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Respiratory virus disease in the Antarctic: Immunological studies

Introduction
This work was begun to investigate reports that men who had recently returned from one-and-a-half to two years isolation in Antarctica suffered severely from acute respiratory infections. It was surmised that this might be due to increased sensitivity to one of the common families of respiratory viruses. Two lines of study were therefore followed. In the first, men were examined at intervals throughout their stay in Antarctica at Stonington Island, and their titres of specific antibody against a wide range of respiratory viruses were measured throughout the isolation period.

The second line of study consisted of three clinical trials in which a dose of a known respiratory virus was given to a number of subjects; the clinical and serological responses were observed and also the spread of the organism within the community. The results of these investigations were compared with those of similar trials in England carried out at the Common Cold Unit at Salisbury.

The choice of viruses to be used was determined by the following characteristics: they had to be representative of the most likely culprits, have mild effects and be readily studied by laboratory techniques; they also had to be viruses which had been tested in volunteers in England so that comparative data were available. The viruses chosen were a myxovirus, an attenuated vaccine strain of Influenza A<sub>2</sub> Leningrad 4/65, an enterovirus, coxsackievirus A<sub>21</sub>, and a rhinovirus (common cold virus), the HGP strain of rhinovirus type 2 (RV2). The latter was a partially attenuated strain which had been adapted to tissue culture.

The objects of the trials were threefold: (1) to determine whether
isolation affected severity of the disease; (2) to determine the persistence and spread of the virus in a small closed community in a situation largely free from intercurrent infections; and (3) to follow the titres of antibody in the serum and nasal secretions throughout the period of isolation.

The procedures followed consisted of compiling continuous clinical records of all subjects and titrating serial serum samples for antibody against a large number of common viral pathogens likely to cause upper respiratory disease. In this way, antibody titres could be followed throughout the year to determine whether or not they declined. Such studies might also reveal which virus or family of viruses were implicated in any outbreak of respiratory disease, whether any silent infections were occurring and, if so, for how long they persisted inside the community.

Methods

The work was carried out at Stonington Island in Marguerite Bay, where the British Antarctic Survey maintains a permanent base. This base is usually isolated for ten months of the year. Local fauna comprise 120 husky dogs, and seals and birds are found in the vicinity during the summer. Conditions for the spreading of virus were good because the base was compact and the bunkroom crowded. The work was carried out in 1968–69, when thirteen men wintered, and in 1971–72 when seventeen men wintered over.

The trials were made during the winter, when the base had been isolated for over three months, to give an adequate period for any wild infections to die out.

Serum, nasal washings and nose and throat swabs were collected throughout the year. Since members of the base were away on sledging trips, collections were irregular. However, blood was obtained at approximately six weeks intervals, and when possible, nasal washings and nose and throat swabs more frequently.

Sera were tested by haemagglutination inhibition or complement fixation tests against influenza A, B C and \( A_2 \) Hong Kong, parainfluenza viruses 1 and 2, coronavirus OC43, respiratory syncytial-virus, and the adenovirus group. Of the viruses used in the clinical trials, the sera were titrated against coxsackievirus \( A_{21} \) by haemagglutination inhibition (HI) and colour neutralisation tests, against
influenza A₂ Leningrad 4/65 by HI and against RV2 by colour neutralisation tests.

Sera were collected from fifty husky dogs by my colleague, Dr. Allen, during the season 1969–70, in an attempt to determine whether these dogs had been acting as vectors in the spread of the virus.

Serum globulin levels were measured in four subjects at intervals throughout the year, to assess whether changes in antibody levels were associated with changes in serum globulin levels.

The virus trials were carried out double blind; the author did not know which subjects received the virus and which received a placebo. The code was sealed and, except following the Leningrad A₂ 4/65 trial, was only opened when the laboratory work in the UK was completed. There were two virus trials in 1968–69 using coxsackievirus A₂₁ and influenza virus A₂ Leningrad 4/65; in 1970–71 the third trial was run with rhinovirus type 2. Seven subjects took part in the trial with coxsackievirus A₂₁, four receiving the virus and three a placebo. Thirteen subjects took part in the influenza trial, which was in two parts. At first, seven men received the virus and six a placebo. Six weeks later, all thirteen had been given virus, since the code was broken and those who had at first received placebo were given virus. In the rhinovirus trial seventeen subjects took part, nine receiving virus and eight a placebo.

**Results**

*Antibody levels*

The changes in antibody levels during the period 1967 to February 1969 for thirteen subjects are shown in Table 1. This period is from the time the members of the base left the UK up to the arrival of the relief ship.

| Table 1. Survey of antibody levels during the period October 1967 to February 1969 |
|---------------------------------|
| **Haemagglutination Inhibiting Antibodies:** |
| **Influenza A₂ Hong Kong 1/68** |
| One significant rise in titre at the time of the Influenza A₂ Leningrad trials. |
Table 1—contd.

Influenza C
All thirteen men had antibody. One significant fall in titre over the isolation period.

Parainfluenza 1
All thirteen men had antibody. Five of the six bled before they left England showed a fourfold or greater increase in titre between leaving the UK and arriving at Stonington Island. This was not shown by complement fixation.

Parainfluenza 2
Ten of the thirteen men had antibody. There were three significant falls in titre over the isolation period.

Coronavirus OC43
Ten out of eleven men tested had antibody. No significant changes.

Complement Fixing Antibodies:
There were no significant changes in titres against the following antigens: influenza A, B and C; parainfluenza 1; and two respiratory syncytial virus (RSV); the adenovirus group.

Coxsackievirus A21 Trial
Seven men took part in this trial. Four men were given virus and five developed mild symptoms, one later than the others, suggesting that the virus was transmitted within the community. The sera were titrated for haemagglutination inhibition (HI) antibody and neutralising antibody. The results are summarised in Table 2. The two men who had no symptoms had pre-existing HI titres of > 12 and were the only ones with detectable levels of neutralising antibody. All five men with symptoms had significant rises in HI antibody titre, but four of these were transient. There was only one rise in neutralising antibody. Those who became infected after inoculation mostly developed mild colds. Those who were apparently infected later showed no symptoms. Increases in HI titres were observed on a number of occasions over the period of September 1967 to March 1969, and are set out in Table 3. Rises in HI titre when unaccompanied by a rise in neutralising antibody appeared to be transient. It was concluded that the virus caused typical colds, then spread in the group, and must have persisted in some way without causing further symptoms.
Table 2. Clinical and serological results of Coxsackievirus A21 trials
(Virus administered on 12th April 1968)

| Subject | Inoculum | Symptom  | Clinical response Severity | DUR | DO  | Antibody titres* |
|---------|----------|----------|---------------------------|-----|-----|------------------|
|         |          |          |                           |     |     | 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 (TCID₅₀) of virus. Sera were collected on 12th April, 15th May and 26th July 1968.
Table 3. 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 July '68 | July '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              | —†                    | —                    | —                     | 4                   | —                   | —                   |
| 7              | —†                    | —                    | 6                     | —                   | —                   | 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 12th 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 trial**

Trials in the UK with influenza A₂ Leningrad 4/65 caused mild symptoms. No symptoms were recorded at Stonington after the first part of the trial in which seven people had received virus. Six weeks later, therefore, when there was still no evidence of overt illness or spread of the virus, the code was broken and the six men who originally received a placebo were given virus. Though, again, no symptoms were observed in these men, nine out of the thirteen subjects had been silently infected as judged by serological results. Of the other four men, three had pre-existing antibody levels which were probably high enough to protect them against infection, and the remaining man (subject No. 8) accidentally swallowed his inoculum before it could be given intranasally. (See Table 4.)

**Table 4. Serological results of influenza A₂ Leningrad trials**

| Inoculum | HI antibody titres in indicated serum* |
|----------|----------------------------------------|
| Subject  | July 26 | Sept. 15 | 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 26th July
  2 " " 15th September
  3 " " 7th October
  4 " " 27th October
  5 " " 2nd December

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

**Rhinovirus Type 2 trial**

The clinical and serological results of this trial are summarised in Table 5. Seven of the nine subjects receiving virus had definite
respiratory symptoms and in four cases these were severe. Of the remaining two subjects, one had insignificant symptoms and the other no symptoms. In a similar trial at Salisbury, using an unattenuated strain, only six out of sixteen subjects developed symptoms.

At Stonington the virus apparently spread in the community. Two of the control subjects (subjects 2 and 7) had no symptoms. Two had

Table 5. Clinical and Serological results of the Rhinovirus type 2 trials (Virus administered 20th August 1971)

| Subject | Inoculum | Symptoms | DO  | DUR | Neutralising* Antibody response |
|---------|----------|----------|-----|-----|---------------------------------|
| 1       | Virus    | xx       | 1,1|     | None                            |
| 6       | Virus    | xxxx     | 1,1|     | 64↑                             |
| 8       | Virus    | x        | 1,1|     | >4↓                             |
| 9       | Virus    | x        | 1,1|     | None                            |
| 10      | Virus    | x/-      | 1,1|     | None                            |
| 11      | Virus    | —        | 1,1|     | 5↑                              |
| 12      | Virus    | xxxx     | 1,1|     | 5↑                              |
| 13      | Virus    | xxx      | 1,1|     | 5↑                              |
| 14      | Virus    | xxxx     | 1,1|     | 5↑                              |
| 5       | Nil      | xxxx     | 1,1|     | 4↓                              |
| 2       | Placebo  | —        | 1,1|     | None                            |
| 3       | Placebo  | xx       | 1,1|     | 6↑                              |
| 4       | Placebo  | x/-      | 1,1|     | None                            |
| 7       | Placebo  | —        | 1,1|     | None                            |
| 13      | Placebo  | x        | 1,1|     | 4↓                              |
| 14      | Placebo  | x/-      | 1,1|     | None                            |
| 17      | Placebo  | xxx      | 1,1|     | 12↑                             |

**Symptoms**

x/- Very mild  
xx Moderate upper respiratory symptoms  
xxx Severe upper respiratory symptoms  
xxxx Severe upper respiratory symptoms with lower respiratory symptoms also present  

**DO** Day of onset after inoculation  
**DUR** Duration of days  

↑ Rise in antibody titre  
↓ Fall in antibody titre  

* The figures are not titres but show the maximum rise or fall in titre i.e. 8 = eight-fold increase
insignificant symptoms (subjects 4 and 14) but virus was recovered at least once from all of them except subject 14.

The clinical data from this trial were compared to data from fifty-six volunteers at Salisbury who had been given "wild" virus. The results are summarised in Table 6. An unattenuated virus would be expected to cause more severe symptoms, but by all criteria the Antarctic infections were more severe.

| Table 6. Comparative results from Rhinovirus type-2 trials |
|----------------------------------------------------------|
| Stoneington Island | Salisbury |
| % of those inoculated who were ill | 78 | 37-5 |
| Mean duration of symptoms | 11-25 days | 9-00 days |
| % of those inoculated with antibody rises | 56 | 35 |
| % of those affected with lower respiratory symptoms | 31 | 0 |
| % of men with malaise | 58 | 28 |
| % of men with pyrexia | 67 | 22 |

Other observations

The serum globulin levels in the four men studied showed only small changes during the year and those changes were not related to fluctuations in antibody titre.

There was no evidence from examination of the sera obtained from husky dogs that they were acting as vectors in the transmission of virus infections.

Discussion

It has become plain during these studies that it is possible to introduce a virus into a closed community and, under primitive conditions, to record in detail its spread and the symptoms that it causes. It has also been shown that preserving and transporting material for subsequent virological investigations, although difficult, is not impossible. It is obvious that complete analysis on the spot is not possible.

The rhinovirus behaved in a more virulent manner than expected, though the other viruses used in the trials produced reactions comparable to those which would be expected in England. Serum antibody levels were largely maintained against all the viruses tested
including the rhinovirus, so if rhinoviruses do cause more severe
disease after a period of isolation, it is probably local protective
mechanisms against them which are disturbed. On the other hand,
the influenza and enteroviruses produced the same effects as in
England, and the reason for the different response to the rhinovirus
requires further investigation.

The unique conditions make it feasible to study the effects of a
single infection and the roles of the various protective mechanisms
against virus diseases where elsewhere their effects are obscured by
intercurrent infection, and though this project has been limited in
scope, even as a series of pilot studies, it has shown that such work is
possible and fruitful.

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