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Variations in the clinical pattern of experimentally induced colds

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Studies of colds experimentally induced in volunteers have been in progress at the Common Cold Research Unit* of the National Institute for Medical Research for many years. This paper is based on an analysis of the results of the first 253 trials, which took place from August 1946 to July 1957.

Conduct of the trials

The organization of the work of the Unit has been previously described (Common Cold Research Unit, 1947; Andrewes, 1949; Lovelock, Porterfield, Roden, Sommerville & Andrewes, 1952). Each trial began with the intake of a number of volunteers who, soon after their arrival at the Unit, were segregated, usually in pairs, for 9 days. They were observed for a few days and medically examined in order to determine their fitness to participate in the subsequent experimental procedures. Any volunteer who showed signs or symptoms suggestive of the common cold during this preliminary period of observation was excluded from the trial, and so was any companion sharing the same living quarters at the Unit. Volunteers were also excluded if they were found to be unsuitable, either on general grounds or by reason of any acute infection, abnormal quantity of nasal discharge or evidence of allergy.

The experimental procedures adopted in any trial were chosen in advance and the numbers of volunteers to be allocated to each procedure were decided at the end of the preliminary period of observation. For the purposes of allocation each segregated group of volunteers was treated as a single unit. The allocation was made by means of random sampling numbers. Neither the volunteers nor the clinical observers were aware of the nature of the experimental materials used until the final clinical assessments had been recorded.

The general conduct of the trials was the same throughout the years under review, though some modifications of detail were introduced from time to time. For example, the upper age limit for volunteers, which was originally 40 years, was raised in 1951 to 45 years. The lower age limit of 18 years was not altered. Until 1952 the preliminary period of observation was usually 3 days, though occasionally a 4-day period was chosen in order to attempt a higher degree of precision by reducing the possibility of the late development of natural infections after the experimental procedures had begun. Subsequently, 4 days became the general routine, except for work on experimentally induced colds of relatively long

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incubation, when a preliminary period of 2 days was adopted. The length of time for which volunteers remained under observation, after the experimental procedures had begun, varied, therefore, from 5 to 7 days.

Every effort was directed to the maintenance of continuity and uniformity of the records. Observations were entered daily on each volunteer's medical examination chart, which listed the main symptoms and signs on which the clinical assessments were based. Mouth temperatures were taken twice daily. Most of the observations and assessments were made by one of four medically qualified men—D. K. M. Chalmers (1946–47), T. Sommerville (1947–51), A. T. Roden (1951–56) and J. W. Field (1956–57). Dr C. H. Andrewes, who was in charge of the programme of scientific work throughout the period, helped to preserve uniformity.

Clinical assessments

Three main categories of assessment were adopted: (i) no cold, (ii) doubtful or abortive cold, and (iii) definite cold, the last being graded as mild, moderate or severe. A system of scoring points for diagnostic symptoms and signs was practised, but no arbitrary levels for the total score were fixed and the final assessment was essentially a clinical judgement.

Analysis of the records

A scheme for the analysis of the records was drawn up in 1955, in consultation with Dr E. R. Bransby, of the Ministry of Health, and Mr S. F. King, of the Treasury. Coded sheets of information suitable for transference to Powers-Samas punch-cards were prepared at the Unit. The punching and sorting of the cards was kindly undertaken by the Statistics Department of the Ministry of Labour and National Service.

A separate punch-card was prepared from the record of each occasion on which a volunteer was admitted to the Unit. It carried the following information:

Identification numbers of the volunteer, the trial and the living quarters occupied.
Number of persons sharing the living quarters.
Number of occasions on which the volunteer had been admitted to the Unit.
Month in which admitted.
Sex of volunteer and of companions, if any.
Age.
History of past frequency of colds.
Length of time since most recent cold.
History of tonsillectomy.
Duration of preliminary observation.
Duration of segregation.
Reason, if any, for exclusion from the trial.
Final clinical assessment.
Incubation period of cold, if any.
Symptoms persisting or developing after discharge from the Unit.
Nature of the experimental procedure.
Clinical observations.

The results given in this paper have been derived partly from a general analysis of the records and partly from a more detailed analysis of the clinical effects of different strains of virus.

Experimental procedures

For the purposes of a general analysis the experimental procedures were classified into three groups: (i) positive control, (ii) negative control, and (iii) other experimental procedures.

The positive control procedures consisted of the administration by nasal instillation of material, presumed to contain common cold virus, from the nasal washings of persons suffering from colds, either naturally acquired or experimentally induced. The negative control procedures consisted of the administration by nasal instillation of some presumably inert fluid. The other experimental procedures comprised the administration of materials under test for the presence of virus and various other forms of exposure to possible risk of infection.

Nasal washings for the positive control procedures were prepared, either by centrifugation, or by filtration through a collodion membrane of average pore diameter 0·65–0·7 μ, and stored at $-76°$ C. The quantity of material available from a single washing was limited and, although such material was often used, it seldom sufficed for administration to more than a total of twenty volunteers. Pooled washings, collected either from several persons or from the same person on more than one occasion, provided larger quantities of material, enough for administration to fifty volunteers or more.

The materials used for the negative control procedures comprised sterile normal saline, sterile broth saline and uninoculated egg or tissue culture fluids.

A wide variety of materials and methods was used for the other experimental procedures and it is not proposed to examine these in detail in this paper.

Strains of virus

During the period under review six strains of virus were propagated by serial transfer from volunteer to volunteer, using either individual or pooled nasal washings. The pedigrees of these strains were as follows:

Strain H.W. was present in a pool of nasal washings collected during an outbreak of minor upper respiratory tract infection at an English public school. This strain was propagated to the eighth serial transfer from the original material without apparent alteration in its pathogenic effects.

Strain C.W. was derived from a volunteer who developed a natural infection in one of the trials during the preliminary period of observation.

Strain R.D. was obtained from a volunteer who developed a cold after the administration of egg culture material.

Strains J.P. and D.C. each originated from a natural infection of a member of the staff of the Unit. The D.C. strain was subsequently propagated in tissue culture (Andrewes, Chaproniere, Gompels, Pereira & Roden, 1953; Tyrrell, Bynoe, Buckland & Hayflick, 1962) as well as by human passage.

Strain G.E. had an unusual history in that it was obtained from a volunteer who

developed a typical common cold after the administration of a culture of Type 1 adenovirus. The strain was propagated by human passage to the third serial transfer and no adenovirus was recovered in nasal washings either from the original volunteer or from others subsequently infected.

In addition to these six pedigree strains various other strains from miscellaneous sources were tested from time to time.

Clinical observations

For the purposes of a detailed analysis the clinical observations, which had been entered daily on the volunteers' medical examination charts, were classified retrospectively according to a uniform plan. Six signs and symptoms—pyrexia, coryza, purulent nasal discharge, nasal obstruction, sore throat and cough—were selected for study and each was graded as absent, slight and transient, slight and persistent, marked and transient or marked and persistent.

A symptom or sign was graded as transient if it was recorded as present during the experimental period of observation on one day only and as persistent if it was recorded as present on more than one day. Mouth temperatures of 98·8° to 99·8° F. were graded as slight pyrexia, and of 100° F. or more as marked pyrexia. Increased nasal discharge up to a maximum of eight paper handkerchiefs (or one fully soiled linen handkerchief) per day was graded as slight coryza and beyond this maximum as marked coryza. The remaining signs and symptoms had already been graded as slight or marked on the medical examination charts. The presence of purulent nasal discharge or of nasal obstruction was an objective finding on the part of the clinical observer. The grading of sore throat depended on the subjective observations of the volunteer and the grading of cough was based partly on subjective and partly on objective findings.

This retrospective classification of symptoms and signs in no way affected the final clinical assessments or the incubation periods already recorded.

RESULTS

In the first 253 trials, 3803 individuals were admitted to the Unit on a total of 5290 occasions, an average of twenty-one volunteers in attendance at each trial. The total number of exclusions was 855, of which 138 were on general medical grounds, 289 for signs or symptoms suggestive of the common cold, 137 by reason of an abnormal quantity of nasal discharge, fifteen on account of acute infections other than the common cold, 220 in contact with common cold or other infection and fifty-six for personal or other reasons.

The total number of volunteers who were included in the trials, counting each re-admission as a new entry, was 4435. Sixty-two of these were excluded from the analysis of the experimental results, either because the preliminary period of observation was less than 2 days or because the period of segregation was less than 9 days. This left a total of 4373, of whom 993 were allocated to positive control, 526 to negative control and 2854 to other experimental procedures.

The proportion of men among the volunteers allocated to each of these procedures was:

Positive control	447/993 (45·0 %)
Negative control	220/526 (41·8 %)
Other experimental	1253/2854 (43·9 %)

The respective proportions of volunteers less than 30 years old were:

Positive control	640/993 (64·5 %)
Negative control	331/526 (62·9 %)
Other experimental	1770/2854 (62·0 %)

The observed differences, in each instance less than twice the corresponding standard error, indicated that the groups were reasonably similar in sex and age composition. There were, however, marked disparities in the seasonal allocations. The proportions for months of admission October to March were:

Positive control	457/993 (46·0 %)
Negative control	166/526 (31·6 %)
Other experimental	1173/2854 (41·1 %)

The observed differences, all more than twice, and as regards the negative control group more than three times, the corresponding standard errors, revealed a bias in favour of positive control and against negative control procedures during the winter months.

Table 1. *Final clinical assessments in relation to experimental procedures*

	Experimental group							
	Positive control		Negative control		Other		Total	
	No. of volunteers	Percentage of group	No. of volunteers	Percentage of group	No. of volunteers	Percentage of group	No. of volunteers	Percentage of total
nal clinical assessment								
Vo cold or other illness	514	51·8	474	90·1	2307	80·8	3295	75·4
Doubtful or abortive cold	89	9·0	34	6·5	194	6·8	317	7·2
Definite cold — Mild	206	20·7	15	2·8	217	7·6	438	10·0
Definite cold — Moderate	159	16·0	3	0·6	91	3·2	253	5·8
Definite cold — Severe	21	2·1	—	—	13	0·5	34	0·8
Other forms of illness	4	0·4	—	—	32	1·1	36	0·8
Total	993	100·0	526	100·0	2854	100·0	4373	100·0

Table 1 shows the final clinical assessments of the 4373 volunteers included in the analysis of the experimental results. In the positive control group 386/993 (38·9 %) developed definite colds, compared with 18/526 (3·4 %) in the negative control group, a most significant difference statistically. The disparity between the two groups increased with the severity of the colds. The incidence of doubtful or abortive colds, 89/993 (9·0 %) and 34/526 (6·5 %) respectively, was not grossly dissimilar. Although the difference is less than twice its standard error, a partition of χ^2, or a discriminant analysis, shows that it is statistically very highly significant ($P < 0{\cdot}001$).

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The attack rates of definite colds in the positive control group were examined in relation to each of the following properties of the individual volunteers: sex, age, past frequency of colds, length of time since most recent cold, history of tonsillectomy, number of persons sharing the same living quarters and number of occasions on which the individual had been admitted to the Unit. No difference exceeding twice the standard error was detected.

Table 2. *Seasonal distribution of experimental colds (positive control group)*

	Month of admission				
	Jan.–Mar.	Apr.–June	July–Sept.	Oct.–Dec.	Total
No. of volunteers	324	256	280	133	993
No. of colds: Definite	111	107	122	46	386
No. of colds: Doubtful or abortive	23	28	21	17	89
Percentage of volunteers who developed colds	41·4	52·8	51·1	47·4	47·9

Table 3. *Seasonal distribution of natural colds (volunteers excluded from trials)*

	Month of admission				
	Jan.–Mar.	Apr.–June	July–Sept.	Oct.–Dec.	Total
No. of volunteers	1417	1436	1553	884	5290
No. of colds (definite or doubtful)	103	64	48	74	289
Percentage of volunteers who developed colds	7·3	4·5	3·1	8·4	5·5

The seasonal distribution of all colds, definite and doubtful, in the positive control group is shown in Table 2. For purposes of comparison Table 3 shows the seasonal distribution of volunteers excluded from trials by reason of signs or symptoms suggestive of the common cold. A substantial number of these volunteers left the Unit soon after the onset of their symptoms and it was not possible, therefore, to classify all the natural colds into definite or doubtful categories. The distribution of the natural colds conformed to the known seasonal incidence of the disease, whereas the attack rate of the experimental colds was distinctly higher during the months April to September than from October to March. The observed difference, which was more than twice the standard error, was accounted for mainly by a lower attack rate of experimental colds among males during the winter months.

The proportions of definite colds among males in the positive control group were 65/221 (29·4 %) from October to March, and 102/226 (45·1 %) from April to September, a difference more than three times the standard error. The corresponding attack rates among females were 92/236 (39·0 %) from October to March and 127/310 (41·0 %) from April to September, a difference which was not

statistically significant. The difference between male and female attack rates from October to March was more than twice the standard error, but there was no statistically significant difference between the respective attack rates from April to September, or for the year as a whole.

Allocation of the virus strains

The different strains of virus, which were used for the positive control procedures, were administered to volunteers over varying periods of time: H.W., 1946–52; C.W., 1947–48; R.D., 1950; J.P., 1951–52; D.C., 1953–56; G.E., 1954–55; miscellaneous, 1946–57. The numbers of volunteers were, respectively: H.W., 282; C.W., 57; R.D., 49; J.P., 114; D.C., 139; G.E., 69; miscellaneous, 283.

There were minor differences as regards sex composition, the proportions of men being:

H.W.	133/282 (47·2 %)	J.P.	61/114 (53·5 %)
C.W.	25/57 (43·9 %)	D.C.	51/139 (36·7 %)
R.D.	20/49 (40·8 %)	G.E.	25/69 (36·2 %)

Miscellaneous 132/283 (46·6 %)

($\chi^2 = 10{\cdot}58$, $n = 6$, $0{\cdot}2 > P > 0{\cdot}1$)

The age composition showed significant disparities between some of the groups, which were attributable to a tendency for the average age of volunteers to rise as the work of the Unit progressed. The proportions of volunteers less than 30 years old were:

H.W.	203/282 (72·0 %)	J.P.	68/114 (59·7 %)
C.W.	42/57 (73·7 %)	D.C.	79/139 (56·8 %)
R.D.	29/49 (59·2 %)	G.E.	35/69 (50·7 %)

Miscellaneous 184/283 (65·0 %)

($\chi^2 = 20{\cdot}15$, $n = 6$, $P < 0{\cdot}01$)

Marked differences in the seasonal allocations were also evident. One strain (R.D.) was tested only during the months of May to September 1950. The remaining strains were tested both in winter and in summer. The allocations for months of admission October to March were:

H.W.	152/282 (53·9 %)	D.C.	56/139 (40·3 %)
C.W.	34/57 (59·6 %)	G.E.	31/69 (44·9 %)
J.P.	30/114 (26·3 %)	Miscellaneous	154/283 (54·4 %)

($\chi^2 = 36{\cdot}70$, $n = 5$, $P < 0{\cdot}001$)

Incidence of doubtful colds

Table 4 shows the final clinical assessments in the positive control group in relation to the strains of virus used. The incidence of doubtful or abortive colds was uniformly less than that of definite colds and, with each strain of virus, the assessments tended to fall into the categories of either no cold or definite cold, with relatively few doubtful cases. Two of the strains (C.W. and G.E.) were associated with an unusually low proportion of doubtful colds, but the numbers involved were small and none of the entries in this row of Table 4 differs from

expectation by more than twice its standard error. Partitioning the total χ^2 shows no significant difference ($P > 0{\cdot}05$) between the virus strains in the proportion of doubtful or abortive colds they produced. Nevertheless, it was the clinical impression that the G.E. strain, at least, gave particularly clear-cut results, although most of the colds associated with it were graded as mild.

Although statistically significant, the meaning of the doubtful or abortive colds was considered to be obscure, since they occurred with considerable frequency in the negative control group (see Table 1). For this reason the comparisons of the virus strains which now follow ignore all assessments in the doubtful category.

Table 4. *Final clinical assessments in relation to strains of virus*

	Strain of virus							Total no. of volunteers
	H.W.	C.W.	R.D.	J.P.	D.C.	G.E.	Miscellaneous	
Final clinical assessment								
No cold or other illness	126	21	25	73	76	27	166	514
Doubtful or abortive cold	31	3	4	8	15	2	26	89
Definite cold — Mild	62	12	15	19	20	28	50	206
Definite cold — Moderate	57	20	5	11	22	11	33	159
Definite cold — Severe	6	1	—	2	6	—	6	21
Other forms of illness	—	—	—	1	—	1	2	4
Total	282	57	49	114	139	69	283	993

Table 5. *Clinical attack rates of experimentally induced colds*

	Strain of virus							
	H.W.	C.W.	R.D.	J.P.	D.C.	G.E.	Misc.	Total
Oct. to Mar.								
No. of volunteers	152	34	Nil	30	56	31	154	457
No. of definite colds	60	20	Nil	7	17	15	38	157
Percentage	39·5	58·8	Nil	23·3	30·4	48·4	24·7	34·4
Apr. to Sept.								
No. of volunteers	130	23	49	84	83	38	129	536
No. of definite colds	65	13	20	25	31	24	51	229
Percentage	50·0	56·5	40·8	29·8	37·3	63·2	39·5	42·7

Incidence of definite colds

The proportions of volunteers who developed definite colds were:

H.W.	125/282 (44·3 %)	J.P.	32/114 (28·1 %)
C.W.	33/57 (57·9 %)	D.C.	48/139 (34·5 %)
R.D.	20/49 (40·8 %)	G.E.	39/69 (56·5 %)

Miscellaneous 89/283 (31·4 %)

These differences were most unlikely to have arisen by chance ($\chi^2 = 35{\cdot}16$, $n = 6$, $P < 0{\cdot}001$).

Table 5 shows the number of colds associated with each of the virus strains during the months October to March and April to September, respectively. Five of the pedigree strains were tested at both these periods of the year. All but one

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(C.W.) gave a higher clinical attack rate during the summer than during the winter and so did the miscellaneous strains. The differences between the attack rates associated with strains other than C.W. were of roughly the same order both in summer and in winter.

Severity of illness

The proportions of colds graded as moderate or severe were:

H.W.	63/125 (50·4 %)	J.P.	13/32 (40·7 %)
C.W.	21/33 (63·6 %)	D.C.	28/48 (58·3 %)
R.D.	5/20 (25·0 %)	G.E.	11/39 (28·2 %)

Miscellaneous 39/89 (43·8 %)

These differences were unlikely to have been fortuitous ($\chi^2 = 16{\cdot}95$, $n = 6$, $P < 0{\cdot}01$).

Some variability in the grading of the severity of illness was shown by different clinical observers, and by the same observer at different times, but the variations were not generally of a gross degree. Most of the assessments associated with the J.P., D.C. and G.E. strains were made by one observer.

Table 6. *Incubation periods of experimentally induced colds*

	Incubation periods (days)						Total no. of colds
	0–	1–	2–	3–	4–	5–	
Strain of virus							
H.W.	12	70	29	10	2	2	125
C.W.	5	10	12	3	2	1	33
R.D.	—	6	8	4	1	1	20
J.P.	6	18	4	1	3	—	32
D.C.	13	28	6	1	—	*	48
G.E.	2	2	14	11	7	3	39
Misc.	21	45	17	4	1	1	89
Total	59	179	90	34	16	8	386

* Volunteers usually no longer under observation (see 'Conduct of the trials').

Incubation period

Table 6 shows the incubation periods, grouped in 24 hr. intervals from 12 noon of the day of inoculation to the day of onset of illness, of the 386 colds which developed in the positive control group. The mean periods, in days, calculated from the central values of the 24 hr. intervals were: H.W., 1·91; C.W., 2·20; R.D., 2·65; J.P., 1·78; D.C., 1·40; G.E., 3·22; miscellaneous, 1·62. (Variance ratio 16·83, for degrees of freedom 6 and 379, $P < 0{\cdot}001$.) The proportions of colds with an incubation period of less than 2 days were: H.W., 82/125 (65·6 %); C.W., 15/33 (45·5 %); R.D., 6/20 (30·0 %); J.P., 24/32 (75·0 %); D.C., 41/48 (85·4 %); G.E., 4/39 (10·3 %); miscellaneous 66/89 (73·9 %).

The original data on the D.C. and G.E. strains, between which the greatest disparity of incubation period existed, were re-examined to determine whether differences in the rate of evolution of symptoms could have influenced the observer's judgement of the day of onset of illness. Two further sets of measure-

ments were compared; from the time of inoculation to (i) the development of the first marked symptom, (ii) the onset of persistent coryza; and in each instance the distinctive distributions of the incubation periods were maintained.

The 125 colds associated with the H.W. strain were examined for a possible seasonal influence, but none was found. The mean incubation periods were 1·73 days from October to March and 2·07 days from April to September. The proportions of colds with an incubation period of less than 2 days were 42/60 (70·0 %) and 40/65 (61·5 %), respectively. Each of these differences was less than twice the corresponding standard error.

Duration of colds

Some of the volunteers recovered completely from experimentally induced colds before their departure from the Unit. Symptoms which developed, recurred or persisted after departure were recorded by the volunteers themselves on follow-up cards, which they were asked to return to the Unit a fortnight later by post. Although the information so obtained was not always complete or accurate, it was found possible in most instances to form a rough estimate of the duration of colds. Table 7 summarizes the findings. It will be seen that the colds associated with the G.E. strain, of relatively long incubation period, tended to be of short duration, only three of thirty-nine being known to have persisted for more than 2 weeks from the time of onset. In contrast, the colds associated with the D.C. strain, of

Table 7. *Estimated duration of symptoms*

	Duration (days) from onset				Total no. of colds
	Less than 7	7 to 13	14 or more	Not known	
Strain of virus					
H.W.	35	25	31	34	125
C.W.	17	5	1	10	33
R.D.	4	8	1	7	20
J.P.	11	11	9	1	32
D.C.	7	16	24	1	48
G.E.	12	23	3	1	39
Misc.	24	21	22	22	89
Total	110	109	91	76	386

relatively short incubation period, tended to be prolonged, no fewer than twenty-four of forty-eight persisting for 2 weeks or more. The duration of colds associated with the J.P. strain occupied an intermediate position. Information on the remaining strains was less complete, but there was evidence to suggest that colds associated with the C.W. strain were usually of short duration.

Respiratory symptoms, which had previously cleared, were reported in five cases to have recurred after departure. Two of these were associated with miscellaneous strains and one each with H.W., C.W. and G.E.

Relative frequency of symptoms

The first symptom to appear was usually soreness of the upper respiratory passages, followed or accompanied by increased nasal exudate. The soreness was referred sometimes to the upper part of the naso-pharynx, sometimes to the region of the fauces and occasionally to a lower level. Precise location was not always possible and the term sore throat was used to cover this symptom in a general way. Inspection of the throat was not particularly helpful. There was often marked reddening without soreness or other evidence of a cold, while a normal appearance was frequently maintained in the presence of obvious evidence of infection.

The most characteristic sign was coryza, or increased nasal discharge, the amount of which could be measured roughly by examining and counting the used handkerchiefs. Some degree of coryza was observed in almost every case assessed as a definite cold. In only five of the 386 colds under consideration was the diagnosis made in the absence of coryza. The exudate usually consisted at first of clear mucus and, in some cases, remained clear throughout the course of the illness. Muco-purulent discharge often developed at a later stage and, occasionally, was present at the time of onset of coryza. A feeling of nasal stuffiness was common and some degree of obstruction frequently detected. Cough was relatively less common and, when it occurred, tended to be a late manifestation.

The grading of severity was largely influenced by the presence of symptoms other than coryza and by the degree of malaise which accompanied them. Malaise, headache or sneezing, all fairly common, were found to have no significance when they occurred as isolated symptoms, and, although their presence might influence the grading of severity, contributed little to the diagnosis. Some signs, such as hoarseness and watering of the eyes, were highly characteristic, but relatively infrequent and invariably accompanied by other evidence of infection.

Slight pyrexia, usually transient, was sometimes observed, either at the onset or at the peak of illness, but many of the colds were completely afebrile. Mouth temperatures exceeding 99·8° F. were recorded in only nine of the 993 volunteers in the positive control group, eight of whom were among the 386 who developed colds.

Comparison of the virus strains, based on the presence or absence of individual symptoms or combinations of symptoms, revealed differences which became more distinct when those graded as slight had been excluded. Table 8 shows an analysis of the 386 colds according to the relative frequency of marked degrees of pyrexia, coryza, purulent nasal discharge, nasal obstruction, sore throat and cough. A partition of the 3-way χ^2, by Lancaster's method (Lancaster, 1951), showed not only that different strains varied in the overall frequency of symptoms they caused, but also that they were very definitely associated with certain symptoms, the second-order interaction χ^2 being highly significant, $P < 0{\cdot}001$.

Colds associated with the H.W. strain, which accounted for nearly one-third of the total, showed a frequency near the average for marked coryza and above the average for other respiratory symptoms. Strain D.C. resembled the H.W. strain

in the frequency of coryza, purulent nasal discharge and cough, but nasal obstruction and sore throat were well below the average. The miscellaneous strains also gave a frequency near the average for coryza, but other respiratory symptoms were either near or below the average.

Table 8. *Relative frequency of marked symptoms*

	No. of volunteers in whom marked symptoms occurred						
Strain of virus	Pyrexia	Coryza	Purulent nasal discharge	Nasal obstruction	Sore throat	Cough	Total no. of colds
H.W.	3 (2·4 %)	87 (69·6 %)	76 (60·8 %)	105 (84·0 %)	75 (60·0 %)	46 (36·8 %)	125
C.W.	—	27 (81·8 %)	15 (45·4 %)	31 (93·9 %)	13 (39·4 %)	5 (15·2 %)	33
R.D.	1 (5·0 %)	16 (80·0 %)	14 (70·0 %)	18 (90·0 %)	13 (65·0 %)	2 (10·0 %)	20
J.P.	2 (6·2 %)	13 (40·6 %)	14 (43·7 %)	18 (56·2 %)	23 (71·9 %)	12 (37·5 %)	32
D.C.	—	31 (64·5 %)	28 (58·3 %)	31 (64·5 %)	19 (39·6 %)	19 (39·6 %)	48
G.E.	—	31 (79·5 %)	6 (15·4 %)	31 (79·5 %)	9 (23·1 %)	4 (10·3 %)	39
Misc.	2 (2·2 %)	60 (67·4 %)	45 (50·5 %)	53 (59·5 %)	43 (48·3 %)	20 (22·5 %)	89
Total	8 (2·1 %)	265 (68·6 %)	198 (51·3 %)	287 (74·3 %)	195 (50·5 %)	108 (28·0 %)	386

Colds induced by the C.W. strain showed the highest frequencies for marked coryza and nasal obstruction, but other respiratory symptoms were below the average. The R.D. strain gave figures almost as high for coryza and nasal obstruction and well above the average for purulent nasal discharge and sore throat, but cough was infrequent. The G.E. strain, also, showed high figures for coryza and nasal obstruction, with exceptionally low frequencies of purulent nasal discharge, sore throat and cough. The comparative rarity of marked cough was a feature of all three of these eminently coryzal strains.

Colds associated with the J.P. strain were peculiar in the relative infrequency of marked nasal symptoms, with cough approximating to the proportions shown by the H.W. and D.C. strains and an exceptionally high frequency of marked sore throat.

Similar differences in the pattern of the clinical effects associated with the various strains were maintained when the records of the volunteers were examined for symptoms, irrespective of the final assessments. The differences appeared to be independent of season. Table 9 shows the number of volunteers who developed marked symptoms after administration of the H.W. strain during the months October to March and April to September respectively. The incidence of all symptoms was higher in the summer than in the winter, but their relative pro-

portions were much the same. The clinical patterns of illness associated with the other pedigree strains were also, as far as could be judged from rather small numbers, not materially affected by season.

Table 9. *Incidence of marked symptoms—H.W. strain*

	No. of volunteers in whom marked symptoms occurred						
	Pyrexia	Coryza	Purulent nasal discharge	Nasal obstruction	Sore throat	Cough	Total no. of volunteers
Oct. to Mar.	2	42	38	64	47	23	152
Apr. to Sept.	1	45	39	69	54	27	130
Total	3	87	77	133	101	50	282

DISCUSSION

The trials were designed with the primary object of testing experimental materials for the presence of common cold virus and, in this respect, were admirably suited to their purpose. The reliability of the observations and the validity of the assessments based on them have been discussed elsewhere (Roden, 1958). The bias which was detected towards the adoption of positive control procedures during the winter months did not invalidate a comparison between the positive and the negative control groups, partly because there was a gross difference of more than tenfold in the respective attack rates of definite colds and partly because it was found that the attack rate in the positive control group was in fact higher during the summer.

The heterogeneous nature of the experimental materials precluded any firm deductions concerning factors likely to have influenced the susceptibility of volunteers to infection. No laboratory method for the detection or titration of common cold virus was available at this time and there was no way either of verifying the identity of the strains propagated by human passage or of estimating the quantity of virus administered. If it were assumed, for the purpose of a general analysis, that equivalent doses of identical viruses were administered to all volunteers in the positive control group, the only factor which appeared to have influenced the number of experimentally induced colds was a seasonal one, associated with a lower clinical attack rate among males during the winter months. The seasonal variation could not be attributed to differences between the strains of virus used, though this was probably the explanation of the varying attack rates which were observed from year to year (Andrewes *et al.* 1953). A seasonal influence on susceptibility was not demonstrated in volunteer studies in the United States (Dowling, Jackson, Spiesman & Inouye, 1958) or by earlier analyses of data from the Common Cold Research Unit. The negative findings as regards the other factors examined must be accepted with reserve, since they may have reflected an experimental design inadequate to reveal differences which could have existed.

The diversity in attack rate, severity, incubation period, duration and clinical pattern of colds associated with different strains of virus was too great to be

attributed merely to errors of sampling. Variations in the dose of virus could be held to have explained the differences in the attack rates, but not the paradoxical finding that those strains (C.W. and G.E.) which gave the highest attack rates were associated with long incubation periods, short durations and low proportions of symptoms other than coryza and nasal obstruction. The season of the year was found to have little or no influence, either on the clinical pattern of colds associated with a particular strain or on the attack rate relative to that of other strains. Disparity in the age and sex of the volunteers was an inadequate explanation of the observed variations in clinical pattern, since strains H.W. and C.W., which differed considerably in their effects, were tested in groups of reasonably similar age and sex composition and so were strains D.C. and G.E. It is possible that some of the longer incubation periods arose from failure to infect volunteers by the experimental procedure, with subsequent cross-infection from a companion, but this could not have accounted for the widely different proportions of colds which developed within less than two days of inoculation. The conclusion was that the findings were explicable only in terms of qualitative differences between the strains. This is consistent with more recent work at the Unit where a number of common cold viruses have been grown in the laboratory, some of which differ in their cultural requirements (Tyrrell, Bynoe, Hitchcock, Pereira, Andrewes & Parsons, 1960) and clinical effects (Tyrrell & Bynoe, 1961). In the absence of laboratory tests there was no assurance that propagation of the same strain was taking place by human passage. The possibilities of coincidental natural infection or of activation of a latent virus must also be considered. On the whole the accidental propagation or activation of heterogeneous viruses would have tended to obscure the observed variations and, for this reason, the occurrence of either of these contingencies would have been unlikely to invalidate the general conclusion that there were differences between the strains under study.

To account for the seasonal variation in the attack rate of colds associated with the miscellaneous strains and at least four of the pedigree strains it is tempting to put forward the hypothesis that the resistance of volunteers to these strains was higher during the winter months as a consequence of recent exposure to natural infection. Reid, Williams & Hirch (1953), in an epidemiological investigation of colds among office workers, obtained evidence of a short-term immunity. A similar conclusion was reached by Lidwell & Williams (1961). Volunteer studies by Andrewes (1950), Jackson, Dowling & Anderson (1958) and Jackson & Dowling (1959) gave results which suggested that resistance to the common cold was of a specific character. Tyrrell & Bynoe (1961) have since shown that infection by cytopathic common cold viruses induces the production of specific neutralizing antibodies, which can be detected in the blood serum. As regards the present data, it is difficult to understand why an increased resistance to infection in the winter months should be restricted to the male sex. An alternative explanation for the findings may lie in the possibility that men who were more susceptible to the common cold tended not to come to the Unit during the winter and that those who did come tended to be excluded from the trials because of symptoms or signs of upper respiratory disease. Whatever the explanation may be there was clearly

some factor, associated with the nature of the volunteer groups, which differentiated the sexes as regards susceptibility to infection during the winter and ceased to operate during the summer; but it would be unjustifiable to infer that this necessarily applied to natural infections in the general community.

Jackson, Dowling, Spiesman & Boand (1958) described variations in the incubation period and duration of experimental colds, which were induced in volunteers by the instillation of nasal secretions collected from different donors, and in the time relationship of the average daily score of symptoms. In contrast with the results of the present analysis these investigators found that neither sex nor season nor the source of the infectious secretion was significantly associated with susceptibility to experimental infection and that there was a striking similarity in the illnesses caused by different secretions.

Future work on the common cold is likely to proceed in conjunction with laboratory tests for known serotypes of virus, which will bring greater precision to the findings. It is improbable, however, that anyone will be able to test specific strains of virus on large numbers of volunteers living in isolation. For this reason it is thought that the present analysis may provide useful background information, help to clear the path for subsequent investigations in this field, and draw attention to some of the difficulties which may arise in the interpretation of data derived from volunteer studies.

SUMMARY

An analysis has been made of the records of 5290 attendances at the Common Cold Research Unit, Salisbury, during the years 1946 to 1957. Materials prepared from the nasal washings of persons suffering from colds were administered to 993 volunteers, of whom 386 (38·9 %) showed definite evidence of infection within a few days. The attack rate of these experimentally induced colds was lower during the months October to March than from April to September. The difference was statistically significant as regards male but not as regards female volunteers.

Six strains of virus were propagated by passage from volunteer to volunteer, in one instance up to the eighth serial transfer from the original material, without apparent loss of pathogenic properties. Comparison of the clinical effects of these strains, and of a number of miscellaneous strains, revealed several differences which were minor in character but, statistically, highly significant. These comprised differences in attack rate, severity, incubation period, duration and relative frequency of symptoms, which could not be ascribed to seasonal or other influences.

It was concluded that the observed variations in the clinical pattern of the experimentally induced colds were attributable to qualitative differences in the strains of virus used.

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REFERENCES

ANDREWES, C. H. (1949). The natural history of the common cold. *Lancet*, i, 71.

ANDREWES, C. H. (1950). Adventures among viruses. III. The puzzle of the common cold. *New Engl. J. Med.* **242**, 235.

ANDREWES, C. H., CHAPRONIERE, D. M., GOMPELS, A. E. H., PEREIRA, H. G. & RODEN, A. T. (1953). Propagation of common-cold virus in tissue cultures. *Lancet*, ii, 546.

COMMON COLD RESEARCH UNIT (1947). Interim report on a transmission experiment. *Brit. med. J.* i, 650.

DOWLING, H. F., JACKSON, G. G., SPIESMAN, I. G. & INOUYE, T. (1958). Transmission of the common cold to volunteers under controlled conditions. III. The effect of chilling of the subjects upon susceptibility. *Amer. J. Hyg.* **68**, 59.

JACKSON, G. G. & DOWLING, H. F. (1959). Transmission of the common cold to volunteers under controlled conditions. IV. Specific immunity to the common cold. *J. clin. Invest.* **38**, 762.

JACKSON, G. G., DOWLING, H. F. & ANDERSON, T. O. (1958). Neutralization of common cold agents in volunteers by pooled human globulin. *Science*, **128**, 27.

JACKSON, G. G., DOWLING, H. F., SPIESMAN, I. G. & BOAND, A. V. (1958). Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. *Arch. intern. Med.* **101**, 267.

LANCASTER, H. O. (1951). Complex contingency tables treated by the partition of χ^2. *J. R. statist. Soc.* B, **13**, 242.

LIDWELL, O. M. & WILLIAMS, R. E. O. (1961). The epidemiology of the common cold. II. Cross-infection and immunity. *J. Hyg., Camb.*, **59**, 321.

LOVELOCK, J. E., PORTERFIELD, J. S., RODEN, A. T., SOMMERVILLE, T. & ANDREWES, C. H. (1952). Further studies on the natural transmission of the common cold. *Lancet*, ii, 657.

REID, D. D., WILLIAMS, R. E. O. & HIRCH, A. (1953). Colds among office workers. An epidemiological study. *Lancet*, ii, 1303.

RODEN, A. T. (1958). Clinical assessment of the common cold. *Proc. R. Soc. Med.* **51**, 271.

TYRRELL, D. A. J. & BYNOE, M. L. (1961). Some further virus isolations from common colds. *Brit. med. J.* i, 393.

TYRRELL, D. A. J., BYNOE, M. L., BUCKLAND, F. E. & HAYFLICK, L. (1962). The cultivation in human-embryo cells of a virus (D.C.) causing colds in man. *Lancet*, ii, 320.

TYRRELL, D. A. J., BYNOE, M. L., HITCHCOCK, G., PEREIRA, H. G., ANDREWES, C. H. & PARSONS, R. (1960). Some virus isolations from common colds. *Lancet*, i, 235.