they showed generalized and widespread disruption of normal cortical activity during photic stimulation. It thus appears that only certain types of discharge, or, perhaps more accurately, discharges in certain areas rather than others, can be important in producing the psychological phenomena associated with epilepsy. The local character of the discharge invalidates the objection made on the grounds of a failure of anticonvulsants to modify such abnormal behaviour and the failure of metrazol to precipitate it. Focal epileptic activity is very difficult to control by drugs, and it is rarely produced by intravenous metrazol (though, of course, topical application may work).

Does the clinical material enable us to say anything about the psychological effects that may be associated with these forms of discharge? One can, I think, find disturbances in many areas that might be related. There is, firstly, the crude and overt expression of sexual and aggressive functions. There are, secondly, the peculiar disturbances of consciousness, the twilight states, the apparently hysterical outbursts, the pseudo-hallucinations, and so on. There is, thirdly, the intellectual dullness and slowness which may be very marked in some patients. All these three changes may be paralleled by the effects of animal experiments, and I suspect there may be some unitary disturbance of function behind all three, the nature of which we cannot at present formulate. One can only speculate on what might be the effects of epilepsia partialis continuans in the amygdaloid regions by analogy with the continuous jerks produced by this type of constant discharge in the motor areas.

The second lecture, together with a list of references, will appear in our next issue.

VIRUSES ISOLATED FROM NATURAL COMMON COLDS IN THE U.S.A.*

BY

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A new group of viruses causing common colds in human volunteers has now been cultivated in the laboratory by altering the conditions of incubation and the content of bicarbonate in the culture medium (Tyrrell *et al.*, 1960; Hobson and Schild, 1960; Tyrrell and Bynoe, 1961). Pending more complete serological definition of these agents, they are being grouped according to the type of tissue culture in which they produce a cytopathic effect. While some grow equally well in both monkey-kidney and human-kidney cultures, others grow only in human-kidney cultures.

The purpose of this report is to present the results of virus isolations from natural common colds in a group of young adults in the United States. These isolations support the aetiological role of such new viruses in natural common colds, together with some previously described viruses (E.C.H.O. 28, respiratory syncytial virus, para-influenza 1 and 2).

Materials and Methods

Study Population.—Student volunteers in the first and second preclinical years of medical training at the University of Chicago were followed throughout the eight-month academic year. The 96 men and 5 women who enrolled in this programme reported to the laboratory at the first sign of a cold and returned in two to three weeks for convalescent specimens. In addition, specimens were taken from the asymptomatic members of the group at about six-week intervals during the entire period. At each visit nasal and throat swabs were taken in duplicate. One set was placed in 0.5% bovine serum albumin in phosphate-buffered saline for inoculation into tissue cultures and the other in blood broth for bacteriological cultures performed by our clinical microbiology laboratory. A blood sample was also drawn each time. The serum was separated and stored at −20° C. The signs and symptoms of each illness and their duration were recorded.

Tissue Culture.—Secondary monkey- and human-kidney cultures were prepared from cells which had been grown in bottles as primary cultures, and were trypsinized and frozen by the method of Stulberg *et al.* (1959). The monkey-kidney tissue was grown in 0.5% lactalbumin hydrolysate with 5% calf serum and maintained in 50% Eagle's medium and 50% medium "199." Both contained 0.2% hyperimmune rabbit antiserum to SV-5 virus. Human-kidney cultures were grown in 0.5% lactalbumin hydrolysate, 20% calf serum, and 10% medium 199 (Hsiung, 1959) and maintained in 50% Eagle's medium and 50% medium 199 with 1% calf serum. Both maintenance media also contained 0.03% sodium bicarbonate. To all media, 100 units of penicillin and 100 μg. of streptomycin per ml. were added. Both the monkey-kidney and human-kidney cultures were incubated at 33° C. on a roller drum. The H.Ep.2 cell line was carried in bottles on Eagle's medium with 10% calf serum. Tube cultures, after initial growth on the same medium, were maintained on Eagle's medium with 5% chicken serum. Incubation was at 36° C. in stationary racks. The specimens were inoculated into tissue cultures of monkey kidney, human kidney, and H.Ep.2 cells the same day on which they were obtained. The cultures were then examined for cytopathic effect three times a week and passages were made of any which appeared abnormal. Monkey-kidney cultures were tested at five-day intervals for the presence of haemadsorbing viruses by the method of Chanock *et al.* (1958).

Identification of Viruses.—Haemadsorbing viruses were identified by means of the haemadsorption-inhibition test with known antiserum (Chanock *et al.*, 1958). Respiratory syncytial virus and E.C.H.O. 28 were identified by neutralization tests with known antisera (Beem *et al.*, 1960; Hamre and Procknow, 1961).

Serological Tests.—Complement-fixation tests with respiratory syncytial virus were performed using 2 units

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of antigen prepared from the Long strain of virus by the method of Chanock *et al.* (1961). Overnight incubation of 0.1 ml. of antigen, 0.1 ml. of serum, and 0.2 ml. of complement (equivalent to 2 exact units) was carried out at 4° C. in small cups of a plastic panel. Sensitized sheep cells were then added and the test was placed in a humidified incubator at 37° C. for one hour. Titres were read as the reciprocal of the highest dilution of serum giving 75–100% fixation. The neutralization test for respiratory syncytial virus consisted of mixing equal volumes of diluted serum with approximately 100 TCID50 of the respiratory syncytial virus strain 21/Chicago/61 originally isolated from one of the student volunteers. After incubation for two hours at room temperature, the mixture was inoculated into H.Ep.2 cultures which had been prepared one day previous to the test. A known positive serum was included in each day's test, and the virus was retitrated. Tests were read for typical cytopathic effect three to four days later.

Results

Isolation of viruses from nose and throat swabs obtained from October 3, 1960, to June 9, 1961, in the student volunteers are summarized bi-weekly in Table I. From the 199 specimens obtained early in the course of a common cold, 53 viruses were isolated. Eight of these were identified as E.C.H.O. 28, all of which were

TABLE I.—*Bi-weekly Summary of the Isolation of Viruses from Nose and Throat Swabs from Young Adults*

1960–1 Week Starting	Viruses Isolated from Specimens										
	Common Cold							Convalescent Visits		Routine Asymptomatic Visits	
	No. of Specimens	E.C.H.O. 28	H.K.*	M.K.†	R.S.‡	Para 1	Para 2	No. of Specimens	H.K.	No. of Specimens	H.K.
Oct. 3	28	3	1	1				0		75	1
,, 17	18	5	3					24		0	
,, 31	5		1					19		0	
Nov. 14	7							9		0	
,, 28	10							9		68	
Dec. 12	5							7		0	
,, 26	4							3		1	
Jan. 9	16					1		7		81	
,, 23	19				7			10		0	
Feb. 6	14		2		4		1	21		0	
,, 20	8							16		74	
Mar. 6	11		2					6		4	
,, 20	12		5	1		1		5		0	
Apr. 3	18		6	2				13		58	
,, 17	9		2	2				16	1	9	1
May 1	8			1				9		0	
,, 15	7		2					9		81	
,, 29	0							7		5	
Totals	199	8	24	7	11	2	1	190	1	456	2

* Human-kidney viruses. † Monkey-kidney viruses. ‡ Respiratory syncytial virus.

isolated during the first two weeks of October, when the incidence of colds was exceptionally high. Twenty-four were unidentified viruses which grew in human kidney and resembled the F.E.B. strain from Salisbury (Tyrrell *et al.*, 1960). Seven were unidentified viruses which grew in both monkey- and human-kidney cultures and resembled the H.G.P. strain from Salisbury. However, these viruses were not neutralized by guinea-pig antiserum to H.G.P. Eleven strains of respiratory syncytial virus, known also as the chimpanzee coryza agent, were isolated in late January and early February. Para-influenza viruses were encountered only sporadically.

Of the convalescent specimens, only one yielded a virus which grew in human-kidney cultures. A similar agent had been isolated from the acute specimen obtained from the same individual two weeks previously.

Only two viruses were isolated in human-kidney cultures from specimens obtained from asymptomatic students. These viruses were present during periods of increased prevalence of colds and when similar agents were being isolated from symptomatic individuals. The remaining 454 specimens obtained during routine sampling were negative.

Nine of the 101 student volunteers were free of colds during the entire season. Otherwise, the number of colds ranged from one to five per student. There did not appear to be any remarkably different clinical pattern associated with the various viruses isolated to classify as specific for a given virus. The signs, symptoms, and duration of illness are correlated with the specific virus isolated as the suspected aetiological agent in Table II. The illnesses associated with the para-influenza viruses are excluded because there were so few. Only six throat swabs obtained from individuals with colds yielded beta-haemolytic streptococci by

TABLE II.—*Summary of Clinical Histories of Cases from which Viruses were Isolated*

Signs and Symptoms	Human-kidney Viruses (24 Cases) %	Monkey-kidney Viruses (7 Cases) %	E.C.H.O. 28 (8 Cases) %	Respiratory Syncytial (15 Cases) %
Coryza	80	86	88	48
Nasal congestion	29	44	12	53
Cough	46	57	37	67
Sneezing	46	71	0	51
Pharyngitis	71	57	88	100
Headache	37	44	50	46
Chilliness	8	14	25	0
Feverishness	16	0	25	20
Malaise	29	28	25	40
Duration (days):				
Average	10·2	7·6	10·2	10·3
Range	4–19	4–14	4–24	2–20

routine bacteriological culture. By contrast, 11 convalescent specimens and 19 routine specimens yielded beta-haemolytic streptococci. In two specimens both a human-kidney virus and beta-haemolytic streptococci were present.

In an attempt to establish a viral aetiology for the common cold, it is notable that human-kidney and monkey-kidney viruses were usually isolated during each period of increased incidence of colds. However, other viruses were often involved also. In October, during the first outbreak of colds affecting 51 individuals, five human-kidney, one monkey-kidney, and eight E.C.H.O. 28 viruses were isolated. Neutralization tests with E.C.H.O. 28 virus on all sera collected during this period revealed rises in titres only in sera from persons from whom the virus had been isolated (Hamre and Procknow, 1961).

During the second outbreak of illness occurring in late January and early February, 33 specimens yielded 11 respiratory syncytial viruses, two human-kidney viruses, and a single para-influenza 2 virus. A flurry of 41 spring colds during March and early April resulted in a most impressive isolation of 13 human-kidney viruses, three monkey-kidney viruses, and one para-influenza 1. Although the incidence of colds declined somewhat after mid-April, an additional four human-kidney viruses and three monkey-kidney viruses were recovered. During the months of November and December no viruses were isolated, although respiratory symptoms were definitely occurring in the student volunteers.

Although all human-kidney viruses, after several passages, were inoculated into monkey-kidney cultures and incubated on a roller drum at 33° C. for 10 days, none produced a cytopathic effect in monkey-kidney cells. When the tissue cultures were incubated stationary at 36° C., none of the human-kidney or monkey-kidney viruses produced more than a slight evanescent cytopathic effect. Under the optimum conditions of rolled cultures incubated at 33° C. these viruses produced a change similar to that described by Tyrrell *et al.* (1960). Foci of rounded refractile cells and large cells with long processes appeared. These cells seemed to contract later on and disappear from the culture. The extent to which these changes involved the culture varied widely with different viruses and was not always improved by passage. Differences in growth of the human-kidney viruses could not be correlated with different batches of human-kidney tissue. Kidneys from term infants (minimum weight of 2,500 g. and length of 50 cm.), premature infants (weight from 1,000 to 2,500 g. and 45 cm. or less in length), and foetuses from abortions or pregnancy interruptions (weight less than 1,000 g.) supported growth of these viruses.

Complement-fixation tests with respiratory syncytial virus have been performed on all sera collected in this study. In addition, neutralization tests with respiratory syncytial virus were carried out on sera from all students who reported with colds during the period from January 23 to February 24. Rises in antibody titre were detected in only those sera collected during the period of a month when the virus had been successfully isolated. All titre increases occurred in persons reporting with cold symptoms. The serological results and respiratory syncytial virus isolations are presented in Table III. It will be noted that a fourfold or greater increase in antibody titre could not be detected in four persons from whom respiratory syncytial virus had been isolated. All had initial neutralizing antibody titres of 1 : 16 or greater. In two subjects from whom viruses were isolated an antibody increase was detected only by neutralization tests and not by complement-fixation tests. Four additional infections with this virus were detected by serological tests alone.

TABLE III.—*Results of Serological Tests with Respiratory Syncytical Virus*

Student No.	Date of Acute Specimen	R.S. Virus Isolation	Complement Fixation		Neutralization	
			Acute	Conv.	Acute	Conv.
186	Jan. 24	+	<8	8	16	32
143	,, 27	+	<8	8	8	32
116	,, 27	+	<8	32	16	>128
131	,, 27	+	8	8	16	32
103	,, 30	−	<8	16	32	128
119	Feb. 1	+	<8	128	<8	>128
211	,, 1	−	<8	256	<8	>128
151	,, 2	+	<8	32	16	64
154	,, 2	−	8	64	32	>128
117	,, 3	+	16	32	>64	128
147	,, 6	−	<8	32	16	64
153	,, 6	+	<8	<8	<8	32
104	,, 7	+	16	64	32	128
124	,, 8	+	<8	<8	>32	16
132	,, 13	+	<8	16	8	>64

Discussion

These data serve to further emphasize and implicate multiple viral agents in the aetiology of the common cold in young adults and to stress the importance of establishing suitable isolation methods for obtaining viruses currently prevailing during respiratory illnesses, It should be pointed out, however, that the aetiological agent was successfully identified in 28% of the colds seen in the present study. Serological methods for detecting infections with some of the new viruses may serve to increase this identification somewhat, but the likelihood that certain viruses causing colds still cannot be cultivated in the laboratory must be borne in mind (Tyrrell and Bynoe, 1961).

The establishment of a specific viral aetiology for the common cold depends upon a close clinical appraisal of these infections. Because it was quite impossible for the student volunteers to be seen at intervals during their colds, it was necessary to rely entirely on a medical history obtained at the time of convalescent sampling. Upon examining these histories in retrospect, it was impossible to exclude any of the colds from the study, although allergic rhinitis or sore throat from non-specific irritants might have been the better diagnosis. Therefore the total figure of 199 specimens obtained at the acute onset of respiratory symptoms includes an unknown number which rightly contained no viral or bacterial pathogens.

While serological data for comparing the human-kidney and monkey-kidney viruses isolated in England with those reported in this study are not yet available, the similarities in host range, optimum growth conditions, and cytopathic effect of these viruses strongly suggest that they belong in the same family.

Results of this study again emphasize the right of the respiratory syncytial virus to be included in the list of common-cold viruses. The importance of this virus in respiratory infections in infants and small children is now well established (Chanock *et al.*, 1957 ; Beem *et al.*, 1960 ; Chanock *et al.*, 1961 ; Parrott *et al.*, 1961 ; Hamparian *et al.*, 1961 ; McClelland *et al.*, 1961 ; Reilly *et al.*, 1961). Although previously isolated from only two adults (Hamre *et al.*, 1961), recent studies in human volunteers (Johnson *et al.*, 1961 ; Kravetz *et al.*, 1961) have demonstrated that this virus can cause mild respiratory disease in older persons. The outbreak which occurred in late January and early February among the student volunteers in the present study coincided with the season of greatest prevalence of this virus in children.

It is also interesting to note certain similarities between the natural infections with this virus and induced infections in human volunteers reported by Johnson *et al.* (1961). Twelve of their volunteers from whom virus was recovered did not develop increased antibody titres. Virus was isolated from four of our student volunteers whose sera failed to show any increase in antibody titre. Their thesis of reinfection with respiratory syncytial virus, based on the high incidence of neutralizing antibody in pre-infection specimens, is supported by similar findings among our students. Neutralization tests on sera obtained from some of these students a month before the outbreak of respiratory syncytial infections had the same titre as the acute specimens shown in Table III, thus ruling out the possibility that these titres represented rapid increase in antibody due to the current respiratory syncytial virus infection.

Summary

In a study of natural common colds occurring among 101 medical students over the period from October 3, 1960, to June 9, 1961, 53 viruses were isolated from 199 specimens. Thirty-one of these viruses required low

temperature and low bicarbonate content of the medium for optimal growth in tissue culture. Twenty-four strains grew only in human-kidney cultures and resembled F.E.B. virus of Salisbury. Seven viruses grew in both human-kidney and monkey-kidney cultures and resembled but were not identical to H.G.P. virus of Salisbury. E.C.H.O. 28 virus, as well as these new viruses, was associated with an outbreak of colds in October, 1960. Another outbreak in late January and early February was caused primarily by the respiratory syncytial virus which was isolated from 11 specimens. Para-influenza viruses 1 and 2 appeared sporadically.

Only one virus was isolated from 191 specimens obtained during convalescence. A similar agent had been isolated from the acute specimen of the same student. The 456 specimens obtained from asymptomatic individuals throughout the study period yielded only two viruses, both growing only in human-kidney cultures.

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On April 16 next year regulations relating to the lead content of food, based on recommendations of the Food Standards Committee, will come into operation in England and Wales. The regulations lay down statutory limits for the lead content of food and beverages imported, sold, or intended for sale for human consumption. The regulations limit, with certain exceptions, the lead content of ready-to-drink non-alcoholic beverages to 0.2 parts per million and of foods to 2.0 p.p.m. One of the exceptions is fish (including crustacea and molluscs or any product containing such fish) for which a natural lead content in excess of 2.0 p.p.m. has been established. The Secretary of State for Scotland is making corresponding regulations to apply in Scotland, and similar regulations will also be made for Northern Ireland. (H.M.S.O., price 5d. net.)

HYPOPLASTIC ANAEMIA TREATED BY TRANSFUSION OF FOETAL HAEMOPOIETIC CELLS

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Aplastic anaemia is an uncommon condition arising from many causes, but most frequently idiopathic. Both course and prognosis are extremely variable. Spontaneous remission has often been recorded, so that any benefit which follows a particular type of treatment must be cautiously interpreted. In 1958 Dameshek, McFarland, and Granville (1960) reported some remissions after bone-marrow transplantation, and it therefore seems pertinent to place on record the results of similar treatment with foetal haemopoietic cells.

Fourteen cases have received foetal liver cells by intravenous injection and have survived more than 48 hours after their administration (see Table). From this series two cases are presented in detail.

Case 9

A white male born in 1923 began to notice symptoms attributable to anaemia in 1954. This was confirmed in September, 1956. Subsequently he required the transfusion of more than 3 pints (1.7 litres) of blood each month. The usual haematinics and cortisone (300 mg. daily initially; a six-weeks course) conferred no benefit. He came of a healthy stock and had not been in contact with known myelotoxic agents.

Examination in July, 1958, revealed a pallid, normally developed man without evidence of a haemorrhagic diathesis or other abnormal physical signs.

Investigations.—Sedimentation rate 50 mm. in first hour (Westergren). Urine analysis normal. Haemoglobin, 7.4 g./100 ml.; M.C.H.C., 31%; M.C.V., 108 cubic microns; reticulocytes, 0.2–0.4%; total leucocyte count, 2,400/c.mm. (neutrophils 528, lymphocytes 824); platelets, 49,000/c.mm. Group O rhesus-positive. Direct antiglobulin test negative. An aspiration biopsy of the marrow showed marked hypoplasia involving all myeloid elements, a finding confirmed by iliac-crest-trephine biopsy (Fig. 1). The red-cell half-life (^{51}Cr method) was slightly reduced (19 days; normal 22–30 days). The half-time of the disappearance of iron from the serum re-utilization was reduced to 40% (normal 90–100%) in 10 days (^{59}Fe method). The Ham and Donath–Landsteiner tests were negative. Erythrocyte osmotic fragility was normal (fresh and incubated). Serum vitamin B_{12} was 700 $\mu\mu$g./ml. Liver-function tests were normal, including the serum albumin (5.3 g.), globulin (1.8 g.), and electrophoresis. The Wassermann reaction was negative and the blood urea 35 mg./100 ml. The serum was fully saturated with iron at 245 μg./100 ml. A histamine-fast achlorhydria was present. The stools were negative for occult blood. The 24-hour urinary excretion of 17-keto-steroids was 8.4 mg. Radiographs of the chest and skeleton