ..	National Institute for Medical Research, London and Salisbury	—	21	2 (2)*
1955–60	"	16	64	14† (2)
1960 ..	Sheffield (selected specimens)	—	9	6
1960 ..	Cirencester	11	13	0 (1)
1960 ..	Epsom	13	3	3 (1)
	Total ..	40	110	25 (6)

* Figures in parentheses denote appearance of specific cytopathic effect which cannot be passed serially.

† Including the three strains previously reported (Hitchcock and Tyrrell, 1960).

The cytopathic effect produced by five viruses was studied in more detail by examining fixed and stained preparations, and was in all cases indistinguishable from that produced by our prototype strain H.G.P. and very

like that of enteroviruses. Thirteen of our strains were also tested at the higher *p*H and temperature of conventional tissue cultures: in no instance was the cytopathic effect as good as in our standard "common cold" conditions, and with seven strains the cytopathic effect was completely suppressed. Most enteroviruses might be expected to grow better, or at least equally well, in cultures at high *p*H and high temperature (Barron and Karzon, 1957).

Two viruses were isolated from 21 specimens collected in 1951. The isolation rate is low, perhaps because of unsatisfactory conditions of storage. It is, however, of interest that viruses of the type were in circulation at that time; it will also be valuable to have these strains for later serological studies. Viruses were isolated more often from the specimens collected between 1955 and

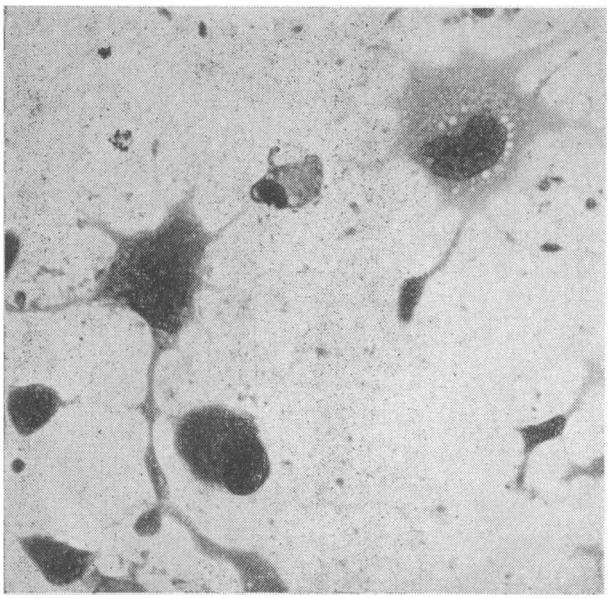

FIG. 1.—High-power view of cells undergoing characteristic degeneration in a culture infected with H.G.P. Cells of this type are the hallmark of virus degeneration when difficulties arise in interpreting changes in human-embryo-kidney-cell cultures. Fixed and stained with haematoxylin and eosin. (×441.)

1960, probably because they were better preserved. The very high rate of isolation from the Sheffield specimens is due to their being selected, and is discussed below. Only one virus was isolated from the nose or throat swabs tested. This might have been because the prevailing virus at the time, particularly in the outbreak at Cirencester, was not cytopathic (see below). On the other hand, we suspect that virus is more readily isolated from nasal washings than from throat or nose swabs; this point is being studied further.

When taken together these results show that viruses cultivable by our modified technique have been current over most of the years since 1951, and that they may be recovered from both children and adults suffering from common colds.* It is of interest that we isolated no adenoviruses or enteroviruses from these washings. The washings D.C., Re., Br., H.G.P.57b, and Gi. were tested for the presence of parainfluenza viruses, using monkey-kidney cells and the haemadsorption technique, but no virus was detected.

*Elsewhere in this paper such viruses are called "cultivable viruses." Viruses found in washings and which cannot be grown in our modified conditions are called "non-cultivable."

Volunteer Studies

We next performed experiments in volunteers to determine whether the washings from which we isolated viruses contained agents which could produce colds. The methods were those used in earlier experiments (Tyrrell *et al.*, 1960), but in most trials we collected nasal washings using Hanks's saline. The washings were taken when a volunteer had suffered for about a day from symptoms which suggested that he was developing a cold. At the same time a washing was taken from the volunteer's companion in isolation, or from another volunteer in the trial who had received the same inoculum and had not developed a cold. It can be seen from Table II that all but one of the washings from which a virus was isolated produced colds in volunteers. (As the specimen from B631 was very small, only four volunteers could be inoculated, and such a result might easily occur by chance with a positive specimen.) We further tested the viruses after they had been passed four times in tissue culture (Table III). This enabled us to test the 1951 and some other strains of which only a little remained in the form of nasal washings. It can

TABLE II.—*Cultivation of Viruses from Cold Washings (Only Those Viruses are Tabulated Which Were Tested Both in Volunteers and in Cultures)*

Strain	Year of Cold	Provisional Strain Designation	No. of Colds in Volunteers given Washings‡	Cytopathic Effect in Human-Embryo-Kidney Cultures
Ri*	1956	—	0/6	0
H.G.P.56	1956	—	0/9	0
Pe.	1959	—	0/10	0
H.S.	1960	—	1/11	0
Ba.§	1960	—	0/7	0
D.C. and derivates	1953	—	7/27	0
Re.*	1956	—	6/8	0
Br.	1956	—	2/7	0
H.G.P.57b	1957	—	3/6	0
Gi.*	1957	—	3/7	0
P.K.†	1956	Salisbury/1/56M	5/11	+
Or.	1956	Salisbury/2/56H	2/4	+
Bo.	1956	Salisbury/3/56H	3/8	+
H.G.P.57a†	1957	Salisbury/1/57M	3/14	+
Th.	1959	Salisbury/1/59H	3/6	+
H.G.P.59	1959	Salisbury/1/59H	4/6	+
16/60 Sheffield	1960	Sheffield/1/60H	2/4	+
30/60 ,,	1960	Sheffield/2/60M	2/7	+
B631 Epsom	1960	Salisbury/1/60H	0/4	+

* These specimens came from volunteers who developed colds after being inoculated with experimental material. Ri. and Re. received organ culture fluid and Gi. washings from a 1955 cold.

† These strains were described in a previous paper (Tyrrell *et al.*, 1960).

‡ The numerator denotes the number of colds occurring and the denominator the number of volunteers inoculated. In parallel with these experiments 101 volunteers received saline or fluids from uninoculated tissue cultures and 2 developed colds.

§ This specimen was a pool of fluids from nose and throat swabs collected from a mother and child developing colds on the same day.

TABLE III.—*Production of Colds by Viruses Passed in Tissue Cultures*

Strain	Year of Cold	Provisional Strain Designation	No. of Passages in Culture	Dose of Virus Given (TCD_{50})	No. of Colds in Volunteers Given Culture Fluids
D.C.	1953	—	2	0	0/7
H.G.P.57b	1957	—	2	0	0/7
Gi.*	1957	—	2	0	0/7
No.	1951	Salisbury/1/51	4	300	3/7
Cl.	1951	Salisbury/2/51	4	3	3/6
Mi.	1956	—	1	?	2/6
Or.	1956	Salisbury/2/56H	4	3,000	3/8
P.K.†	1956	Salisbury/1/56M	2	?	5/24
H.G.P.57a.†	1957	Salisbury/1/57M	2	?	6/24
F.E.B.†	1958	Salisbury/1/58H	8	?	4/16
Th.	1959	Salisbury/1/59H	4	1,000	1/13
16/60	1960	Sheffield/1/60H	4	1,000	4/6
30/60	1960	Sheffield/2/60M	4	<10	2/7

* † See notes to Table II.

be seen that six out of seven strains tested in this way produced colds in volunteers. There is little evidence of attenuation of the viruses except in the case of the strain Th., and we found no correlation between the number of tissue culture doses of virus inoculated and the clinical result.

Proportion of Colds Yielding Cultivable Viruses

We wished to know what proportion of cold viruses we were able to grow, and for this purpose we studied our most plentiful specimens from the second collection and two specimens from Cirencester. These were diluted 1/10 and inoculated into volunteers, and it can be seen from Table II that about half of them contained a cold-producing virus. We made repeated attempts, using several washings, to demonstrate a cytopathic effect of the D.C. strain, the apparent cultivation of which was reported in 1953 (Andrewes *et al.*, 1953); we also tested tissue culture fluids in human volunteers. All these experiments gave negative results, and it does seem that some further substantial modification of the conditions will be necessary before such a virus can be cultivated. Some modification of the culture procedure will also be needed to propagate strains such as Mi. On two occasions a typical cytopathic effect was seen in cultures inoculated with these washings, and fluid from such cultures produced colds in volunteers (Table III). It was not possible, however, to detect a definite cytopathic effect on further passage. Five other strains showed a similar behaviour; they are for the time being listed as "virus isolations" but enclosed in parentheses in Table I.

Clinical Features

We have considered critically the clinical features of the colds produced by these materials (see Table IV). It is clear that all of the colds, whether from washings or culture fluid, were typical "common colds," the characteristic features of which were coryza, frequently becoming mucopurulent, nasal obstruction, and sore throat. There was a cough in a proportion of cases. Our experimental colds have in the past been characterized by the absence of pyrexia, but in these experiments some degree of fever—maximum 101.6° F. (38.7° C.)—occurred in 12 of 63 volunteers with colds. The incubation period was in most cases between one and three days, except for one strain, Re., in which the incubation period was from three to six days. The Re. strain was also distinguished by the unusually high percentage of "takes" (six colds in eight volunteers) and by the marked degree of malaise. The No. strain produced colds in three out of seven volunteers; two of these three volunteers had nausea and diarrhoea, and so did one other, without symptoms of a cold. However, in this particular trial two control volunteers also suffered from symptoms involving the alimentary tract.

TABLE IV.—*Frequency or Average Magnitude of Selected Symptoms and Signs in Colds Produced by Certain Viruses*

Strain	Malaise	Injected Fauces	Cough	Mean of Peak No. of Handkerchiefs Used per Day	Incubation Periods (Days)
H.G.P.	1/12*	1/12	9/12	15	2·2
Kelly	0/9	0/9	6/9	12	2·3
Re.	5/6	1/6	1/6	16	3·8
16/60	1/6	0/6	1/6	10	2·3
30/60	0/4	0/4	1/4	4·7	2
Th.	1/4	2/4	2/4	26	2
Or.	3/5	4/5	3/5	15	2

* Numerator = Number of volunteers with symptoms or sign. Denominator = Number of volunteers with colds.

Although all volunteers with colds complained of sore throat there were usually no abnormalities on examination of the throat. The throat was, however, injected in three of five colds produced by Or. virus. The average number of paper handkerchiefs used at the peak of nasal discharge was between 10 and 16 a day, but the average for colds produced by strain 30/60 was 4.7 a day, while that produced by strain Th. was 26 a day. Though not conclusive, these results suggest that not only is the individual volunteer's response to a cold virus variable, but that the average clinical picture produced by some strains is different from that produced by others. Some strains produce marked constitutional upset, while others produce either a "streaming" or a relatively "dry" cold. Other workers have reported differences in the clinical picture produced by certain cold viruses (Pereira and Roden, unpublished; Jackson *et al.*, 1958). This matter will be the subject of further study.

Re-isolations of Viruses from Experimental Colds

It seemed desirable to obtain more evidence that the cytopathic viruses were the cause of the colds we were producing in volunteers. For this purpose we attempted

TABLE V.—*Recovery of Viruses from Volunteers Inoculated with Infected Washings or Culture Fluids*

Virus Inoculated	Cytopathic Virus Recovered from Volunteers	
	With Colds	Without Colds
Cultivable strains:		
No.	1/2	0/3
Cl.	1/3	0/1
Mi.	0/2	—
Or.	4/6	1/5
Bo.	3/3	1/2
Th.	4/4	1/7
16/60	5/5	—
30/60	1/3	0/4
Total ..	19/28	3/25
Non-cultivable strains:		
Re.	0/5	—
Br.	0/2	0/1

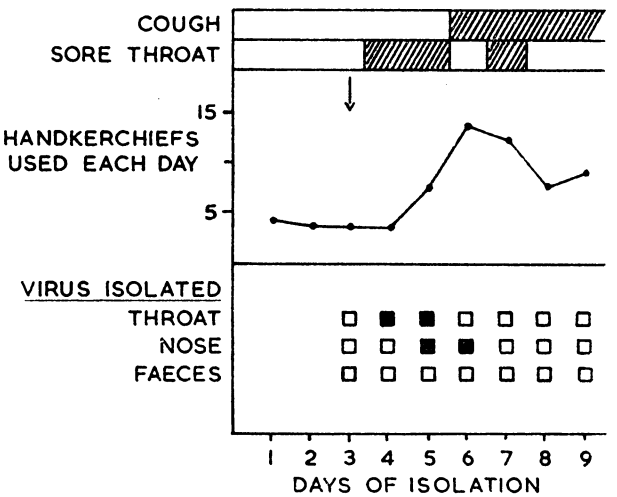

FIG. 2.—Clinical and laboratory findings in a well-studied cold produced by inoculation of washings containing H.G.P. virus. During the illness there was a 28-fold rise in homologous neutralizing antibodies. Virus was isolated separately from the nose and throat by swabs, and specimens of faeces were collected daily. Filled squares represent recovery of virus. Hatched areas indicate presence of a symptom. The handkerchief count is shown.

to isolate cytopathic viruses from volunteers with colds and from volunteers who had not developed colds after inoculation with the same virus. The results are shown in Table V. It can be seen that the rate of isolation apparently varied with the strain used, though the numbers were small. The rates of isolation were similar whether the virus had been passed in tissue culture or not. However, there were far fewer viruses isolated from the volunteers who had no colds than were isolated from those with colds. Altogether we tested washings from 28 volunteers who had developed colds after inoculation of eight different cytopathic viruses. Virus was isolated from 19 of these volunteers, but from only 3 or 25 volunteers without colds, and from none of seven volunteers who had colds induced by non-cultivable viruses. The rate of isolation from these volunteers probably represents the maximum possible at the moment. A fairly large number of volunteers have now been inoculated with H.G.P. virus, and it is clear that virus cannot always be isolated from infected volunteers, especially if specimens are collected long after the onset of symptoms (Fig. 2).

Serological Studies

We would like to have been able to present here a classification of these viruses into serological groups. At the moment, however, this is impossible. We have prepared immune sera in rabbits against our prototype viruses H.G.P. and F.E.B. The former neutralizes the P.K. virus previously described, and one strain, Ha. (from a washing collected in 1955), which, like H.G.P. and P.K., is pathogenic for monkey-kidney cells. This serum fails to neutralize all the other monkey-kidney pathogenic strains (M strains) which have been isolated, and all those pathogenic only for human embryo kidney (H strain) which we have tested so far. It is possible that only one out of seven H strains tested with the F.E.B. serum is neutralized by it. It seems, therefore, that there is definite evidence that the viruses we have isolated form a heterogeneous group both in their cell affinity and in their serological properties. This heterogeneity may hold part of the answer to the problem of the frequent recurrence of clinical colds in many individuals as may be seen in Table VI.

It will be seen from this table and Fig. 2 (legend) that there was a well-marked antibody response to natural and experimental infection with the H.G.P. strain. The antibody levels in this subject slowly declined during the course of two other colds due to viruses which could be clearly distinguished from H.G.P. by their different behaviour in tissue culture. Jackson and Dowling (1959) have shown that after experimental colds produced by inoculation of nasal secretions volunteers fail to develop colds on reinoculation with the same washing, but may develop colds if a different washing is inoculated. Antibody responses such as those shown in Table IV may be the basis of the resistance of volunteers to further infection, but this has yet to be proved.

TABLE VI.—*Study of Certain Spontaneous Colds in Subject H.G.P.*

Tests on Nasal Washings		Tests on Serum		
Date Collected	Results of Tests	Date Collected	Neutralization Titre *versus* H.G.P. 22/2/57 M Type	Neutralization Titre *versus* H.G.P. 15/12/59 H Type
		14/4/56	<5	
		8/5/56	<5	
7/12/56	No virus in T.C.*	7/12/56	5	
	No colds in volunteers			
22/2/57	M type virus.	22/2/57	5	<8
	Colds in volunteers			
		8/3/57	80	<8
		16/5/57	40	<8
26/7/57	Colds in volunteers.*	27/7/57	80	
	No virus in cultures			
15/12/59	H type virus.*	16/12/59	20	<8
	Colds in volunteers			
		31/12/59	30	16

* See Table II.

Discussion

The results show that it is often possible to cultivate a cytopathic virus from washings collected from cases of common colds, and that these viruses, when given to man, cause colds. We therefore feel it reasonable to conclude that they are the causative agents. We can thus confirm our earlier work, and further virus isolations have also been made by others in human-embryo-kidney cells (Hobson and Schild, 1960) and monkey-kidney cells (Hamre, personal communication). On the other hand, we have only begun to scratch the surface of many of the problems raised by these results. On the clinical side we need to investigate the range of diseases in which these viruses play a part. In this laboratory it will be necessary to prepare animal-immune sera and make thorough serological comparisons between the strains isolated and other viruses, and this will be a slow procedure while the source of the antigen consists of cultures of a tissue such as human embryo kidney not readily available in quantity. It is also clear that we should explore in more detail both the serological responses to infection in patients and volunteers, and also the nature of that resistance to infection which so many volunteers show.

In addition, the nature of the virus itself is worth further study, and we have made a start by developing a microplaque assay method (Parsons and Tyrrell, 1961). We intend to look for further modifications to our technique, including the use of susceptible lines of continuously cultivable cells; in this way we may be able in the future to cultivate more virus strains, do more serological work, and obtain higher virus titres than we can at the moment. Our results so far suggest to us that the strains we are growing cover what might be called a "spectrum of cultivability" ranging from those much like JH and 2060, which will grow readily in human and monkey kidney, via strains which will grow in human cells only, to others which will grow only imperfectly in human-kidney cells.

Although we have, for obvious reasons, given most attention recently to viruses which we *can* grow, we would like to know what proportion these represent of all circulating cold viruses. Existing data do not permit us to answer this question; they may well mislead us into making estimates that are too low or too high. We are, we feel, justified in ignoring for this calculation results based on materials which had been stored for nine years and also those based on swabs rather than washings. The figures in Table I then give an isolation rate of 17 definitely cultivable viruses from 80 washings (21%). This figure is probably too low, for two reasons: (*a*) the washings doubtless included a number which contained no virus of any kind and would have given no colds in volunteers; and (*b*) it appears from Table III that virus can be recovered from only about two out of three washings from people inoculated with known cytopathic viruses and developing colds thereafter.

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

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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