

## Coronavirus antibody titres in sera of healthy adults and experimentally infected volunteers

BY A. F. BRADBURNE AND B. A. SOMERSET

*Common Cold Unit, Clinical Research Centre, Salisbury, Wilts.*

(Received 13 September 1971)

### SUMMARY

Six coronaviruses isolated in the U.S.A. have been inoculated into volunteers and all produced colds. Between 10 and 20% of infected volunteers developed heterologous antibody responses after these and other experimental infections with coronaviruses. The haemagglutination-inhibition test with the OC43 virus strain was found to detect antibody rises after infection with a variety of strains.

Studies on normal adult sera taken between 1965 and 1970 revealed a high frequency of neutralizing antibody to one strain (229E) and a frequency of HI antibody to strain OC43 which fluctuated from year to year. Complement-fixing antibodies to these two viruses were also found, revealing an apparent increase in the activity of coronaviruses in the general population of the U.K., during the winter of 1968-9.

### INTRODUCTION

During the last decade a number of viruses have been isolated from cases of human upper respiratory infection which, though ether-labile, and probably containing RNA, were serologically unrelated to any known human respiratory pathogens (Tyrrell & Bynoe, 1965; Hamre & Procknow, 1966; McIntosh *et al.* 1967*a*; Tyrrell, Bynoe & Hoorn, 1968; Kapikian *et al.* 1969). It has been shown since that many of these newly isolated strains have the same characteristic morphology as that of the virus of avian infectious bronchitis (McIntosh *et al.* 1967*a*; Tyrrell, 1967; Almeida & Tyrrell, 1967) and they have been classified together with murine hepatitis (Almeida & Tyrrell, 1967) and porcine transmissible gastro-enteritis (Tajima, 1970) as coronaviruses. These viruses are in many cases related to each other serologically (McIntosh *et al.* 1969; Bradburne, 1970).

In previous studies of experimental and natural infections with human coronaviruses, rises in heterologous antibody titres have been found (McIntosh *et al.* 1969; Bradburne, 1970). The antigenic relationships which have been demonstrated between some human coronaviruses and mouse hepatitis viruses probably account for the presence in human sera of antibody, detected by Hartley, Rowe, Bloom & Turner in 1964, which reacts with the murine viruses in both complement-fixing and neutralization tests.

This paper deals firstly with the heterologous serological responses in human sera after experimental infection with various coronaviruses and secondly with a survey of sera of adults collected in Britain over 5 years.

Table 1. *The history of coronaviruses used for inoculation of volunteers*

Virus	Reference of isolation	Passage history (after original specimen)
229 E	Hamre & Procknow (1966)	3 × OC + 1, 2 or 3 human
LP	Tyrrell <i>et al.</i> (1968)	Human only, or 4 × OC
B 814	Tyrrell & Bynoe (1965)	3 × OC or human only
EVS	Tyrrell <i>et al.</i> (1968)	3 × OC or 3 × OC + 1 human
OC 38 } OC 43 }	McIntosh <i>et al.</i> (1967a)	3 × OC or 3 × OC + 1 human
No. 691 (OC 44) } No. 703 (OC 48) } No. 501 (OC 16) } No. 663 (OC 37) }	McIntosh <i>et al.</i> (1967a)	3 × OC

'OC' = organ culture passage.

'Human' = passage in human volunteers.

Table 2. *The production of colds in volunteers inoculated with the 'OC' strains of McIntosh*

Virus	Volunteers inoculated	No. of colds produced	Classification of cold		
			Mild	Moderate	Severe
690 (OC 43)	6	3	2	1	0
664 (OC 38)	7	7	1	5	1
691	9	6	5	0	1
703	7	7	2	4	1
501	8	2	1	1	0
663	12	10	7	2	1

#### METHODS

Volunteers were isolated and cared for as described elsewhere (Tyrrell, 1963). They were inoculated with various strains of coronaviruses which had been passed serially in organ cultures of human nasal or tracheal epithelium, maintained by the methods of Tyrrell & Blamire (1967). Alternatively, nasal washings taken from volunteers with colds were passaged serially to other groups of volunteers. The passage histories of the various viruses are listed in Table 1. Certain of the viruses (the 'OC' strains of McIntosh *et al.* (1967a)) had not previously been given to volunteers and their pathogenicities for man were unknown. Typical 'coronavirus-type' colds were induced similar to those produced by the 229 E and B 814 viruses (Bradburne, Bynoe & Tyrrell, 1967). The numbers of colds induced and the severity of the disease appeared to vary with the virus strain used (Table 2), but this may be a reflexion of variation in dose of virus given and the immune state of the volunteer rather than the differences of virulence.

Serum samples were taken from volunteers on arrival at the Unit and again 3 weeks later and were stored at  $-20^{\circ}$  C.

Table 3. *Summary of the preparation of CF antigens used*

Virus	Antigen prepared in	Concentrated	Filtered on Sepharose 4B	Final titre (units/ml.)
229 E	L 132 cells	× 50	Yes	640
OC 43	Suckling mouse brain	—	Yes	2560
MHV <sub>3</sub>	Suckling mouse	—	No	1280

All antigens tested against 8 units of animal antiserum.

### *Serological procedures*

*Neutralization tests.* Antibody to virus 229E was estimated by 90% plaque reduction in L132 cells (Bradburne & Tyrrell, 1969).

*Haemagglutination-inhibition tests (for OC 43 virus).* The OC 43 strain had been isolated by McIntosh *et al.* (1967*a*) and had been shown to be indistinguishable from another isolate named OC38. The OC43 strain adapted to the brain of suckling mice (McIntosh, Becker & Chanock, 1967*b*) was used for haemagglutination inhibition tests as described by Kaye & Dowdle (1969).

Sera for use in the haemagglutination-inhibition (HI) test were diluted in phosphate buffered saline and inactivated by heating at 56° C. for 30 min. Sera and antigens were mixed in 0.025 ml. drop volumes in a microtitre system using 4 agglutinating units of antigen. This antigen was a 10% suspension of infected brain homogenized in phosphate-buffered saline (pH 7.2) which had been clarified by light centrifugation. Antigen and serum mixtures were held at ambient temperatures for 1 hr. and then 0.025 ml. of 1% fowl erythrocytes were added and allowed to settle for 50 min. at 4° C. before the tests were read.

*Complement-fixation tests.* Virus 229E grown in L132 cells was purified on Sepharose 4B (Pharmacia) and concentrated by dialysis; such preparations contained over 1000 antigenic units/ml. (Bradburne, 1970). OC 38-43 and MHV<sub>3</sub> viruses were cultivated in suckling mouse brain and 10% suspensions of infected brain tissue were used. A summary of the preparation of each antigen used is shown in Table 3.

Low doses of complement were necessary for the optimum detection of antibody and from 1.6 to 1.75 units of complement were employed, using overnight fixation at 4° C. Anticomplementary activity was removed, without impairing antibody titres, by inactivation at 65° C. for 30 min.

Before starting this survey, large batches of CF antigens for the 229E, OC43 and MHV<sub>3</sub> viruses were prepared, and one batch only for each virus was used throughout; each batch was subdivided and stored at -20° C. In each test 8 units of antigen were used.

## RESULTS

### *Heterologous antibody rises in volunteer sera after experimental infection*

The proportion of heterologous rises in neutralizing antibody to coronaviruses has been found to be quite small after natural (McIntosh *et al.* 1969) or experi-

Table 4. *Rising titres of HI antibody against OC 43 in paired sera from volunteers who developed colds after inoculation with various coronaviruses*

Virus given	No. of serum pairs tested	No. of rises (4-fold or greater) in HI antibody to OC 43
229E	13	3
LP	7	0
B 814	6	0
EVS	8	2
OC 38 + OC 43*	13	6
691*	9	5
663*	12	4
501*	8	0
703*	7	0

\* N.I.H., Bethesda 'OC' strains from McIntosh.

Table 5. *Rises in CF antibody titres to OC 43, MHV<sub>3</sub>, and 229 E in paired sera from volunteers who developed colds after inoculation with various coronaviruses*

Virus given	No. of serum pairs tested	No. of 4-fold or greater rises in CF antibody to:		
		OC 43	MHV <sub>3</sub>	229 E
229E	13	1	2	5
LP	7	1	2	3
B 814	6	1	0	0
EVS	8	1	1	0
OC 38 + OC 43*	13	3	4	2
691*	9	1	1	3
663*	12	1	2	3
501*	8	0	4	2
703*	7	0	2	0

\* 'OC' strains of McIntosh.

mental (Bradburne, 1970) infections. Similar studies of HI and CF antibody made after experimental infections have not been reported.

Paired sera taken from volunteers infected with all the viruses listed in Table 1 were tested by HI against OC 43. These sera were all selected from volunteers who developed colds after inoculation (see Table 2). Except for volunteers given 229E and LP, virus isolation was not attempted; however, viruses were isolated from every individual with a cold induced by 229E or LP and it is therefore likely that all these volunteers were infected.

Significant rises in the levels of HI antibody were detected mainly in volunteers given the OC 38 or OC 43 viruses, or strains nos. 691 or 663, and a few in volunteers given other strains. The results are shown in Table 4. Of 70 paired sera obtained from persons given viruses other than OC 38 and OC 43, 14 (20%) had fourfold or greater antibody rises in the HI tests.

These same sera were tested by complement-fixation against three different coronavirus antigens. To ensure that the optimum dose of complement was used

Table 6. *The numbers of heterologous antibody rises to one or more strains of coronaviruses in human volunteers*

	Volunteers given	
	229E, LP, OC 38, OC 43 (nos.)	Other coronaviruses (nos.)
Total pairs of sera tested	33	50
Pairs showing rises to homologous strain	19	—
Pairs showing rises to:		
1 heterologous virus	10	15
2 heterologous viruses	3	7
3 heterologous viruses	0	2

for each antigen the tests were performed at three different dilutions of complement, estimated at 1.5, 1.65 and 1.8 units/drop volume. Significant rises detected in these CF tests are summarized in Table 5. There are several examples of heterologous antibody rises.

The 229E and LP viruses have been found to be antigenically closely related (Bradburne & Tyrrell, 1969) and cross-react completely by CF tests using animal antisera. The number of significant CF antibody rises to the 229E antigen in the sera of volunteers given viruses other than 229E or LP was 10 out of 63 pairs tested (16%). The mouse hepatitis (MHV<sub>3</sub>) antigen reacted with several sera and 18 out of 83 pairs showed significant rises in antibody titre. Some volunteers developed rises to more than one coronavirus antigen (Table 6). The actual antibody titres obtained in the HI and CF tests varied considerably amongst the 63 pairs, but generally heterologous antibody rises occurred in those with a low titre before inoculation against the heterologous virus. However, several individuals with high pre-inoculation antibody developed significant rises in antibody titres.

#### *A survey of the frequency and titres of coronavirus antibody in normal human sera*

Serum samples were taken from volunteers on arrival at the Unit. These sera represent a collection of random specimens from 'normal' healthy adults aged between 18 and 50 years and living in all parts of the U.K. Sera taken over the previous 5 years were studied. For most of this period approximately 20 sera per month were available; about 10% of these specimens represented serial samples from the same individuals, taken at minimum intervals of 6 months.

The sera were tested as follows:

- (1) At a dilution of 1/20 for their capacity to neutralize 90% or more of 229E plaques produced in L 132 cell monolayers.
- (2) At a dilution of 1/10 for their ability to completely abolish the agglutination of fowl erythrocytes produced by four agglutinating doses of OC 43 virus antigen.
- (3) At twofold dilutions (1/10 to 1/80) for their capacity to fix at least 1 unit of complement with 4 units of either 229E or OC 43 antigens.

The results, as shown in Figs. 1 and 2, were grouped into 3-monthly periods, representing accumulated tests on a maximum of 60 sera. It can be seen in Fig. 1

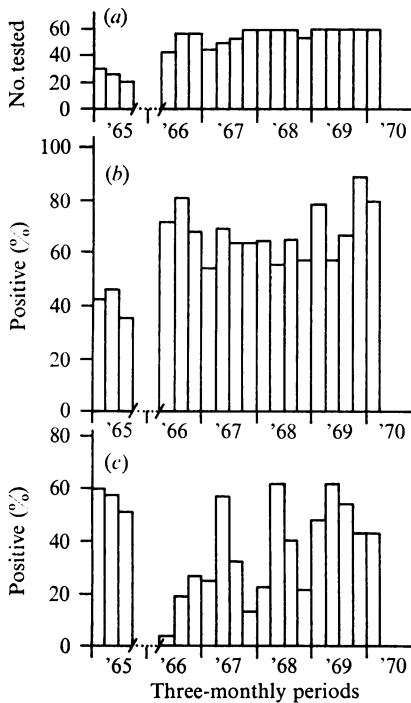


Fig. 1

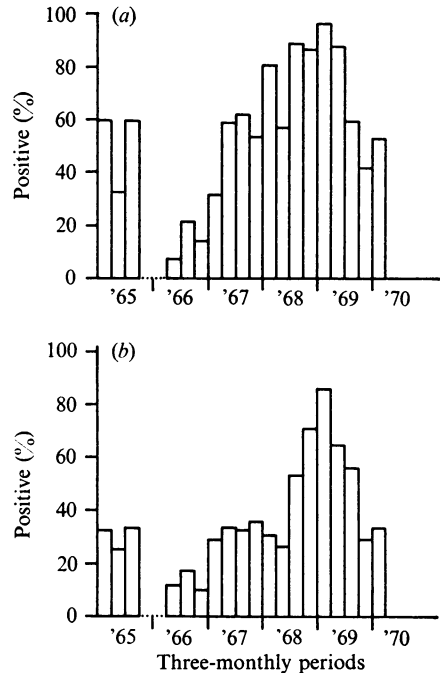


Fig. 2

Fig. 1. The numbers of normal human sera tested (a) and the proportion of those found to neutralize 229E virus (as a 90% plaque reduction) (b) when diluted to 1/20. The proportion of sera which inhibited the effects of four agglutinating doses of OC 43 virus, at a serum dilution of 1/10, are shown in the lower histogram (c). All results are divided up into 3-monthly periods, between 1965 and 1970. The break in the horizontal axis indicates a period when no sera were available.

Fig. 2. The proportion of normal sera (same specimens as Fig. 1) which react, at a dilution of 1/10 or greater, with 229E (a) or OC 43 (b) complement-fixing antigens.

that relatively few sera were available during 1965, and none at all during the last quarter of 1965 and the first quarter of 1966.

In Fig. 1 (b) it is seen that of sera collected in the first three quarters of 1965, 40% neutralized 229E virus. This could reflect a deterioration of the sera after prolonged storage, but this is not borne out in the pattern of HI antibody to OC 43 virus detected during 1965 and in later years (see below). From 1966 there was a fairly high proportion of positive sera, which remained high with possible peaks in the first and last quarters of 1969.

In Fig. 1 (c) the frequency of HI antibody against OC 43 is quite different from that of neutralizing antibody against 229E virus. There was a high frequency during 1965 and again in the first half of 1969. It was much lower in the intervening years except for prominent peaks in the second quarters of 1967 and 1968.

Fig. 2 summarizes the rates of detection of CF antibody to both 229E and OC 43 viruses. Again there are no peaks of occurrence of 229E antibody, but a low frequency in 1966, unlike that seen with neutralizing antibody. Antibodies against OC 43 likewise fell in 1966, but instead of three peaks, one peak of high incidence

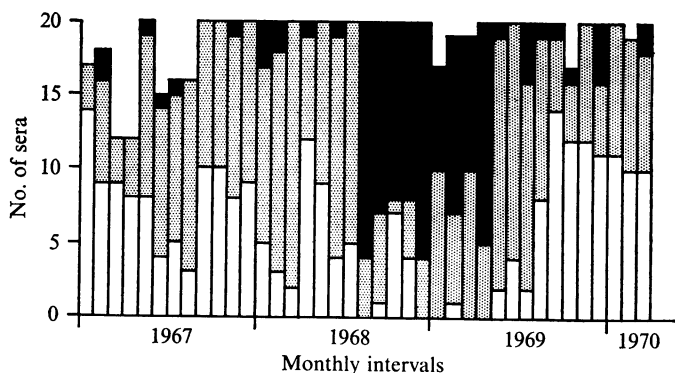


Fig. 3. The actual monthly pattern of complement-fixing antibody titres to the 229E virus in normal human sera obtained between January 1967 and March 1970. Titre range: ■, greater than 1/80; ▨, 1/10 to 1/80; □, less than 1/10.

is seen in the last two quarters of 1968 and the first three of 1969. The frequency of CF antibodies against both viruses fell towards the end of 1969. The results of CF antibody titration against the 229E antigen are analysed in more detail on a monthly basis in Fig. 3. Each column shows the number of sera without antibody, those with titres from 1/10 to 1/80, and those greater than 1/80. It can be seen that from August 1968 to April 1969 the proportion of sera with high titres of CF antibody increased considerably. Also around this period there were raised incidences of neutralizing antibody to 229E and of CF and HI antibody to OC43 virus (Figs. 1, 2).

The maximum incidence of CF antibody, found in the first quarter of 1969, were 98% for 229E virus and 89% for OC43 virus; these contrast with the lowest values detected in this survey of 9% for 229E and 14% for OC43. The maximum and minimum incidences of neutralizing antibody detected against 229E virus were 91% and 37% respectively. The corresponding values for OC43 virus HI antibody were 61% and 5%.

## DISCUSSION

### *Heterologous responses in volunteers*

The induction of the symptoms of the common cold in volunteers with the 'OC' strains of McIntosh *et al.* (1967*a*) and the passage of the infectious agents from one volunteer to another fulfils the third clause of Koch's postulates for these coronaviruses. However, for practical reasons rates of virus isolation and patterns of virus excretion from infected volunteers were not determined. Similarly, direct serological tests, using the viruses administered, were not feasible. The heterologous responses detected in the paired sera taken from experimentally infected volunteers demonstrated that some antigenic interrelationship may exist between these new virus strains, even though some of the viruses are distinct on the basis of neutralization tests (Kapikian *et al.* 1969). As yet there is no direct evidence of common or group antigens.

It is possible that the heterologous serological responses may be the result of

antigenic recall after previous exposure to a related coronavirus. Specific cross-reactions between these viruses have been demonstrated with animal antisera (McIntosh *et al.* 1969; Bradburne, 1970) using several different serological tests, and heterologous rises have been found in human sera by other workers (McIntosh *et al.* 1969; Kapikian *et al.* 1969; McIntosh *et al.* 1970). Haemagglutination-inhibition antibody rises to the OC 43 virus in sera from persons given viruses serologically similar to the 229E strain have not been demonstrated before, and this may affect attempts to separate these viruses into serological groups (Kapikian *et al.* 1969; McIntosh *et al.* 1970). The majority of the coronavirus strains seem to be capable of producing a heterologous antibody rise to mouse hepatitis virus (MHV<sub>3</sub>) as detected by complement-fixation.

Two of the 'OC' strains of McIntosh (OC 38 and OC 43) have been reported to be identical (McIntosh *et al.* 1967*b*). Viruses designated nos. 691 and 663 also produce a high proportion of HI responses to OC 43, but were not adaptable from human tracheal organ cultures to suckling mice, unlike OC 38 and OC 43. The other viruses react with both MHV<sub>3</sub> and 229E antigens by complement-fixation. There is some correlation between these results and those reported by McIntosh *et al.* (1970), but in the latter studies very few heterologous rises were detected.

#### *The incidence of antibodies in random human sera*

In the light of the heterologous responses detected in volunteer sera, the antibody responses that were detected to the two coronavirus antigens used in the serological survey cannot be interpreted as being specifically due to these particular viruses. In other surveys (Kapikian *et al.* 1969; Cavallaro & Monto, 1970) the results of neutralization and complement-fixation tests have been assumed to be relatively specific to the antigens used in the serological tests. A specific assumption that the 229E and OC 43 viruses are unrelated was held by Cavallaro and Monto and that serological responses to the 229E virus represented infections with a 229E-type virus. Such an assumption may be valid while studying sera from a relatively small community, but probably does not apply to the sera studied in this report, which were taken from persons living all over the U.K. It is notable that the antibody titres, particularly in the complement-fixation test using a 229E antigen, were considerably higher, as was the rate of detection, than those reported in previous studies. This is probably the result of using a combination of concentrated CF antigens and a low (1.5–1.75 units) complement dosage.

Some of the detected variations in the incidence and titres of antibody in adult human sera were probably not significant but the larger variations may reflect genuine changes in the frequency of antibody in the general population. The occurrence of HI antibody to OC 43 virus demonstrated this particular point. The peak frequency in 1967–9 occurred in the second quarter of each year, the quarter after the 3-month period in which coronaviruses have been most often isolated from man.

Complement-fixing antibody to both viruses was not common during 1966, but increased to a peak in the winter of 1968–9, declining again over the following year. This makes it less likely that the rising frequency of antibody, detected



before 1969, was an artifact, resulting from a deterioration of sera after long-term storage. There was a significant peak in the incidence of neutralizing antibody to 229E during the first quarter of 1969. As sera positive to OC 43 by the HI test became more numerous at this time, it seems reasonable to assume that there was a wave of infection passing through the country caused by a related virus or viruses.

Epidemiological observations by Cavallaro & Monto (1970) have led them to propose that coronavirus complement-fixing activity has a relatively short half-life. In our studies it was observed that gross variations in the prevalence of antibody did not occur, but there were more marked variations in the percentage of sera with high antibody titres (see Fig. 3). This suggests that a less-sensitive CF test would only pick up such high-titre antisera, or antisera with greater avidity, resulting from recent infection with a virus closely related to that used in the CF test. It would not take into account those antisera displaying a low titre and possibly less strain-specific activity. Such sera may constitute the greater proportion of a random selection of sera taken during non-epidemic periods.

Such serological responses may not necessarily involve upper respiratory tract infection. Cavallaro & Monto (1970) obtained a significant relationship between upper respiratory disease and CF antibody responses. However, McIntosh *et al.* (1970) found a significantly negative correlation between the development of serological responses to OC 43 or MHV (strain A-59) viruses and respiratory tract disease. It is interesting that the coronavirus infections of other animals involve more generalized illnesses, and coronavirus-like particles have been detected in non-respiratory diseases of man (Zuckerman, Taylor & Almeida, 1970; Friedmann & Bird, 1969). If such viruses were related serologically to respiratory coronaviruses, they might also figure in the serological responses detected in these studies.

The authors would like to acknowledge the co-operation of the volunteers, and the assistance given by various members of the Staff of the Unit responsible for the clinical evaluation of the experimental infections. We would also like to thank Drs K. McIntosh and D. Hamre for supplying original virus isolates.

#### REFERENCES

- ALMEIDA, J. D. & TYRELL, D. A. J. (1967). The morphology of three previously uncharacterized respiratory viruses that grow in organ cultures. *Journal of General Virology* **1**, 175.
- BRADBURNE, A. F. (1970). Antigenic relationships amongst coronaviruses. *Archiv für die gesamte Virusforschung* **31**, 352.
- BRADBURNE, A. F., BYNOE, M. L. & TYRELL, D. A. J. (1967). Effects of a 'new' human respiratory virus in volunteers. *British Medical Journal* **iii**, 767.
- BRADBURNE, A. F. & TYRELL, D. A. J. (1969). The propagation of 'Coronaviruses' in tissue culture. *Archiv für die gesamte Virusforschung* **28**, 133.
- CAVALLARO, J. J. & MONTO, A. S. (1970). Community-wide outbreak of infection with a 229E-like coronavirus in Tecumseh, Michigan. *Journal of Infectious Diseases* **122**, 272.
- FRIEDMANN, I. & BIRD, E. S. (1969). Viruses in a malignant lymphoma. (Corresp.) *Lancet* **ii**, 1013.
- HAMRE, D. & PROCKNOW, J. J. (1966). A new virus isolated from human respiratory tract. *Proceedings of the Society for Experimental Biology and Medicine* **121**, 190.

- HARTLEY, J. W., ROWE, W. P., BLOOM, H. H. & TURNER, H. C. (1964). Antibodies to mouse hepatitis viruses in human sera. *Proceedings of the Society for Experimental Biology and Medicine* **115**, 414.
- KAPIKIAN, A. Z., JAMES, H. D. JR., KELLY, S. J., DEES, J. H., TURNER, H. C., McINTOSH, K., KIM, H. W., PARROT, R. H., VINCENT, M. M. & CHANOCK, R. M. (1969). Isolation from man of 'avian infectious bronchitis virus-like' viruses (Coronaviruses) similar to 229E, with some epidemiological observations. *Journal of Infectious Diseases* **119**, 282.
- KAYE, H. & DOWDLE, W. R. (1969). Some characteristics of haemagglutination of certain strains of 'IBV-like' viruses. *Journal of Infectious Diseases* **120**, 576.
- McINTOSH, K., DEES, J. H., BECKER, W. B., KAPIKIAN, A. Z. & CHANOCK, R. M. (1967*a*). Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. *Proceedings of the National Academy of Sciences of the USA* **57**, 933.
- McINTOSH, K., BECKER, W. B. & CHANOCK, R. M. (1967*b*). Growth in suckling mouse brain of 'IBV-like' viruses from patients with upper respiratory tract disease. *Proceedings of the National Academy of Sciences of the USA* **58**, 2268.
- McINTOSH, K., KAPIKIAN, A. Z., HARDISON, K. A., HARTLEY, J. W. & CHANOCK, R. M. (1969). Antigenic relationships among the coronaviruses of man and between human and animal coronaviruses. *Journal of Immunology* **102**, 1109.
- McINTOSH, K., KAPIKIAN, A. Z., TURNER, H. C., HARTLEY, J. W., PARROT, R. H. & CHANOCK, R. M. (1970). Seroepidemiologic studies of coronavirus infection in adults and children. *American Journal of Epidemiology* **91**, 585.
- TAJIMA, M. (1970). Morphology of transmissible gastroenteritis virus of pigs: a possible member of coronaviruses. *Archiv für die gesamte Virusforschung* **29**, 105.
- TYRRELL, D. A. J. (1963). The use of volunteers. *American Review of Respiratory Disease* **88**, 128.
- TYRRELL, D. A. J. (1967). Common colds and related diseases. *British Medical Bulletin* **23**, 124.
- TYRRELL, D. A. J. & BLAMIRE, C. J. (1967). Improvements in the method of growing respiratory viruses in organ cultures. *British Journal of Experimental Pathology* **48**, 217.
- TYRRELL, D. A. J. & BYNOE, M. L. (1965). Cultivation of a novel type of common cold virus in organ cultures. *British Medical Journal* **i**, 1467.
- TYRRELL, D. A. J., BYNOE, M. L. & HOORN, B. (1968). Cultivation of 'difficult' viruses from patients with common colds. *British Medical Journal* **i**, 606.
- ZUCKERMAN, A. J., TAYLOR, P. E. & ALMEIDA, J. D. (1970). Presence of particles other than the Australia SH antigen in a case of chronic active hepatitis with cirrhosis. *British Medical Journal* **i**, 262.