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Studies of respiratory viruses in personnel at an Antarctic base

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SUMMARY

Thirteen men wintering on an Antarctic base were isolated from other human contact for 10 months. During this period Coxsackievirus A21 and later influenza A2 virus were administered to some of the men. Serum samples were collected from each of the men at monthly intervals.

Coxsackievirus A21 produced symptoms and apparently spread to uninoculated men. It also appears that repeated re-infections occurred and that the virus persisted in this small community for most of the period of isolation. HI antibody responses in the absence of neutralizing antibody responses seem to be transient.

The vaccine strain of influenza virus induced antibody responses but did not cause symptoms. There was no evidence of spread to uninoculated men.

Antibody titres against influenza C, parainfluenzaviruses 1 and 2 and coronavirus OC43 did not fall significantly during isolation.

An outbreak of respiratory illness occurred at the end of isolation and its origin was traced. No causative agent was detected.

INTRODUCTION

Stonington Island is a British Antarctic Survey base situated on the south-west coast of the Antarctic Peninsula. The men who spend the winter there are isolated from contact with other personnel for periods of 10 or more months a year. Such an isolated population provides a good group in which to study the spread of respiratory viruses in the absence of extraneous infections by other agents, and to study the persistence of antibody against respiratory viruses in the absence of any likelihood of exposure to them. It is also of interest to examine the possible effects of a cold environment on the symptoms produced by respiratory viruses.

This paper describes two trials at Stonington in which men were inoculated with Coxsackievirus A21 and influenza A2 virus, strain Leningrad/4/65. Clinical

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symptoms of the men and their antibody status against these and other respiratory viruses were studied throughout their stay at the base, from February 1968 to March 1969.

MATERIALS AND METHODS

Population studied

The wintering party at Stonington in 1968 consisted of thirteen men. Eight men left England in September and October 1967, and arrived in February 1968. They were joined, on the way, by one man from a sub-antarctic base. The other four men had already spent 1 year at Stonington. The thirteen men were isolated from other humans until an aeroplane landed on 9 December 1968. Between 13 March and 8 July 1968, six men were trapped on a small ice shelf to the north (the Jones Ice Shelf) and were therefore not available for study. The base hut was compact, particularly the bunkroom, which was divided into three cubicles, 8 ft. × 7 ft. × 7 ft. high, each accommodating four men. Over 100 husky dogs were also kept on the base.

Viruses

Coxsackievirus A21 in the form of tissue culture fluid from human embryo kidney cells infected with nasal washings from volunteers at the Common Cold Unit, and influenza A2/Leningrad/4/65 in the form of lyophilized allantoic fluid, were transported and stored at temperatures between −7 and −15° C. The influenza virus was a vaccine strain originally obtained from Professor A. A. Smorodintsev, Leningrad. It had one passage in a human volunteer and two passages in eggs at Salisbury (Beare, Bynoe & Tyrrell, 1968).

Administration

On 12 April 1968, after two months in isolation, Coxsackievirus A21 was given to four of the seven men then at the base. The others were given a placebo consisting of phosphate buffered saline (PBS) containing flour and red ink. Administration of virus and placebo was double blind. Virus was diluted 1/10 in PBS and 2 ml. inoculated intranasally. The original virus pool had a titre of 10^4 TCD50 per ml. in human embryo kidney cells before leaving England, on 12 September 1967.

Influenza A2 virus was given to seven of the 13 men on base on 26 July 1968. The remainder were given placebo (PBS containing powdered milk). Each ampoule of virus was reconstituted in 10 ml. PBS and 2 ml. inoculated intranasally. Administration of virus was again double blind. When no symptoms developed, the code was broken and the six men who received placebo on 26 July were given virus on 15 September. Each ampoule of virus contained 10^6 EID50 when titrated in embryonated eggs before leaving England.

Samples of each virus were brought back to the U.K. in July 1969 and shown to be viable. Coxsackievirus A21 was recovered in Hela cells and A2/Leningrad/4/65 in embryonated eggs.

Collection of sera

Sera were collected from six men before leaving England and from the other seven soon after arrival at Stonington. Thereafter sera were collected at intervals of 4–6 weeks, throughout the following 12 months. Sera were stored at 4° C. in a temperature-controlled box at the base, and at −9 to −15° C. on the return voyage.

Serological tests

Sera were tested for haemagglutination-inhibiting (HI) antibody against Coxsackievirus A21 by the microtitre method, using human foetal group O cells (Chapple, 1966) and for neutralizing antibody by a microtitre colour test (Stott & Tyrrell, 1968). Also using the microtitre method, HI antibodies were estimated against influenza A2/Leningrad/4/65, influenza A2/HK/1/68, influenza C, parainfluenza viruses 1 and 2 and coronavirus OC 43. Complement fixing (CF) antibodies were titrated against influenza viruses A, B and C, parainfluenza virus 1, respiratory syncytial virus (RSV) and the adenovirus group. Sera for HI tests were treated with cholera filtrate, absorbed with chicken red cells and inactivated at 56° C. for 30 min, except in the case of the OC 43 tests, where they were diluted with PBS and inactivated at 65° C. for 30 min. Sera for neutralization and CF tests were inactivated at 56° C. for 30 min.

Serum globulin estimations

Serum globulin values (IgG, IgA, IgM) were estimated in the sera collected throughout the year from four subjects, using a gel-diffusion technique (Immunoplate, Baxter Laboratories).

RESULTS

Clinical trials during isolation

Coxsackievirus A21 trial

On 12 April 1968, four of the seven men on the base were given Coxsackievirus A21; the remainder received a placebo (Table 1). All four men given virus and one of the men given placebo developed symptoms. The onset of illness in the man given placebo (36 hr. after the others) suggests transmission of infection from one of the men given virus. The two men who did not develop symptoms (subjects 6 and 7) were the only men in this group who had detectable neutralizing antibodies and who had HI titres higher than 12 before inoculation. Four-fold or greater rises in HI antibody titres were detected in all five men who became ill, but a significant rise in neutralizing antibody titre was found only in subject 10. The incidence of symptoms in the men who were infected is shown in Table 2.

Influenza A2/Leningrad/4/65 trials

Seven of the 13 men on base were given influenza A2 virus on 26 July (Table 3). The other six received placebo. None of the men developed any symptoms, but three of those given virus (subjects 1, 9 and 13) developed four-fold or greater rises

Table 1. *Clinical and serological results of Coxsackievirus A21 trials*

(Virus administered on 12 April 1968.)

		Clinical response				Antibody titres*					
						HI			Neutralizing		
Subject	Inoculum	Symptom	Severity	DUR	DO	Apr.	May	July	Apr.	May	July
4	Virus	URTI	Mild	6	1·5	< 6	12	72	< 4	< 4	< 4
5	Placebo	URTI	Mild	3	3	6	24	6	< 4	< 4	< 4
6	Placebo	None	—	—	—	48	24	48	256	256	256
7	Placebo	None	—	—	—	18	18	36	16	32	64
8	Virus	URTI	Mild	5	1·5	< 6	48	6	< 4	4	4
10	Virus	URTI	V. mild	4	2·5	12	48	9	4	16	32
13	Virus	Diarrhoea	V. mild	3	2·5	6	24	18	< 4	< 4	< 4

DUR = duration in days,
DO = day of onset after inoculation,
URTI = upper respiratory tract illness.

* Expressed as reciprocal of highest dilution of serum showing 50 % inhibition of haemagglutination of 10–100 50 % tissue culture infecting doses (TCID 50) of virus. Sera were collected on 12 April, 15 May and 26 July 1968.

in HI antibody titre during the following 7 weeks, indicating that they had been infected. Two men (subjects 2 and 6) had antibody rises of < 6 to 6 eventually rising to 12. The remaining two men who were given virus (subjects 7 and 11) had pre-inoculation titres of 48 and 144 respectively. None of the men given placebo developed significant rises in antibody titre.

Table 2. *Incidence of symptoms on Coxsackievirus A*21 *trial (5 men infected)*

Symptom	No. of men with symptom
Coryza	4
Blocked nose	4
Headache	4
Sore throat	1
Pyrexia	2
Malaise	4
Myalgia	2
Abdominal colic	2
Diarrhoea	4

Table 3. *Serological results of influenza A*2 *Leningrad trials*

	Inoculum		HI antibody titres in indicated serum*				
Subject	26 July	15 Sept.	1	2	3	4	5
1	Virus	Placebo	< 6	24	24	—	24
2	Virus	Placebo	< 6	6	6	—	12
3	Placebo	Virus	< 6	< 6	24	—	24
4	Placebo	Virus	< 6	< 6	24	—	12
5	Placebo	Virus	12	12	48	—	48
6	Virus	Placebo	< 6	6	—	12	12
7	Virus	Placebo	48	36	36	—	48
8	Placebo	Virus	6	6	—	6	6
9	Virus	Placebo	< 6	24	24	—	18
10	Placebo	Virus	< 6	< 6	—	9	9
11	Virus	Placebo	144	96	96	—	96
12	Placebo	Virus	12	24	24	—	36
13	Virus	Placebo	< 6	18	—	24	18

*1 serum collected 26 July,
2 serum collected 15 September,
3 serum collected 7 October,
4 serum collected 27 October,
5 serum collected 2 December.

Titres expressed as reciprocal of dilution of serum showing 50% inhibition of haemagglutination.

On 15 September, the six men given placebo on 26 July were given virus, and the other seven were given placebo. Again, none of the men developed any symptoms. Three of these (subjects 3, 4 and 5) developed significant antibody responses. One (subject 10) had an antibody rise from < 6 to 9 eventually reaching 12. The

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remaining two subjects (8 and 12) showed no rises. Subject 12 had a pre-inoculation titre of 24 and subject 8, who had a pre-inoculation titre of 6, misunderstood instructions and swallowed his inoculum before it could be given intranasally.

Survey of antibody titres and globulin values during isolation

Influenza A2/Hong Kong/68

Only one man (subject 11) had detectable HI antibody at the beginning of the period of isolation. His initial titre of 8 was maintained throughout the year. One other man (subject 7) apparently had a rise in HI antibody titre from < 8 to 16 between July and September 1968. No other significant changes were detected.

Influenza C

All 13 men had HI antibody (titres 8 to 128) against influenza C at the beginning of isolation. Titres stayed constant for the ten months in all the men except subject 12, whose titre fell from 64 to 16.

Parainfluenza 1

Five of the six men who were bled in England before they left showed four-fold or greater rises in HI antibody titre by the time they arrived at Stonington. This suggests that there was an outbreak of parainfluenza 1 on the ship, though no clinical symptoms were reported. All 13 men had HI antibodies against parainfluenza 1 (titres 8 to 256) at the beginning of isolation, and only subject 4 showed a significant fall in titre (256 to 64) during the following 10 months.

Parainfluenza 2

Three men had no detectable HI antibody in March 1968. Three of the 10 men (subjects 7, 8 and 11) who had antibody showed a four-fold reduction in titre by December. In subject 7, the fall in titre occurred in April; in the other two cases, titres fell in the last three months of isolation.

Coronavirus OC 43

Coronavirus HI antibody was present in 10 out of 11 subjects examined (titres 10 to 80). All of these 10 subjects maintained their titres without significant change.

Coxsackievirus A 21

Two men (subjects 2 and 12) had eight-fold rises in HI antibody titre between leaving England and arriving in Antarctica. Apart from these two cases and the five antibody responses directly associated with the trial, there were ten significant HI rises: a six-fold rise in subject 8 between July and September; four- to eight-fold rises in subjects 3, 5, 11 and 12 between September and October; four-fold rises in subjects 1, 10 and 13 between December 1968 and February 1969; six-fold rises in subjects 7 and 8 between February and March 1969 (Table 4).

A four-fold or greater fall in HI antibody titre was detected on 12 occasions. Ten

of these falls occurred in eight of the nine subjects whose neutralizing antibody titres were four or less. Two falls were detected in the four men who had neutralizing antibody titres of eight or more, and in both cases these were accompanied by falls in neutralizing antibody.

Table 4. *Haemagglutination-inhibition titre rises against Coxsackievirus A21 in the personnel at Stonington Island, September* 1967 *to March* 1969

Subject number	Sept. 67 to Feb. 68	Apr. 68 to Jul. 68	Jul. 68 to Sept. 68	Sept. 68 to Oct. 68	Dec. 68 to Feb. 69	Feb. 69 to Mar. 69
1	—	—	—	—	4	—
2	8	—	—	—	—	—
3	—	—	—	4	—	—
4	—	> 12*	—	—	—	—
5	—	4	—	4	—	—
6	—†	—	—	—	—	—
7	—†	—	—	—	—	6
8	—†	> 8*	6	—	—	6
9	—†	—	—	—	—	—
10	—	4*	—	—	4	—
11	—	—	—	8	—	—
12	8	—	—	6	—	—
13	—†	4*	—	—	4	—

* Subjects given virus during the Coxsackie A21 clinical trial on 12 April 1968.
† No preliminary serum taken in September 1967.
The figures are not titres, but show maximum rise of titre; i.e. 8 = eight-fold rise.

Influenza A2/Leningrad/4/65

Apart from changes in HI antibody titres which could be associated with the trials described above, there were no significant changes in titre during the seven months after the trials.

Serum globulins

Serum globulin estimations were made on serial sera from four men to see if fluctuations in globulin were related to any of the antibody titres measured. Two sets of sera were taken from men spending a second year in Antarctica and two from men spending their first year. One of each pair was with a party which was partially starved for 6 weeks. Although some low values and fluctuations were recorded, they bore no obvious relation to the antibody titres of any of the four subjects.

Clinical symptoms during isolation

Apart from the symptoms associated with the Coxsackievirus A21 trial, respiratory symptoms occurred in only one man during the period of isolation: subject 11 had intractable purulent catarrh and, on two occasions in the spring, developed a left maxilliary sinusitis which was relieved only by puncture and irrigation of the sinus.

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Upper respiratory disease after isolation period

Epidemiology

Details of visitors to Stonington after the 10-month period of isolation (Table 5) show that no respiratory illness developed in men on the base until the arrival of the R.R.S. *John Biscoe* on 13 February. The next day an epidemic began among

Table 5. *Details of visitors to Stonington base at the end of 10-month isolation period*

Date	Transport	No. persons	Origin	Duration of visit	Result in base personnel
Dec. 1968					
9	Aircraft	2*	Adelaide Is.	0·5 hours	Isolation broken, no symptoms
16	Helicopter	6	HMS *Endurance*	6 days	No symptoms
Jan. 1969					
28	Aircraft	2	Adelaide Is.	1 hour	No symptoms
Feb. 1969					
13	Ship	3†	See Table 6	1 year	On 14th and 15th upper respiratory infection in 7 of 10 men originally on base

* Aircraft crew had themselves been isolated for about 7 weeks.
† All three men convalescing from respiratory illnesses.

Table 6. *Spread of upper respiratory tract infection carried on R.R.S.* John Biscoe

Date	
Jan. 1969	
31	Argentinian ship, *Bahia Aguirre*, visits Argentine Is. (300 miles north of Stonington). First contact of island with outside world for 4 months.
Feb. 1969	
1	Ship (R.R.S. *John Biscoe*) collects man from Argentine Is.
2	Man on ship develops upper respiratory symptoms.
3	Ship arrives Adelaide Is. (70 miles north). Upper respiratory symptoms develop in most of base personnel on board and in half the officers, but none of the crew.
5	All base personnel at Adelaide Is. develop respiratory symptoms. Aircraft from Adelaide Is. visits Fossil Bluff (300 miles south).
7	Respiratory symptoms develop in men at Fossil Bluff.
13	Ship arrives at Stonington. Three men disembark, all recovering from respiratory illness. Ship leaves with three base personnel from Stonington.
14	Respiratory symptoms develop in six of ten base personnel remaining at Stonington.
15	Ship returns to Stonington. Two of three men collected on 13th disembark. Both have respiratory symptoms. Ship leaves. Symptoms also develop in seventh man on base.
17	HMS *Endurance* stays 4 hr. at Stonington. Two days later respiratory symptoms develop on board.
19	Ship returns to Stonington. Third man collected disembarks with severe upper respiratory symptoms.
25	Clinical observer leaves for Adelaide Is. All ill men are now convalescent.

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the 16 men who were on the base. Three of them had landed the previous day from the ship. All three were recovering from respiratory infections. Ten of the 13 men who had wintered at Stonington became ill. The three who did not were all completing the second year of their tour. The symptoms experienced by three of the 13 affected were clinically consistent with 'influenza', seven with a 'severe' cold and the remaining three with a 'moderate' cold (Tyrrell, 1965). The movements of the ships and aircraft involved in the spread of respiratory illness which eventually arrived at Stonington can be followed in Table 6 and the map of the Antarctic peninsula (Fig. 1).

Complement-fixing antibody levels

A limited number of the sera taken in February 1969 were tested for the presence of CF antibody against influenza viruses A, B and parainfluenza 1, respiratory syncytial (RSV) and an adenovirus. This was done in the hope of identifying the

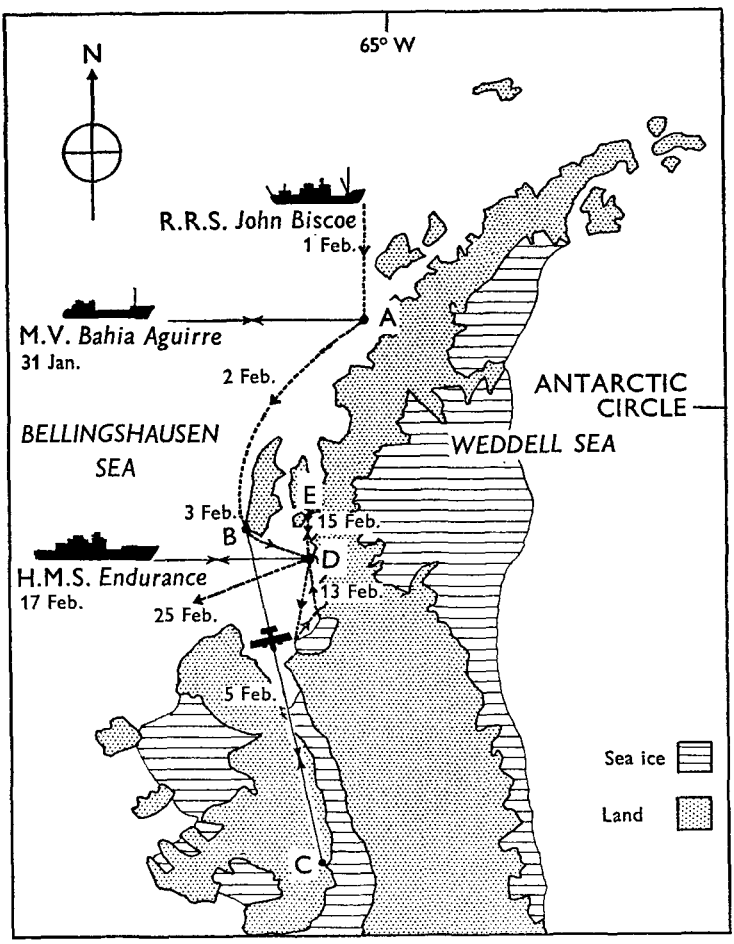

Fig. 1. The Antarctic Peninsula: Movements of ships and aircraft between 31 January 1968 and 25 February 1969. Key. A. Argentine Is.; B. Adelaide I.; C. Fossil Bluff; D. Stonington I.; E. Jones Ice Shelf.

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agent responsible for the epidemic of respiratory disease which occurred after the isolation period and before the relief ship left. The sera were uniformly negative, but they were taken only a week after the start of the epidemic.

DISCUSSION

The Coxsackievirus A21 trial showed that men at Stonington Island were infected with similar frequency, and had similar symptoms, to volunteers infected in England and the United States (Parsons, Bynoe, Pereira & Tyrrell, 1960; Spickard, Evans, Knight & Johnson, 1963; Buckland, Bynoe & Tyrrell, 1965). The unusually high incidence of gastro-intestinal symptoms parallels observations made during an outbreak of respiratory disease at an Australian Antarctic base (Cameron & Moore, 1968). Here they were attributed to the diet, but a high incidence of gastro-intestinal symptoms has also been recorded during outbreaks of respiratory disease by other polar workers (Abs, 1930; Paul & Freese, 1933).

The infection of subject 5, the only man on base at that time who had no detectable neutralizing antibody and who received placebo, suggests that the virus could spread readily under the conditions at Stonington. Buckland *et al.* (1965) noted that this virus only spread successfully under conditions of the barrack room or in crowded communities (Fukumi, Nishikawa, Sonoguchi & Shimizu, 1962; Chapple, 1966). Living conditions at Stonington were more confined than those of the barrack room. The high neutralizing antibody titres of subjects 6 and 7 appear to be protective. This is probably the reason why they were the only men on base at the time of the trial who did not become infected. It is noteworthy that subject 6, who had the highest titre of neutralizing antibody, had recently spent two years in New Guinea, and in a world-wide survey Micronesia was the area found to have the highest percentage of people with antibody to Coxsackievirus A21 (Chapple, 1966). Illness due to Coxsackievirus A21 is usually confined to those without neutralizing antibody, but such antibody does not prevent further symptomless infection (McDonald, Miller, Zuckerman & Pereira, 1962; Johnson, Bloom, Mufson & Chanock, 1962; Spickard *et al.* 1963; Oei & van der Veen, 1967).

Our findings indicate that a rise in HI antibody, when unaccompanied by a rise in neutralizing antibody, may be transient, and does not appear to protect against further symptomless infection; evidence of such transience was recorded without comment by Buckland *et al.* (1965). Furthermore a single infection, in subjects without pre-existing antibody, appeared to be insufficient to stimulate the production of much neutralizing antibody; the same conclusion was drawn by Parsons *et al.* (1960). Other studies, however, have shown that significant rises in neutralizing antibody may occur after infection in 60–70 % of people with no pre-existing neutralizing antibody (Spickard *et al.* 1963; Oei & van der Veen, 1967).

Only one HI antibody rise occurred against influenza A2/HK/1/68 and none against influenza C, parainfluenzaviruses 1 and 2 or coronavirus OC 43 during the period of isolation. Thus the HI antibody rises against Coxsackievirus A21 which were detected after July (three months after the trial) appear to be specific and most probably due to infections with this virus. The fact that variations in

globulin values did not correlate with antibody titres is further evidence for the specificity of the rises in antibody titres. The five antibody rises which occurred after December 1968 could have been due to infection from outside contact. However, the five antibody rises which occurred during isolation suggest that infections and reinfections continued to occur, or that prolonged faecal carriage stimulated delayed antibody response. Volunteer studies in men with no pre-infection antibody have shown that virus is excreted from the throat for up to 40 days after infection (Johnson *et al.* 1962). Such a long period of excretion would allow time for reinfection by the respiratory route. Although Coxsackievirus A21 is most frequently isolated from the throat, it is also found in faeces (Lennette, Fox, Schmidt & Culver, 1958; Parsons *et al.* 1960; Spickard *et al.* 1963). Hence virus could spread by the faecal-oral route. It is also possible that the coprophagic habits of the husky dogs could have caused them to become infected and involved as vectors in the epidemiology of Coxsackievirus A21 at Stonington. This latter possibility is currently under investigation. It is unlikely that prolonged faecal carriage stimulated a delayed antibody response, since in several men antibody titres fell before showing a rise and virus has only been found in the faeces of infected men for a few days (Spickard *et al.* 1963).

In the trials with influenza A2/Leningrad/4/65, men were infected when their pre-infection HI antibody titre was 12 or less, yet there was no evidence of any illness or spread of the virus. In similar trials at Salisbury with the same pool of virus, 13 of 18 volunteers with HI antibody titres of 24 or less were infected (Beare *et al.* 1968). However, nine of the infected volunteers at Salisbury developed symptoms. The influenza virus failed to spread because it was vaccine strain attenuated by passage in eggs (McDonald, Zuckerman, Beare & Tyrrell, 1962).

Although it is difficult to compare clinical results obtained by different observers at Salisbury and Stonington, the available evidence suggests that fewer symptoms were produced by the infection in the Antarctic. Evidence from animal experiments suggests that sudden exposure to cold results in more severe virus infection (Pasteur, Joubert & Chamberland, 1878; Walker & Boring, 1958). However, there are reports that Coxsackievirus infections were less severe in mice adapted to cold over several weeks than in controls (Marcus, Miya, Phelps & Spencer, 1963; Marcus & Miya, 1964).

Antibody against the other respiratory viruses appeared to persist with little fluctuation in most cases. The suggestion that men returning from the Antarctic experience severe respiratory disease because their antibody titres have fallen is not supported by our results. Antibody titres against rhinoviruses were not measured because of the multiplicity of serotypes and the limited quantity of serum. The occurrence of subclinical infections with parainfluenzavirus 1 while the ship was travelling south supports the results of Parrott *et al.* (1962). They showed that, while pre-existing antibody mitigates symptoms, it does not preclude reinfection. More recent evidence suggests that the presence of nasal antibody protects against the appearance of symptoms (Smith, Purcell, Bellanti & Chanock, 1966).

The occurrence of a respiratory epidemic after the end of a period of isolation is a common observation by those studying remote communities (Paul & Freese, 1933; Cameron & Moore, 1968). In this case a causative agent could not be identified. Unfortunately the latest that sera could be collected was only 7 days after symptoms appeared in the men, and this is rather early to expect definite changes in antibody titres. However, some indication of infection might have appeared and, since there was no evidence that the common myxovirus agents were implicated, it is possible that the disease was caused by a rhinovirus. Proof of this would require virus isolation results, and so far it has proved impossible to store and transport the necessary specimens in a suitable condition for study.

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