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Studies of experimental rhinovirus type 2 infections in polar isolation and in England

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SUMMARY

After five months of total isolation a wintering party of seventeen British Antarctic Survey (BAS) personnel was inoculated under double blind conditions with placebo, or rhinovirus type 2 which had been propagated in tissue culture. The clinical and virological responses of these subjects were compared with those of volunteers in England who received a similar dose of the same strain. The virus used was apparently partly attenuated for man; at the dosage used its effects in England were similar to a smaller dose of an unattenuated strain, but in the Antarctic it caused relatively severe infections. Both the symptoms and the laboratory evidence of virus infection appeared to be more pronounced in the BAS subjects than in the volunteers in England who received the same challenge. In the former group the infection readily spread to those who were originally given placebo. In the BAS subjects serum antibody titres were well maintained during the isolation period but a significant fall in nasal immunoglobulin concentration was recorded during the 5 months of isolation after the virus challenge. Possible mechanisms for the increased sensitivity to rhinovirus of subjects who have been totally isolated in a small closed community are discussed.

INTRODUCTION

When the isolation of a closed community is broken, it is frequently swept by a severe epidemic of respiratory disease (Holmes & Allen, 1973). These epidemics

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are less likely to occur when the people who come into contact with the community have themselves been isolated for six weeks or more (Woolley, 1963). Polar communities are especially susceptible to these epidemics, which are rapid in onset, affect all or nearly all of the community and are often clinically indistinguishable from influenza (Holmes, 1973). The people arriving from outside may often be symptom-free (Holmes & Allen, 1973), and the severity of symptoms in the isolated community is directly proportional to the duration of the isolation period (Paul & Freese, 1933). However, while the community remains in complete isolation, respiratory disease is confined to rare, mild, limited outbreaks often accompanied by equally mild abdominal symptoms (Cameron & Moore, 1968; Paul & Freese, 1933; Abs, 1930).

A recent study in which men at an Antarctic base were experimentally infected with an influenza virus and an enterovirus during the isolation period showed that in both these cases the symptoms were mild, and similar to those produced in volunteers in England. A severe epidemic of respiratory disease occurred when isolation was broken by the arrival of the relief ship, but there was no evidence that it was caused by a myxovirus, paramyxovirus, enterovirus or a coronavirus (Holmes, Allen, Bradburne & Stott, 1971).

In continued pursuit of the pathogens responsible for these epidemics, this study describes clinical trials in which volunteers at Stonington Island, a British Antarctic Survey base, and volunteers at the Common Cold Unit, Salisbury, were experimentally infected with similar doses of rhinovirus type 2. The results of challenge in isolated and non-isolated persons were compared.

MATERIALS AND METHODS

Subjects

The first group studied was the wintering party of 1971–1972 at Stonington Island, a BAS base on the Antarctic Peninsula in Marguerite Bay. There were 17 men aged between 21 and 38 who were in total isolation from February until late December 1971. A second group, healthy volunteers of both sexes aged 18–50 at the Common Cold Unit in Salisbury, were studied during late 1972 and early 1973.

The Stonington group slept in a crowded bunkroom. Indoor temperature and humidity approximated to those in temperate climates but outdoor temperatures were lower, ranging from $-48°$ C. to $+2°$ C. Salisbury volunteers lived singly or in groups of two or three in spacious isolation quarters for the period of the experiments, 10 days, at indoor temperatures of about 20° C. and outdoor temperatures seldom below 0° C.

Viruses

Two preparations of rhinovirus type 2 (RV2) strain HGP were used. The first, pool DP29, had had eleven passages in tissue culture. It was grown in WI-38 cells, and contained $10^{5 \cdot 5}$ TCID50/ml. when titrated in rhinovirus-sensitive HeLa cells (Ohio HeLa). Samples of this preparation were transported to the Antarctic

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at $-40°$ C. and stored there at $-28°$ C. Samples brought back to England 18 months later had a titre of $10^{4.5}$ TCID50/ml. in Ohio HeLa cells. The second preparation (Hu_4) of the same strain, originating from the same isolate, had had four experimental passages in man, as nasal washings from infected volunteers, and had not been passed in tissue culture. It had a titre of $10^{4.5}$ TCID50/ml. in Ohio HeLa cells.

Procedures at Stonington

Inoculation

BAS subjects were not isolated from each other. On 20 August 1971, after 5 months of polar isolation, 9 of the 17 men were given 1 ml. of nasal drops containing approximately 10^5 TCID50 of RV2 DP29. Seven men were given a placebo of phosphate buffered saline (PBS) containing powdered milk and red ink. One man did not receive an inoculation. The antibody status of the subjects before inoculation was not known.

Samples

Sera were collected from BAS subjects either before leaving England or on arrival at Stonington. Thereafter sera were taken at intervals of 6–8 weeks with an extra sample 3 weeks after virus inoculation.

Both throat and nasal swabs were taken at the same times as the sera except during the clinical trial, when they were taken at approximately 2 day intervals for 3 weeks. Swabs were broken off into a HEPES-buffered transport medium (Chaniot, Holmes, Stott & Tyrrell, 1974) and were stored at $-28°$ C.

Nasal washings for the measurement of antibody were taken coincidentally with nasal swabs. Twenty ml. PBS containing 100 i.u./ml. penicillin and streptomycin were instilled into the nasopharynx, held for 20 sec. and expelled into a sterile universal bottle. Each washing was tested for the presence of blood with 'Haemastix' and those giving a positive reaction were discarded. The nasal washings were then dialysed against distilled water, lyophilized, reconstituted at one tenth of their original volume in PBS and stored at -28 °C.

Clinical evaluations

Clinical effects in the BAS men were evaluated double blind. Symptoms and body temperature were scored and recorded daily but it was not possible to assess coryza by counting the paper handkerchief tissues used each day, as is customary at Salisbury. Observations were continued until all symptoms had subsided except in the case of two men who left on a sledging trip before they were fully recovered. Using these records, an attempt was made by the Salisbury observer to grade each clinical response according to the criteria used at the Common Cold Unit.

Procedures at Salisbury

Inoculation

Volunteers were isolated singly or in groups of two or three and had contact only with the clinical and virological staff. After a 3-day quarantine period they

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were given 1 ml. of nasal drops containing the calculated dose of RV2 (DP29 or Hu_4) by the same technique as at Stonington. Isolation was maintained for a further 6 days.

Samples

Sera were collected before virus challenge and three weeks later. Throat and nasal swabs were taken by the same method as at Stonington except for storage at $-70°$ C., instead of at $-28°$ C.; nasal washings for virus isolation were collected before inoculation and on the 3rd, 4th, 5th and sometimes the 6th day after inoculation and were stored, mixed with an equal volume of nutrient broth, at $-70°$ C.

Clinical evaluations

Responses were evaluated double blind (Tyrrell, 1963), signs and symptoms, including body temperature being scored and recorded each day for 3 days before and 6 days after virus challenge. The degree of coryza was assessed each day by counting the paper handkerchiefs used. At the end of the isolation period a clinical score was assigned to each volunteer, grading their reactions as nil (or doubtful), mild, moderate or severe. Where symptoms persisted longer than the 6-day post-challenge period, their duration was assessed by records returned from the volunteers by post.

Laboratory methods

Rhinovirus sensitive HeLa cells (Ohio HeLa) were used for virus isolation and for serum neutralization tests (Stott & Tyrrell, 1968; Strizova, Brown, Head & Reed, 1974). Samples of 0·2 ml. of the transport medium into which the nasal and throat swabs had been expressed, or of nasal washing fluid were inoculated into tubes containing Ohio HeLa monolayers. Cultures were incubated at 33° C. on a roller drum and examined for cytopathic effect at intervals up to a week after inoculation. In the case of the Stonington volunteers a single isolation from each individual who shed virus was confirmed as RV2 using a rabbit antiserum. Serum antibodies were titrated by a microneutralization colour test (Stott & Tyrrell, 1968). Each test was run in duplicate, and where the two results were not identical the neutralization endpoint was estimated as the intermediate value. Concentrated nasal washings were titrated for the presence of anti-RV2 antibody by a semi-micro plaque inhibition technique using Ohio HeLa cells grown in 1·5 cm. wells in plastic plates (Linbro). The method was based on that described by Stott & Tyrrell (1968), using 0·4 % agarose in the overlay medium. The dilution of nasal washing inhibiting 50 % of plaques was taken as its neutralization titre. The total immunoglobulin content of the washings was assessed using the radial immune diffusion technique of Lundkvist & Ceska (1972) with the following modifications. Instead of agarose, 3 % agar soaked in saline for 30 min. and autoclaved, with the subsequent addition of 2 % carbowax 6000 MW and 1 % sodium azide was used. Secondly, instead of the protracted washing technique used by Lundkvist and Ceska, the gels were washed for 4 hr. with 20 % acetic acid in saline and stained

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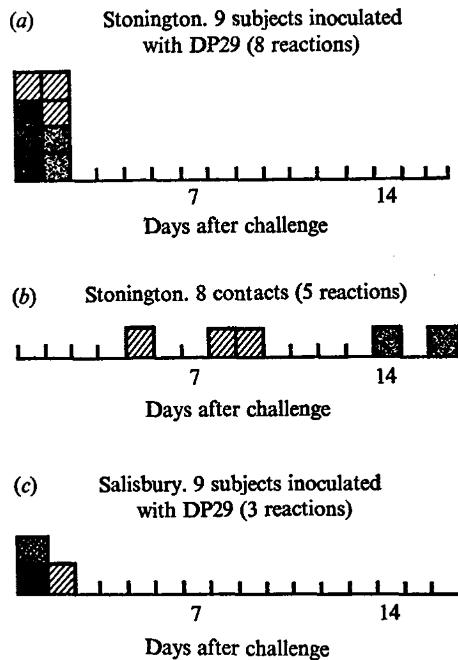

Fig. 1. Day of onset of symptoms and their severity, (*a*) after challenge with 10^5 TCD50 of DP29 or (*b*) after contact infection at Stonington, and (*c*) after challenge with 10^5 TCD50 of DP29 at Salisbury. ▨, mild reaction; ▦, moderate reaction; ■, severe reaction.

with 0·2 % Ponceau's stain in 3 % trichloracetic acid. The stain was leached out from the gel with 5 % acetic acid for 1 hr., and the gels rinsed and photographed at a magnification of three diameters.

RESULTS

Clinical responses

Stonington Island

Of the 9 BAS men given virus, 8 developed symptoms within 48 hr. Of the 8 contacts given placebo or no inoculum, 5 developed symptoms from 5 to 16 days after the inoculation (Fig. 1*a*, *b*). Neither the clinical observer nor all details of the method of evaluation at Stonington were the same as in the studies at Salisbury, but it should be emphasized that the Stonington records were later assessed by the Salisbury observer and were graded as far as possible on the Salisbury system. According to this classification, 3 of the 9 men given a virus challenge developed severe reactions, and pyrexia also started on the day after inoculation in 3 of the 9. Tracheitis and some further lower respiratory involvement (chest pain and rhonchi) also developed in 3 inoculated subjects, and in the one individual who received no inoculum but who became ill 16 days after his colleagues were challenged.

In Table 1 the symptoms experienced by the volunteers at Stonington are compared with data obtained from records made in Salisbury prior to 1965 on

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Table 1. *Symptoms at Stonington after inoculation of RV2, DP29 compared with records of earlier studies using RV2 at Salisbury* (*Bradburne* et al. 1967)

	Stonington	Salisbury
Presence of symptoms [a]	13/17 (76%)	78/213 (37%)
Duration of symptoms	11·25 days	9 days
Malaise [b]	8/13 (62%)	22/78 (28%)
Pyrexia over 99 °F. [b]	9/13 (69%)	11/78 (14%)
Lower respiratory involvement [b]	4/13 (31%)	Rare

a {Numerator: number of subjects who developed colds.
Denominator: number of subjects exposed to virus.

b {Numerator: number of subjects affected.
Denominator: number of subjects with colds.

volunteers inoculated with the same strain of RV2 (HGP) (Bradburne, Bynoe & Tyrrell, 1967; Tyrrell, 1965). Relatively twice as many volunteers at Stonington as at Salisbury suffered significant illness. Stonington subjects had more constitutional effects and lower respiratory involvement and their symptoms lasted longer.

Salisbury studies

The dose of tissue-culture-adapted virus (DP29) given to the BAS volunteers, approximately 10^5 TCID50, exceeded the normal challenge doses of the unadapted virus (up to 200 TCID50), used in the early Salisbury studies described above. It was therefore decided to investigate the effect on Salisbury volunteers of the high inoculum of DP29 used at Stonington. After preliminary studies with smaller doses, reported below, 9 volunteers were given 10^5 TCID50 of DP29. Only 3 developed symptoms, and their clinical responses are shown in Fig. 1*c*. One reaction was graded as 'severe', but this man's symptoms were atypical, appeared within 18 hr., without coryza, and may have been psychogenic or allergic in origin; virus was detected in only 1 of his 4 nasal washings. None of the 9 volunteers had lower respiratory symptoms or pyrexia.

Laboratory evidence of infection

Virus shedding

Table 2 shows that 15 of the 17 BAS men shed virus on one or more occasions between inoculation on 20 August and 6 September, 17 days later. No virus was found in swabs collected at any other time during the isolation period. Recovery of virus generally correlated closely with the presence of symptoms. Of the 2 men from whom virus was not recovered, 1 had received a virus challenge and 1 placebo. Their pre-inoculation serum antibody titres were 1/192 and 1/24 respectively. In 2 subjects who received virus, shedding continued for 2 weeks or more. Virus was isolated from 79 out of 286 specimens taken during this period and the titre of virus in medium expressed from the nasal and throat swabs attained 10^3 TCID50 or more in 7 instances. Virus was recovered less frequently from the contact-infected subjects, but the duration of virus shedding in these people could not be

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Table 2. *Symptoms, virus shedding and serum antibody responses in relation to virus challenge in* 17 *BAS personnel*

Inoculum	Subject number	Symptoms	Virus shedding on indicated days after challenge: 2	4	6	9	11	13	15	17	Serum antibody response*	Nasal antibody response*
DP29	1	Moderate	+	+		+	+	−	−	−	6→4	2→8
DP29	6	Severe	+	+		+	+	+	−	−	<4→128	<2→8
DP29	8	Mild	−	+		−	−	−	−	−	3072→1024	>16→>16
DP29	9	Mild	−	+		+	−	−	−	−	32→24	<2→8
DP29	10	Mild	−	−		−	−	−	−	−	192→256	<2→16
DP29	11	Nil	+	+		−	+	−	−	−	24→128	4→>16
DP29	12	Severe	+	+		+	+	−	+	−	<4→12	<2→16
DP29	15	Moderate	+	+		+	+	+	+	−	6→32	4→>16
DP29	16	Severe	+	+		+	+	+	−	−	<4→96	<2→>16
Nil	5	Moderate	−	−		−	−	−	−	+†	4→192	<2→8
Placebo	2	Nil	−	−		−	−	−	+	+	8→<4	<2→>16
Placebo	3	Mild	−	−		+	+	+	+	−	4→24	<2→>16
Placebo	4	Mild	−	+		−	−	−	−	−	4→6	<2→>16
Placebo	7	Nil	−	−		−	−	−	−	−	24→12	<2→8
Placebo	13	Mild	−	−		−	+	+	+	−	32→8	8→8
Placebo	14	Doubtful	−	−		−	−	−	+	−	12→16	<2→>16
Placebo	17	Moderate	−	−		−	+	−	+	+†	4→24	<2→4

* Reciprocal of the neutralizing antibody titre against RV2 before and after infection.
† Later assessment not possible.

adequately assessed since most of them left the base on the main summer sledging journeys 20 days after inoculation, including 2 men who still had symptoms.

In the case of the Salisbury volunteers who received 10^5 TCID50 of DP29, nasal washings were collected from the 3rd to the 6th day after inoculation, and virus was recovered from 15 of these 33 specimens. The recovery rate from throat and nasal swabs was similar to that from nasal washings. The total duration of virus excretion could not be assessed at Salisbury because 2 volunteers were still shedding virus on the 6th day after challenge when they left the Unit.

Serum neutralizing antibody

While the presence of pre-inoculation serum neutralizing antibody in the Stonington men was associated with some degree of protection, the correlation was incomplete (Table 2). Those with the lowest titres tended to have the most severe symptoms, but surprisingly, a pre-challenge titre of 1/3072 did not entirely prevent virus shedding or mild symptoms. Five of the 9 men given virus and 3 of the 8 given placebo had 4-fold or greater rises in serum antibody titres, all associated with virus shedding. Although some falls in serum antibody titres were recorded, there was no general decline towards the end of the isolation period (Fig. 2).

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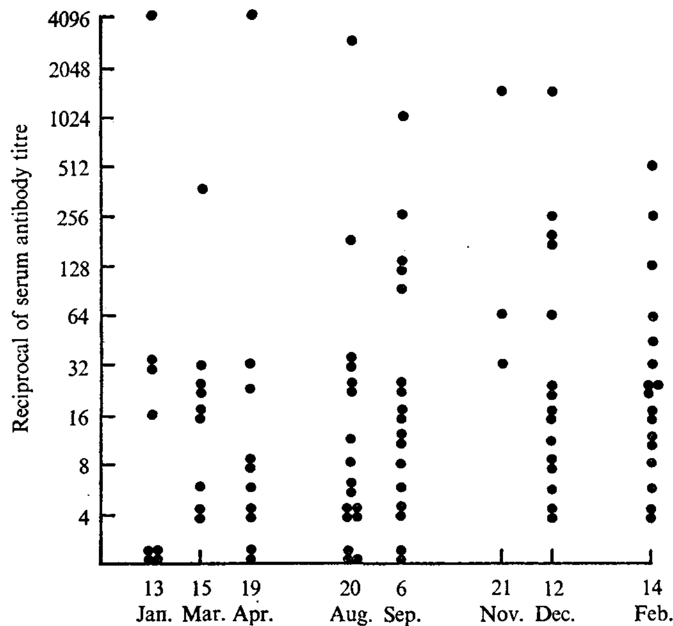

Fig. 2. Distribution of serum antibody titres against RV2 in BAS subjects 1971–1972. Virus inoculation was carried out on 20 August 1971.

Nasal antibody

The pre-inoculation titres of anti-RV2 nasal antibody measured by viral plaque inhibition, ranged from less than 1/2 to greater than 1/16 in the Stonington men (Table 2). Twelve had no detectable antibody. Fourteen of the 17 subjects showed 4-fold or greater rises in local antibody titres between inoculation on 20 August and 4 September. The one man (No. 7) who had no symptoms, no seroconversion and from whom no virus was isolated, was amongst this group and had an 8-fold increase in nasal antibody titre. Of the 3 men who did not have a significant rise in local antibody levels during this period, 1 already had a pre-inoculation titre of 1/8 (No. 13) and 1 had a titre of greater than 1/16 (No. 8). The third was the individual who received no inoculum (No. 5), but this man's symptoms appeared later than in the others and his nasal antibody titre increased 8-fold between 4 September and 12 December. Virus was isolated on one or more occasion from all three subjects. The correlation between the presence of pre-inoculation local antibody and severity of symptoms was similar to that between pre-inoculation serum antibody and symptoms, but rather less well defined.

The 12 men who had undetectable nasal antibody titres at the time of the clinical trial included all those with lower respiratory symptoms, all of the men spending their second consecutive year at Stonington and 4 of the 5 first year men known to possess local antibody at the time of their arrival.

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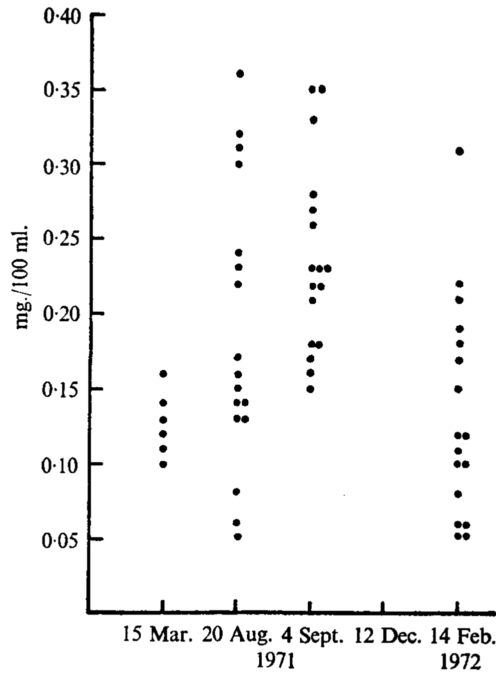

Fig. 3. Total nasal immunoglobulin concentrations during the isolation period at Stonington Island. March 1971–February 1972. For the mean downward trend between 4 September 1971 and 14 February 1972, $P = < 0{\cdot}001$ (paired t-test).

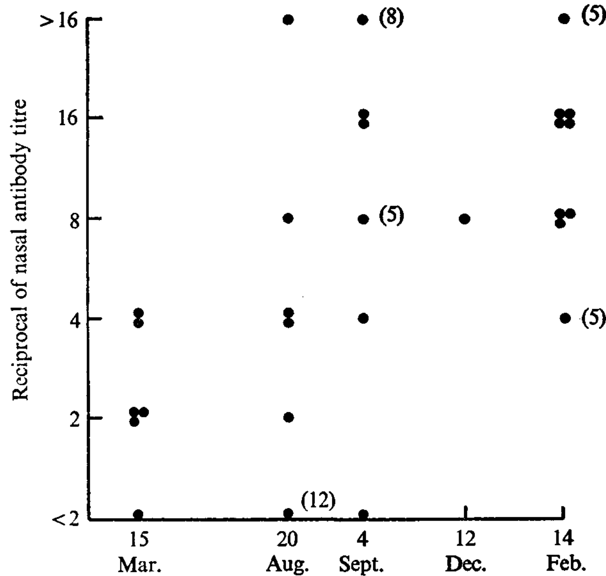

Fig. 4. Distribution of nasal antibody titres against RV2 in BAS subjects 1971–1972. Virus inoculation was carried out on 20 August 1971. Numbers in brackets are substituted when more than four points coincide.

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Table 3. *Laboratory findings in subjects at Stonington or at Salisbury who received DP29 virus challenge* (10^5*TCD50*), *and in the contacts of the Stonington subjects*

	Stonington		Salisbury
	Virus challenge	Contacts	Virus challenge
Pre-inoculation serum antibody titre of 1 in 8 or less	5/9 (56 %)	5/8 (63 %)	7/9 (78 %)
Number of subjects shedding virus [a]	8/9 (89 %)	7/8 (88 %)	7/9 (78 %)
Number of specimens yielding virus [b]	14/18 (78 %)		9/18 (50 %)
Serum antibody response [c]	5/9 (56 %)	3/8 (38 %)	3/9 (33 %)

a Proportion of individuals in whom virus shedding was detected once or more.

b Proportion of nasal swabs or washings taken on days 2 and 4 after challenge (Stonington) or on days 3 and 4 after challenge (Salisbury) which yielded virus on culture.

c Proportion of individuals showing a fourfold or greater rise in serum antibody titre after the inoculation.

Total nasal immunoglobulins

There were a number of fluctuations in the nasal immunoglobulin measurements, but since it is hard to define the degree of change which is significant in an individual, the results are presented as a whole (Fig. 3). There was a tendency for immunoglobulin concentrations to rise in the period immediately following the clinical trial, the mean values being 0·181 mg./100 ml. on 20 August and 0·235 mg./100 ml. on 4 September, though this could not be considered statistically significant ($P = 0{\cdot}06$ by paired t-test). There was, however, a significant drop in mean immunoglobulin concentration from 0·235 mg./100 ml. on 4 September to 0·134 mg./100 ml. by the end of the isolation period on 14 February the following year ($P = < 0{\cdot}001$ by paired t-test). The same trends are apparent in the nasal antibody titres against RV2 (Fig. 4), though here the rises after the clinical trial are highly significant and the falls later in the year are not.

Comparison of Stonington and Salisbury studies

Table 3 shows a summary of the laboratory studies on virus-challenged subjects and contacts at Stonington compared with the Salisbury subjects who received 10^5 TCID50 of RV2 DP29. Retrospective assessment of the pre-inoculation serum antibody status of these three groups shows that they were reasonably similar in this respect, although more of the subjects at Stonington than at Salisbury had antibody titres of more than 1/8 before the challenge. Despite this, laboratory evidence of infection was somewhat more pronounced in the Stonington subjects. It was not possible to compare the pre-inoculation nasal antibody status of the two groups. Virus shedding was slightly more common and probably more protracted at Stonington than at Salisbury. Nine of 18 nasal washings taken from Salisbury volunteers on days 3 and 4 after inoculation yielded virus, whereas 14 of 18 samples taken from the virus-challenged BAS men on the 2nd and 4th days after inoculation were positive. It must also be remembered that specimens from the Antarctic for virus isolation had been stored at far from optimum tem-

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Table 4. *The effect of varying doses of RV2 (DP29) or RV2 (Hu_4) in volunteers at Salisbury*

Inoculum per volunteer	Pre-inoculation antibody titre: All levels — Clinical reactions	All levels — Infections*	1 in 8 or less — Clinical reactions	1 in 8 or less — Infections*
DP29				
10^2–$10^{3\cdot 7}$ TCD50	0/13†	5/13	0/10‡	4/10
10^5 TCD50	3/9	7/9	2/7	6/7
All doses	3/22	12/22	2/17	10/17
Hu_4				
$10^{2\cdot 0}$–$10^{2\cdot 1}$TCD50	4/8†	4/6	4/6‡	4/5

Numerators: number of positive responses observed.
Denominators: number of subjects.
* Infection: virus shedding or fourfold rise in serum antibody, or both.
The differences between the sets of figures marked with the same symbol are significant.
† $P = < 0{\cdot}05$ (chi-squared test). ‡ $P = < 0{\cdot}02$ (chi-squared test).

peratures for 6 months and had suffered some temperature fluctuations during this time. They had then been transported many thousands of miles and were a year old when they were titrated, so that the results obtained almost certainly underestimate the true extent of virus shedding. Four-fold or greater rises in serum antibody titre were commoner in the Stonington subjects. However, the differences between the responses in Stonington and Salisbury volunteers who received the same inoculum were most clearly seen when symptoms rather than laboratory findings were assessed (Fig. 1*a*, *c*). Comparisons of nasal immunoglobulin concentrations and nasal neutralizing antibodies were not possible since, though sera could be collected from the Salisbury volunteers after they had left the unit, nasal washings could not.

Attenuation of the RV2 DP29 inoculum: Salisbury studies

In an attempt to compare the infectivity for man of the DP29 preparation and the human-adapted (Hu_4) virus, Salisbury volunteers were inoculated with varying doses, between 10^2 and 10^5 TCID50 of DP29 or 10^2 TCID50 of Hu_4. Doses of DP29 up to $10^{3\cdot 7}$ TCID50 produced no significant symptoms in any of 13 volunteers, although laboratory studies showed that some of them were subclinically infected. On the other hand, higher doses (10^5 TCID50) of DP29, when given to Salisbury volunteers, produced effects similar to much lower doses of the Hu_4 preparation (Table 4). It appeared, therefore, that the DP29 preparation was partially attenuated for man as judged by its effects in Salisbury volunteers, i.e. those from an open community.

DISCUSSION

While the men at Stonington Island and the volunteers at Salisbury given the same dose of RV2 were infected with similar frequency, the clinical response of

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the Stonington subjects was more rapid, severe, widespread and prolonged. Laboratory studies also indicated that the Stonington infections were more severe, with a higher percentage of sero-conversions, and heavier virus shedding. Fever, malaise and lower respiratory symptoms are not common in rhinovirus infections in an open community, although these viruses have been found to cause relapses in patients with chronic bronchitis (Eadie, Stott & Grist, 1966; Stenhouse, 1967), or to be associated with severe lower respiratory or diarrhoeal disease in infants (Stott, Eadie & Grist, 1969; Urquhart & Stott, 1970).

Another striking feature of the Stonington infections was the apparent ease with which they were transmitted. In fact, though adequate proof of infection is available for only 15 of the men, all 17 may have been infected, since of the remaining 2, one, given placebo, had a significant rise in nasal antibody, and the other, given virus, had a significant clinical response. By contrast, attempts to transmit rhinovirus by aerosol or other forms of contact under controlled conditions using volunteers drawn from an open community have rarely succeeded (Tyrrell, 1965; D'Alessio, Dick & Dick, 1972).

Three features which could have been relevant to the unusual severity of the infections at Stonington are the virulence of the virus itself, the environment of the subjects, and the state of their immunity mechanisms. However, the inoculum used at Stonington did not produce unusually severe infection in Salisbury volunteers, and this indicates that the nature of the virus challenge was not the relevant factor. In the course of the Salisbury studies it was also shown that the DP29 virus strain was partially attenuated, presumably by serial passages in tissue culture, and a large challenge dose of this virus in terms of tissue culture infectious units was roughly equivalent to a thousand-fold lower dose of the wild strain. A strain of RV15 has also been shown to be attenuated by tissue culture passage (Douglas & Couch, 1969).

The environmental differences merit more consideration as a cause of the unusual responses at Stonington. The men were crowded inside a small hut which almost certainly aided the spread of infection. Spread of Coxsackievirus A21 has been demonstrated experimentally under somewhat similar conditions (Couch *et al.* 1970; Holmes *et al.* 1971). On the other hand, though the climate was inclement it has not been possible to show a correlation between cold stress and increased incidence or severity of symptoms (Tyrrell, 1965; Douglas, Lindgren & Couch, 1968). The Stonington men were in any case cold-adapted.

Although the evidence that ascorbic acid intake has any relevance to colds is equivocal (Leading Article, 1973), it is worth mentioning that the BAS men's daily dietary supplement was between 60 and 150 mg. in addition to their normal intake in canned citrus juices and large quantities of fresh seal liver. This gave a high daily intake while the men were at the base, though on sledging journeys they were limited to a dietary supplement of 60 mg. daily in tablet form.

A further environmental difference between the group at Stonington and an open community is the absence of intercurrent infections. Interference between viruses has been shown in the human respiratory tract (Cate, Douglas & Couch, 1969; Tyrrell & Reed, 1973) and repeated virus infections in an open community

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may therefore reduce the severity of experimental infection. The mechanism is unknown although it may be immunological. The third factor mentioned above, the state of the immune mechanisms of the Stonington men after a period of isolation, is therefore worth closer examination.

The pre-inoculation serum antibody titres in the Stonington men were, on average, slightly higher than in the Salisbury subjects, so the greater susceptibility of the former cannot be accounted for in this way. Thus local resistance mechanisms in the upper respiratory tract must be considered, including interferon, local cell-mediated immunity and local secretory antibody. The problems of collecting material for interferon or cellular immunity studies at Stonington were insuperable so these responses could not be assessed, but the local antibody specimens yielded results of some interest. Serial specimens for local antibody studies could be collected only at Stonington so no comparative results from the open community were available, though other workers have studied them in some detail.

The nasal secretions from healthy people may contain neutralizing antibody to any rhinovirus serotype under investigation, and there is some evidence that protection against illness following rhinovirus challenge depends more upon pre-existing local antibody than serum antibody (Perkins *et al.* 1969). Buscho *et al.* (1972) found that homologous nasal neutralizing antibody persisted at least 300 days after a rhinovirus challenge in volunteers from an open community. However, none of the BAS men spending their second consecutive year at Stonington had nasal antibody against RV2 and this may be relevant to the severity of the clinical responses. The Stonington data suggest the possibility that nasal antibodies decline more rapidly in isolation than in normal life, which may mean that in an open community local immunological memory is reinforced by a factor or factors which are absent in polar isolation. It is conceivable that the repeated antigenic stimuli resulting from intercurrent subclinical infections provide such reinforcement. The outpouring of IgA in nasal secretions described by Butler *et al.* (1970) as occurring during the early stages of viral respiratory infection could be an aspect of this.

In contrast to the present findings using rhinovirus challenge, illnesses produced by inoculation with coxsackievirus A21 or a live vaccine strain of influenza virus A2 were not more severe in Antarctic volunteers than in those from an open community (Holmes *et al.* 1971). Unfortunately during neither of these studies could specimens be taken for the assay of local antibodies. However, the challenge with coxsackievirus A21 took place after only two months of isolation, perhaps too soon for the impairment of local defence mechanisms to have developed, though the same could not be said for the influenza virus challenge, which took place after six months of isolation. Here, changes in local resistance may in any case have been of less clinical significance since, in influenza, circulating antibody may be more important than local mechanisms in preventing infection (Tyrrell, 1975).

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