are plotted in the same way, an almost identical distribution is obtained. This shows that no selection was made according to the time of day of patients from whom samples were obtained.

Fig. 2 shows the blood-alcohol levels of the 121 road-traffic-accident drivers (aged 18 years and over) in our series according to the time of the accident in which they were involved. Between 2200 and 0200 hours there were 19 road-traffic-accident drivers (18 years of age and over); 11 (58%) had blood alcohol levels above 50 mg./100 ml., 8 (42%) above 100 mg./100 ml., 3 (16%) above 150 mg./100 ml.

We divided the road-traffic-accident drivers in our series into two groups: those involved in accidents in which no other vehicle or pedestrian was concerned ("single accidents") and those involved in "double accidents." The number of drivers involved in single accidents with blood-alcohol levels under 50 mg./100 ml., over 50 mg./100 ml., over 100 mg./100 ml., and over 150 mg./100 ml. were 16 out of a total of 99 (16%), 11 out of a total of 22 (50%), 10 out of a total of 15 (66.6%), and 6 out of a total of 9 (66.6%) respectively. These figures show a dramatic increase in the ratio of single to double accidents when the blood-alcohol level rises above 50 mg./100 ml. These results agree with the findings of Jeffcoate (1958), who in a survey of 376 fatalities in road accidents showed that the driver who had been drinking tended to be involved relatively more frequently in single accidents, suggesting lack of attention or control.

In our series the overall proportion of drivers involved in road traffic accidents with blood-alcohol levels above 50 mg./100 ml. irrespective of the time of day is 18.2%. This figure agrees closely with the statistical survey of Jeffcoate (1958), who found that in 17% of all accidents it was known that someone involved had been drinking alcohol. This percentage is, however, somewhat lower than in the series of Cassie and Allan (1961).

It is clear from our results that a significant number of the drivers of vehicles involved in accidents have higher levels of alcohol in their blood than the British Medical Association Committee on Relation of Alcohol to Road Accidents regard as compatible with the ability to control a vehicle safely.

Summary

Blood-alcohol levels were determined on 121 drivers of vehicles involved in road traffic accidents in the Birmingham area who were admitted to a hospital casualty department between 15 February and 5 May 1964. The percentage of those drivers with blood-alcohol levels above 50 mg./100 ml., irrespective of the time of day, was 18.2%; between 2200 and 0200 hours 58% of drivers had blood-alcohol levels above 50 mg./100 ml. The number of drivers involved in accidents in which no other vehicle or pedestrian was concerned ("single accidents") was 16 out of a total of 99 (16%) in the group with blood-alcohol levels below 50 mg./100 ml. In the group with blood-alcohol levels above 50 mg./100 ml. the proportion of "single accidents" increased to 11 out of a total of 22 (50%).

We wish to thank Mr. A. B. Watson, Mr. J. C. Fulford, and Mr. M. H. M. Harrison for their co-operation and for access to patients under their care in the casualty department. We would especially thank the staff of the casualty department for their help in collecting blood samples. We are grateful to Dr. R. Gaddie for helpful criticism.

REFERENCES

- British Medical Association (1954). *Recognition of Intoxication*.
— (1960). *Relation of Alcohol to Road Accidents*.
Cassie, A. B., and Allan, W. R. (1961). *Brit. med. J.*, 2, 1668.
Holcomb, R. L. (1938). *J. Amer. med. Ass.*, 111, 1076.
Jeffcoate, G. O. (1958). *Brit. J. Addict.*, 54, 81.
Lucas, G. H. W., et al. (1955). *Proceedings of Second International Conference on Alcohol and Road Traffic*, p. 139. Toronto.
Vamossi, M. (1961). *Traffic Safety Res. Rev.*, 4, 8.

Serological Study of Respiratory Syncytial Virus Infections in Infancy and Childhood

P. S. GARDNER,* M.D., DIP.BACT.; F. M. ELDERKIN,† M.B., B.S., M.R.C.P.ED., D.C.H.;
A. H. WALL,* M.B., B.S., B.SC.

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The recent work of many workers both in the United States and in this country has shown how important respiratory syncytial virus (R.S.V.) is as an aetiological agent in the production of respiratory infection in young children (Chapock *et al.*, 1961; McClelland *et al.*, 1961; Holzel *et al.*, 1963; Andrew and Gardner, 1963). This present study was undertaken to elucidate a number of outstanding questions. It was thought useful to investigate how early in an infant's life an antigenic response could be obtained to virus infection. Though a number of workers (Parrott *et al.*, 1961; Sandiford and Spencer, 1962; Moss *et al.*, 1963) have studied children with bronchiolitis and pneumonia, there was still very little information regarding the antibody response of infants under 3 months of age. Moreover, we wished to study the effect of the presence of maternal antibody on the antigenic responses. We wanted to see whether infection could take place in the presence of pre-existing antibody and also to see, by serological tech-

niques, whether R.S.V. was an important aetiological agent every year. Finally we wished to investigate a report (Jensen, 1962) of a relationship between this virus and measles.

Materials and Methods

Over the last three winters (1961–2, 1962–3, 1963–4) suitably paired sera had been collected from children admitted with acute respiratory illnesses to paediatric units of a number of Newcastle hospitals and stored for studies of this kind. It is normal practice in these units for infants under 1 year of age to be barrier-nursed in cubicles to minimize the risk of cross-infection. Paired sera were taken at an interval which averaged 17 days, the first blood as soon as possible after admission and the second either before the child was discharged or at the first follow-up appointment. Sufficient blood could be obtained by heel stab, and the vast majority of specimens were obtained in this way. Material for virus isolation was taken from many of the cases, and this aspect of the investigation of the 1963–4 winter will be reported elsewhere. Our experience with the

* Department of Microbiology, Royal Victoria Infirmary and University of Newcastle upon Tyne.

† Department of Child Health, Royal Victoria Infirmary and University of Newcastle upon Tyne.

isolation of R.S.V. for the winter of 1962–3 has already been published (Andrew and Gardner, 1963).

Sera were examined by complement-fixation test with the following antigens: R.S.V., measles, influenza A, B, and C, Sendai, adenovirus, and *Mycoplasma pneumoniae*. The complement-fixation test used was carried out in Perspex plates by overnight fixation at 4° C. using 0.1-ml. quantities of reagents. All sera were tested for R.S.V. and measles in this way. When serum was available this method was also used for testing against other antigens. Where very small amounts were available the method based on the technique of Fulton and Dumbell (1949) as described by Gardner *et al.* (1960) was used. The antigens and standard sera used were kindly supplied to us by the Standards Laboratory, Central Public Health Laboratory, Colindale. Four pairs of sera were kindly supplied by Dr. A. P. Goffe, of Burroughs Wellcome, from children who had been recently vaccinated against measles.

Results

Over the three years of the investigation suitably paired sera were received from 148 children with acute respiratory illness; 125 of these were under 1 year of age, 89 of whom were less than 16 weeks and 68 less than 12 weeks old. Table I shows the types of illness studied during these three winters. It

TABLE I.—Clinical Categories in 148 Children Admitted to Hospital From Whom Paired Sera Were Obtained

Winter	Infection of Upper Respiratory Tract	Croup	Bronchitis	Bronchiolitis	Pneumonia
1961–2 ..	0	0	1	17	8
1962–3 ..	0	1	0	19	9
1963–4 ..	4	3	5	67	14
Totals	4	4	6	103	31
Seropositives	1 (25%)	0 (—)	3 (50%)	63 (61%)	17 (55%)

TABLE II.—Serological Results and Virus Isolations of Serologically Negative Cases in the 148 Children

	Total No.	Positive Serology		Virus Isolation, but Negative Serology	Total Percentage Positive
		No.	%		
1961	26	11	42	1	46
1962	29	18	62	3	72
1963	93	55	59	10	70

TABLE III.—Serological Responses in Four Clinical Categories of Acute Respiratory Illness

	Infection of Upper Respiratory Tract	Bronchitis	Bronchiolitis	Pneumonia
Total No. of children ..	4	6	103	31
Antigen:				
R.S.V. ..	0	3	61	15
Measles ..	0	0	0	3 [§]
Adenovirus ..	1 [†]	0	2	1 [‡]
Influenza A ..	1	0	0	0
<i>Mycoplasma pneumoniae</i>	0	0	2*	0

* Associated with a rising titre of antibodies to adenovirus in one case and with R.S.V. in another.

† Associated with a rising titre of antibodies to influenza A.

‡ Associated with a rising titre of antibodies to R.S.V.

§ Two of these rising titres were associated with rising titres of antibodies to R.S.V.

TABLE IV.—Proportion of Fourfold Rising Antibody Titres and Pre-existing Antibody Titres to R.S.V. in Relation to Age

No. of cases ..	Age:	Weeks												Years			
		4	8	12	16	20	24	28	32	36	40	44	48	1	2	3	4+
		No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
Rising antibody titres ..	{	10	27	31	21	11	7	10	2	2	1	1	2	6	9	4	4
	%	0	9	14	16	11	3	10	2	2	0	1	1	5	4	1	0
		0	33	45	76	100	43	100	100	100	0	100	50	83	44	25	0
Antibody titres greater than 1/8 ..	{	9	17	5	0	0	2	0	1	0	0	1	1	1	7	3	4
	%	90	63	16	0	0	29	0	50	0	0	100	50	17	78	75	100

also shows the number of illnesses in which a diagnosis was reached based on at least a fourfold rising titre of antibodies when the first specimen of serum was compared with the second. In only one child was a high non-rising antibody titre in both sera accepted as a serological diagnosis, and this was because the patient was admitted to hospital sufficiently late after the onset of illness to account for the presence of high titres of antibody. One case of bronchiolitis and one of pneumonia occurred in June 1961 and were counted with the 1961–2 group, and two cases of bronchiolitis which occurred in August 1962 were arbitrarily placed in the 1962–3 group.

Over the three years in question attempted virus isolations were made in most cases. Table II gives the serological results for each year and also the isolation of viruses in those who were serologically negative. The isolation of virus without an antibody response occurred only in infants below the age of 3 months. Virus isolations with a positive serological response in the same patient were not recorded separately.

Table III includes only the antigens which gave a positive reaction and also illustrates the cases in which multiple antigenic responses occurred. It should be noted that on two occasions a rising titre of measles antibody was associated with a rising titre of R.S.V. antibody.

Four pairs of sera were obtained from children prior to measles vaccination and one month after; all showed an excellent antibody response to measles antigen but no response to R.S.V.

Table IV demonstrates the relation between the age of the children and the response to R.S.V.; all the 148 children are included. It also shows those with antibody titres greater than 1/8 in the first specimen of serum taken at the onset of illness from the same series of children. These sera reflect either the presence in the very young child of maternal antibody or in the older children of pre-existing R.S. antibody. Table IV shows clearly the disappearance of maternal antibody by 12 weeks and the gradual appearance in older children of antibody, which suggests previous exposure to R.S.V.

In some children there were rises of titres of antibody in spite of the presence of previous complement-fixing antibodies to R.S.V. In one child aged 12 weeks a titre of 1/8 maternal antibody was found, and in a second child aged 5 weeks a titre of 1/16; these were converted to 1/32 and 1/64 respectively by R.S.V. infection. The same was true of some older children with previous experience of R.S.V. antibody; four children between the ages of 10 months and 2 years showed rising titres—three from 1/16 to 1/64 or greater and one from 1/8 to 1/64. It should be emphasized, particularly in the older children, that blood was taken from them as soon as they were admitted, and from the four children just mentioned it was unlikely that the antibody in the first specimen of sera was a product of the present infection, as their illness had not been present long enough.

The Chart shows a comparison of titres of antibody to R.S.V. in the first and second specimens of blood expressed in mean units. It shows the original maternal antibody falling to a low level, from birth to 3 months of age, but in the older children of 7 months or more the first specimens show evidence of acquired antibody. The second specimen of serum constantly contained more antibody than the first specimen, but the amount increased with age. The maximum differences between

the two sera occurred at about 24–28 weeks; after this the gap again narrowed because of the occurrence of antibody in the first serum due to previous exposure to R.S.V.

Discussion

This communication describes a serological study in children of the occurrences of R.S.V. infection in the Newcastle area over the past three years. It is clear that R.S.V. is a common cause of acute respiratory illness, and while this is true for other communities there are certain aspects of our own experience to which we would draw attention. There is a marked difference in the part played by R.S.V. in the first winter as compared with the two subsequent years. Tables II and III show that R.S.V. was the main aetiological agent for the years 1962–4 and accounted for about 60% of cases of bronchiolitis and pneumonia. The winter of 1961–2, however, was different in that only 40% of infections appeared to be due to this virus. This difference may be accounted for by the presence of viruses not as yet detectable by our serological tests.

Although our work tended to diminish during the summer months, there was serological evidence of R.S.V. infection in June and August which showed that this virus was present throughout the year.

Relation of mean antibody titres to age. Titres below $1/8$ were arbitrarily given a value of 4, while any titres greater than $1/64$ were still valued at 64 units.

It has been assumed for many years that infants under the age of 3 months are immunologically less mature than older children (Smith, 1960). Table IV shows that from 4 weeks onwards there is an increasing percentage of children capable of responding immunologically to R.S.V., reaching a maximum by 20 weeks; below 4 weeks of age no children were found who responded to R.S.V. The question arises whether the increasing number of positives is due to the increased immunological competence of the infants or that bronchiolitis and pneumonia below 3 months is not often due to R.S.V. Table II shows a number of virus isolations in which there were no serological responses; seven of these were R.S.V., isolated from infants below 3 months of age with no antibody responses. This would support the view that this virus does cause bronchiolitis and pneumonia in young infants, and that the relative failure to detect serological response in this group, as shown in Table IV, is due to immunological immaturity.

An attempt was made to relate the presence of maternal antibody to immunological responses in the child. Table IV shows the rapid fall of maternal antibody and its disappearance by the twelfth week. This fall in maternal antibody coincided with the increased number of children showing rising titres of antibodies when infected with R.S.V. Two infants of 5 and 12 weeks of age, however, acquired a fourfold or greater rising titre of antibody in spite of the presence of maternal antibody. In a similar way four older children showed a rising titre to R.S.V., although their first sera, taken at the beginning of their illness, already had antibody present.

The Chart illustrates the antibody responses at various ages and shows the increased number of responses as maternal antibody disappeared; at a later age there was a decrease in the number of responses as naturally occurring antibody made its appearance.

Hambling (1964) showed the presence of neutralizing as well as complement-fixing antibodies in cord blood, and though in our series there was insufficient serum to embark on neutralization tests the conclusion can be drawn that neutralizing antibodies were present in our infants' sera and R.S.V. infection could take place despite it.

One of the unusual features of R.S.V. infection from our own observation and those of others (Moss *et al.*, 1963; Hambling, 1964) is the high percentage of adult sera with appreciable levels of R.S.V. complement-fixing antibody; this is reflected in our sera by the high percentage of infants up to 4 weeks old with R.S.V. antibody. Most complement-fixing antibody titres fall rapidly and adults rarely show high titres to most of the common virus antigens unless recently infected. It would therefore seem either that R.S.V. antibodies are unusual in being long-lasting possibly with the persistence of virus, or that there is a group of viruses with the common antigen maintaining a high level of antibody, or, finally, that repeated reinfection with R.S.V. occurs. The latter seems to be the most likely explanation from American work on adult infection (Johnson *et al.*, 1961; Kravetz *et al.*, 1961).

Jensen (1962) drew attention to the possibility of an antigenic relationship between measles and R.S.V., children with measles showing increased antibody titres to R.S.V. as well as to measles. This is not in accordance with our own observations, but this aspect is still being studied. Of the 79 children in this present survey with a rising titre of R.S.V. antibodies, only two had rising titres of measles antibody. Both these children had pneumonia and one had a very recent history of measles. However, the second child had no rash, but the siblings at home had measles. It is possible to suggest either an antigenic relationship or a dual infection in these two children, but we had a third child with a rising antibody titre to measles (1/2–1/16) and a falling titre to R.S.V. (1/128–1/32). The history was that of a serious respiratory infection a few weeks previously which resolved, to be followed by the present acute pneumonia. These findings suggest that on occasions measles virus can cause pneumonia without rash, an observation previously made by Enders *et al.* (1959). Examination of four pairs of sera from measles-susceptible children who were given measles vaccine showed no increase in antibody titre to R.S.V. though the measles antibodies showed a good increase.

Cross-infection occurs in institutions, and the importance of R.S.V. in relation to this needs to be fully investigated. It probably played no great part in these children, as most were under 1 year of age and therefore barrier-nursed. Table III shows that six children had dual infections. Of those infants barrier-nursed, only two out of 125 had serological evidence of dual infection, while four out of 23 children over the age of 1 had evidence of multiple infection. Although this may be suggestive of infection acquired in the wards, it is also consistent with multiple infection before admission. It might conceivably be due to an anamnestic response.

Our conclusion is that R.S.V. is the commonest viral cause of serious infection of the lower respiratory tract in infants and

young children in our community at the present time. After the first four weeks of life good antibody responses can be expected. It is also possible that in some years R.S.V. recedes in importance as a cause of respiratory infection and that adenovirus, influenza, and other viruses then play a bigger part. Finally there is apparently no close antigenic relationship between measles and R.S.V., though this point needs further investigation.

Summary

A serological investigation has been carried out on paired sera from 148 children over the last three years in Newcastle upon Tyne. The great majority of these children had serious infection of the lower respiratory tract, mainly bronchiolitis and pneumonia. Serological evidence is presented that about 60% of severe respiratory infections in the children are due to respiratory syncytial virus. The immunological responsiveness of infants to R.S.V. infection increases with age; this is directly related to the decrease in maternal antibody, which disappears by 3 months and is replaced by acquired antibody. Few of the older children showed antibody responses, and this coincided with high acquired antibody titres to R.S.V. Evidence is presented which suggests that an antigenic relationship between measles and R.S.V. is unlikely.

Professors S. D. M. Court and C. A. Green have been closely associated with the investigation. The hospitals taking part in the survey were: Royal Victoria Infirmary and Babies' Hospital, the Fleming Memorial Hospital, Walkergate Hospital, and Newcastle

General Hospital, and we are indebted to Drs. E. G. Brewis, G. Davison, W. D. Elliott, R. H. Jackson, and F. J. W. Miller for free access to patients under their care and for the use of clinical records. Our thanks are also due to Dr. E. G. Knox, Miss J. McQuillin, and Miss A. White for their assistance in a variety of ways. This work has been partly aided by a grant from the Scientific and Research Subcommittee, for which we are grateful.

REFERENCES

- Andrew, J. D. and Gardner, P. S. (1963). *Brit. med. J.*, **2**, 1447.
 Chanock, R. M., Hyun, W. K., Vargosko, A. J., Deleva, A., Johnson, K. M., Cumming, C., and Parrott, R. H. (1961). *J. Amer. med. Ass.*, **176**, 647.
 Enders, J. F., McCarthy, K., Mitus, A., and Cheatham, W. J. (1959). *New Engl. J. Med.*, **261**, 875.
 Fulton, F., and Dumbell, K. R. (1949). *J. gen. Microbiol.*, **3**, 97.
 Gardner, P. S., Stanfield, J. P., Wright, A. E., Court, S. D. M., and Green, C. A. (1960). *Brit. med. J.*, **1**, 1077.
 Hambling, M. H. (1964). *Ibid.*, **1**, 1223.
 Holzel, A., Parker, L., Patterson, W. H., White, L. L. R., Thompson, K. M., and Tobin, J. O'H. (1963). *Lancet*, **1**, 295.
 Jensen, K. E. (1962). In *American Review of Respiratory Disease, Conference on Newer Respiratory Disease Viruses*, p. 194.
 Johnson, K. M., Chanock, R. M., Rifkind, D., Kravetz, H. M., and Knight, V. (1961). *J. Amer. med. Ass.*, **176**, 663.
 Kravetz, H. M., Knight, V., Chanock, R. M., Morris, J. A., Johnson, K. M., Rifkind, D., and Utz, J. P. (1961). *Ibid.*, **176**, 657.
 McClelland, L., Hilleman, M. R., Hamparian, V. V., Ketler, A., Reilly, C. M., Cornfeld, D., and Stokes, J. (1961). *New Engl. J. Med.*, **264**, 1169.
 Moss, P. D., Adams, M. O., and Tobin, J. O'H. (1963). *Lancet*, **1**, 298.
 Parrott, R. H., Vargosko, A. J., Hyun, W. K., Cumming, C., Turner, H., Huebner, R. J., and Chanock, R. M. (1961). *J. Amer. med. Ass.*, **176**, 653.
 Sandiford, B. R., and Spencer, B. (1962). *Brit. med. J.*, **2**, 881.
 Smith, R. T. (1960). In *Ciba Foundation Symposium on Cellular Aspects of Immunity*, edited by G. E. W. Wolstenholme and M. O'Connor, p. 348. Churchill, London.

Folate Deficiency in Acute Tropical Sprue

W. O'BRIEN,* M.D., M.R.C.P.; N. W. J. ENGLAND,* M.B., B.SC., M.R.C.P.ED., M.C.PATH.

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Anaemia has long been recognized as a feature of tropical sprue. Manson (1879–80) wrote that when the disease is of some standing the patient is anaemic and sometimes the anaemia is profound. Thin (1897) noticed that the anaemia closely resembled pernicious anaemia, and Mackie and Fairley (1929) found megaloblasts at necropsy in the limb bones of two patients. Anaemia has been particularly prominent in the descriptions of sprue from the Caribbean, where the syndrome appears to have been prevalent in a population whose nutritional state was precarious. Rodriguez-Molina (1939) found macrocytic anaemia in 90 out of 100 patients, and Perez-Santiago and Butterworth (1957) described frank megaloblastic change in 71% and mild megaloblastic change in a further 23% of bone-marrow taken from another large series of Puerto Rican patients with tropical sprue. On the other hand, tropical sprue in British soldiers has not been associated with such a high incidence of anaemia. Macrocytic anaemia was reported in under a quarter of such patients in India. Marriott (1945), however, stated that though a sprue-like syndrome was seen in both British and Indian troops anaemia was almost confined to the Indians. Gardner (1960) thought that the incidence of anaemia merely reflected the duration of the disorder and the previous nutritional state of the patient.

Fairley (1932) found that the megaloblastic anaemia of tropical sprue responded well to liver. Rhoads and Miller (1934) and Castle *et al.* (1935) also demonstrated a full response

but warned that the dose of liver required may be much larger than that needed in pernicious anaemia. Spies *et al.* (1946) and Suárez *et al.* (1947) reported a spectacular haematological response to folic acid.

Low serum vitamin-B₁₂ levels were reported by Perez-Santiago and Butterworth (1957) and confirmed by others. Though there might be a moderate rise in these levels after a haematological response to folic acid, in many patients the serum vitamin-B₁₂ level remained low, often in the range seen in pernicious anaemia. Using small doses of folic acid and vitamin B₁₂ Sheehy *et al.* (1961b) found that some patients would not respond to folic acid but responded to vitamin B₁₂. Malabsorption of vitamin B₁₂ was demonstrated by Rodriguez-Rosado and Sheehy (1961) and Rivera and Bernabe Preda (1962). Sheehy *et al.* (1961a) concluded that malabsorption of vitamin B₁₂, a low serum level, and a haematological response to that vitamin constituted evidence of vitamin-B₁₂ deficiency in tropical sprue.

Mollin (1961), reporting on work jointly undertaken by him and Dr. C. C. Booth, described cases with chronic tropical sprue, severe megaloblastic anaemia, gross malabsorption of vitamin B₁₂, and a serum vitamin-B₁₂ level in the range seen in pernicious anaemia. There was a haematological response to folic acid, but vitamin B₁₂ malabsorption and the low serum level remained unaffected. One patient later had a haematological relapse with evidence of damage to the central nervous system. There was a satisfactory response to vitamin B₁₂, though initially this was somewhat slower than in uncom-

* Lieutenant-Colonel, R.A.M.C., British Military Hospital, Singapore.