OUTBREAK OF ADENOVIRUS INFECTION IN THE PORTSMOUTH NAVAL COMMAND, 1958

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The Commission on Acute Respiratory Diseases of the United States Army (1946) postulated the existence of a special disease of the upper respiratory tract affecting chiefly young recruits during their first four weeks of military training. A virus agent was eventually isolated by Hilleman and Werner (1954) from cases of atypical pneumonia and acute respiratory disease, and found to be similar to a "masked" virus recovered from adenoid tissue removed at operation from young children (Rowe *et al.*, 1953).

Adenovirus infection in military recruits causes febrile upper respiratory catarrh with sore throat and cough. About half the patients develop an exudate on the tonsils, and up to 16% may show radiological evidence of pneumonitis (Hilleman, 1956). These infections are of great importance to American Service authorities, and their cost has been estimated at nine million dollars a year.

Outbreaks in this country have been reported from Army and R.A.F. establishments (Tyrrell *et al.*, 1956; Andrews *et al.*, 1956) and from residential schools (Kendall *et al.*, 1957).

The virus has at least seventeen serotypes (Rowe *et al.*, 1958), and to avoid confusion the term "adenovirus" was proposed to cover the group. Types 3, 4, and 7 are responsible for most military cases (Hilleman, 1956); types 1, 2, and 5 cause respiratory infections in young children; and types 6, 8, and 10 are mainly implicated in eye infections (*J. Amer. Med. Ass.*, 1957).

The outbreak reported here became manifest in the Portsmouth Naval Command in February, 1958. It was soon realized that this outbreak was larger than any previously reported from Service sources in this country, and it was decided to investigate its effect on the personnel in five of the larger establishments (A, B, C, D, and E).

Laboratory Methods

Paired sera were taken in the first ten days of illness and two weeks or more later. They were taken routinely from patients admitted to hospital with upper respiratory infections from January and from patients in sick quarters in centres A, B, and C from February until April.

Convalescent sera, taken after the first ten days of illness, were obtained from patients in centres A, B, C, D, and E. They were frozen until tested by complement-fixation against antigens of influenza A, B, and C, *Rickettsia burneti*, adenovirus, and the psittacosis-L.G.V. group of viruses described by Andrews *et al.* (1956).

These antigens were obtained from the Standards Laboratory for Serological Reagents, Colindale. The tests were performed in "perspex" plates of the type recommended for haemagglutination tests (W.H.O., 1953). 0.1 ml. each of serum dilution, antigen at optimal dilution, and complement ($2\frac{1}{2}$ M.H.D.) were mixed and incubated for 75 minutes at 37° C. 0.2 ml. of a 1% suspension of sensitized sheep erythrocytes was then added and incubated at 37° C. for a further 30 minutes, when the plates were cooled to 4° C., left overnight at that temperature, and read. The serum titre was the highest initial serum dilution which showed 75% or more red cells unlysed.

With paired sera a fourfold or greater rise in titre between the acute and convalescent samples was regarded as diagnostic. With a single convalescent serum, obtained from establishments where diagnostic rises in titre were found, a titre of 1 in 20 or greater was considered significant. Titres below 1 in 20 were regarded as negative.

Specimens of faeces, and throat swabs taken by a method similar to that described by Hawkins *et al.* (1956), were frozen. When required for testing, the specimens were thawed and 0.2 ml. of the fluid was inoculated into stationary HeLa cell tissue cultures in a maintenance medium comprising 0.5% lactalbumin in Hanks's buffered salt solution with added 4% fowl serum and enough penicillin and streptomycin to give a final concentration of 100 units of each per millilitre. The tubes were observed for seven days without change of medium or until degeneration occurred, and 0.2 ml. of the fluid was inoculated into fresh tubes and the process repeated until 21 days had elapsed.

Virus isolations were confirmed by complement-fixation tests, using the culture fluids as antigens and a known pool of positive human serum. Neutralization tests were performed in HeLa cell tissue cultures, using rabbit antisera prepared against adenovirus types 1 to 8.

Results

From 511 patients admitted to hospital between January and July 445 pairs of sera were obtained. An analysis of the laboratory findings in these patients is shown in Table I. In addition, 80 pairs of sera and 373

TABLE I.—*Monthly Analysis of Laboratory Findings in Patients Admitted to Upper Respiratory Wards, 1958*

	Jan.	Feb.	Mar.	Apr.	May	June	July	Total
No. of patients admitted	80	111	127	67	51	32	43	511
Streptococcal and Vincent's infection	13	20	9	12	11	10	19	94
Glandular fever	1	2	—	3	4	1	2	13
No. of paired sera tested	61	103	126	57	39	29	30	445
Influenza A	22	8	—	2	—	—	—	32
Adenovirus	3	36	82	18	9	2	—	150
Influenza C	1	1	1	1	—	—	—	4
Influenza A and C	1	—	—	—	—	—	—	1
Influenza A and adenovirus	—	1	1	—	—	—	—	2
Q Fever	1	—	1	—	—	—	—	2
Negative	33	57	41	36	30	27	30	254

single convalescent sera were obtained from patients in centres A to E. All 525 pairs of sera were tested, and 201 were found to have rising titres against adenovirus. The distribution of these 201 rising titres is set out in Table II. The distribution of titres against adenovirus found in the 373 single convalescent sera is shown in Table III. In addition, over 900 sera taken from

TABLE II.—Distribution of Fourfold or Greater Rises in Titre Against Adenovirus Found in 201 of 525 Pairs of Sera Tested

	<1/2-1/8	<1/2-1/16	1/2-1/8	1/2-1/16	1/4-1/16	<1/5-1/10	<1/5-1/20	<1/5-1/40 or Greater	1/5-1/20	1/5-1/40 or Greater	1/10-1/40 or Greater
January ..		1			1	3	1	20		1	
February ..	1	1	1	1		23	4	48	2	2	2
March ..						4	36	9	1	16	4
April ..						3	4	9	1	1	
May ..						2	2	4			1
June ..						1		1			
Total ..	2	2	2	2	1	32	47	82	4	20	7

TABLE III.—Distribution of Titres Against Adenovirus Found in 373 Single Convalescent Sera

C.F. Titre against Adenovirus	<1/10	1/10	1/20	1/40 or Greater	Total
No. of single convalescent sera	90	73	81	129	373
% of single convalescent sera	24	20	22	35	101

civilians in the area during this outbreak and 767 pairs of sera from this Command in 1957 were examined. Less than 5% of these had titres of 1 in 10 or greater.

Adenovirus type 3 was isolated from 3 of 40 throat swabs and 1 of 16 specimens of faeces taken in the first three days of illness from patients subsequently found serologically to have had adenovirus infection.

Seven patients with streptococcal or Vincent's infection were also serologically positive for adenovirus.

Nearly all the patients admitted to hospital complained of sore throat and cough and were febrile. They appeared more distressed than those seen in the previous six months, when an outbreak of influenza A had occurred (Fraser *et al.*, 1958).

For 132 consecutive cases of serologically proved adenovirus infection the average stay in hospital was 8.1 days, as compared with 5.2 days for the influenza outbreak. The average age of the patients was 19 years, with extremes of 16 and 39, and the average length of service in the Navy was 1 year and 9 months, with extremes of 1 month and 15 years. An analysis of their symptoms and complications is given in Table IV.

TABLE IV.—Analysis of Clinical Findings in 132 Consecutive Cases of Confirmed Adenovirus Infection

Total cases	132
Sore throat	129 (98%)
Cough	95 (73%)
Exudate on tonsils	17 (13%)
Clinical pneumonia	8 (6%)
Radiological evidence of pneumonitis on discharge	7 (5%)
Gastro-enteritis	7 (5%)
Stevens-Johnson syndrome	2 (1%)

One case of otitis media and one of sinusitis were seen. Two patients had angina and ulceration of the mouth and eventually progressed to the full picture of the Stevens-Johnson syndrome. Despite extreme chemosis of the conjunctivae there was no photophobia or corneal scarring. Resolution was complete in both patients.

During the investigation 4 out of 12 cases of pneumonia admitted to the general medical wards were proved to be adenovirus infections. Three of eight cases of suspected tuberculosis found by mass miniature radiography were also shown to have adenovirus infections.

The ward routine and treatment were unchanged from the influenza outbreak of 1957, previously mentioned (Fraser *et al.*, 1958). As before, antibiotics were given

only for complications. The two cases of Stevens-Johnson syndrome were treated with cortisone and oxytetracycline, and after a week on this treatment nystatin was added to suppress a superimposed monilial infection.

The 511 patients admitted to hospital between January and July suffering from febrile upper respiratory illnesses came from all parts of the Command.

Centres A to E largely represent the different living conditions and drafting arrangements found in the

FIG. 1.—Weekly pattern of respiratory illnesses in centre A. Number of fresh cases reported to Saturday of each week. x—x, Acute respiratory disease. ●—●, Serologically confirmed cases of adenovirus infection.

FIG. 2.—Analysis of respiratory illnesses related to length of stay in centre A. White columns show percentage of each class attacked by acute respiratory disease (all forms). Black columns show percentage of each class attacked by adenovirus infection, as determined serologically.

Command. It was possible to obtain records of those reporting sick with acute respiratory disease in each of them, and, as already stated, a further 80 paired sera and 373 single convalescent sera were taken and tested.

Centre A was a boys' training establishment where recruits joined in groups at about five-weeks intervals. They remained together for the first few weeks and subsequently mixed with the older residents. Their ages ranged from 15 to 16 years. Of 662 boys at risk during the spring term (January to March), 262 had an upper respiratory infection, 149 of these cases being considered serologically to be due to adenovirus.

Upper respiratory illnesses accounted for 1,301 days' sickness, 825 of which were thought to be due to adenovirus infection. The number of patients with respiratory illnesses who reported each week at Centre A is shown in Fig. 1.

Adenovirus infection in military life has hitherto been regarded as a hazard of the very new recruit (Hilleman, 1956). Fig. 2 shows the incidence of infection related to the length of stay in this recruit centre. It is seen that length of service up to 12 months had no apparent effect on the proportion of patients suffering from adenovirus infection.

Centre B has several courses of training, and each course remains as a unit for up to several years although there is a common meeting-ground in the canteen and cinema.

In one group of 379 there were 56 upper respiratory infections, of which 47 were found serologically to be due to adenovirus. In another group of 602, of 84 cases of infection 55 were shown to be due to adenovirus. Upper respiratory infections accounted for 666 days' sickness, of which 470 were due to adenovirus.

Centre C takes young men from other establishments for specialized training. During the quarter, 86 upper respiratory infections were reported, of which 41 were found serologically to be due to adenovirus. Upper respiratory infections accounted for 458 days' sickness, of which 261 were due to adenovirus.

Centre D is a ship where, during March, 23 of 42 upper respiratory infections were considered serologically to be due to adenovirus. Upper respiratory infections caused 234 days' sickness, of which 146 were due to adenovirus.

Centre E holds several short training courses, and there is considerable movement of personnel in and out of the establishment. Serological evidence of adenovirus infection was found in 20 out of 28 cases examined.

Discussion

Although adenoviruses can be isolated from about half the tonsils and adenoids removed surgically from children (Rowe *et al.*, 1953), they appear to cause little respiratory illness in the civil population. In a study of respiratory infections in families (Jordan *et al.*, 1956) very few cases were found. Similar results were obtained from a student health service (Evans, 1957). Outbreaks of moderate severity have occurred in residential schools, but, in general, adenovirus infections have been regarded as diseases of the Services, particularly of recruit units.

From Table I it will be seen that influenza in this Command had abated by the end of February. The

adenovirus outbreak which began in February lasted until June. Most cases were seen in February and March.

The appearance of several foci of adenovirus infection within a short period of time suggested that the outbreak had arisen *de novo*; however, examination of the hospital records for 1957 showed that fourfold or greater rises in titre were found in 5 out of 767 pairs of sera tested. Although this suggests that the infection may have been present in latent form, it is not justifiable to assume that the same type was responsible for the sporadic cases in 1957 and the large numbers of cases this year, since no virus was isolated until the present outbreak.

The figures obtained from centres A to E represent bed cases only. Since many patients were treated as ambulant cases or at home these figures underestimate the real incidence by about a half, as suggested by the numbers treated on the attending list.

This outbreak did not seem to follow the pattern reported in the U.S. Armed Forces (Hilleman, 1956), in that infection was widespread throughout the Command and cases occurred in all age groups of a predominantly young population. It did, however, resemble that described by Munro-Ashman *et al.* (1958) in that, although type 3 adenovirus was presumed to be the cause in both, conjunctivitis was seldom seen. No similar outbreaks were reported from the rest of the country during the period.

As reported above, examination of 767 pairs of sera from this Command in 1957 and over 900 civilian sera from this area during the outbreak showed that a titre of 1 in 10 or greater against adenovirus occurred in less than 5%. It was in view of this that titres of 1 in 20 or greater in single convalescent sera were considered significant if obtained from centres where fourfold or greater rises in titres had also been found.

The low isolation rate of the virus from throat swabs and faecal specimens may have been due to the storage of samples above -20°C ., which may have led to loss of virus. Medication of the throats with gargles four hours before sampling may have had a similar effect. Subsequent tests with the strains from this outbreak did not indicate that the maintenance medium was inhibitory.

Summary

The influenza A (Asian) epidemic of 1957 in this Command ended in February, 1958. It was followed by a widespread outbreak of adenovirus infection.

The chief symptoms were febrile sore throat with cough. Of 525 patients whose paired sera were tested, 201 showed fourfold or greater rises in titre, and evidence of recent infection was found in 210 of 373 patients whose single convalescent sera showed titres of 1 in 20 or greater. Adenovirus type 3 was isolated from throat swabs of three patients and from the faeces of another.

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ANTIBODIES TO ASIAN INFLUENZA AND INFLUENZA B: EDINBURGH, 1958

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The pandemic of Asian influenza reached England in August, 1957, and spread to the north by September. In Scotland experience of the infection was heavy, as is shown by returns of the Regional National Insurance Offices for increased claims for sickness benefit during that period. A baseline for claims is estimated on the average for the months from April to December of the previous year, and any rise of more than 250% over that baseline or of more than 30% over the previous week is considered significant. There was a marked general increase in claims throughout the country over the weeks from September 10 to November 5. During this time the figures in Scotland peaked to 613% over the baseline, N.W. England being next with 526%, and S.W. England with 525% (Brunyate, 1958).

It was of interest, therefore, to estimate by means of haemagglutination inhibition tests the level of antibodies against the Asian influenza virus in a cross-section of the population living in the Edinburgh area. Information about the extent of the infection in the community during the epidemic in the autumn of 1957 would make it possible to assess the level of susceptibility should a second wave occur in the spring of 1959. At the same time the collected sera were tested for antibody titre against influenza B virus which had been responsible for an epidemic in Edinburgh in December, 1954.

Materials and Methods

Sera.—For our purpose it was felt that sera from blood donors would be reasonably representative of the adult community. These were collected for us through the courtesy of the Blood Transfusion Service (S.E. Scotland). The bacteriology department of the Royal

Infirmery, Edinburgh, supplied sera from the antenatal clinics and V.D. department and from patients in the medical and surgical wards. Small numbers of sera were also obtained from the City Fever Hospital and from general practitioners. Our aim was to collect 100 samples of sera (50 male, 50 female) in each 5-year age group from birth to 80 years of age during August and September, 1958. For the ages between 16 and 60 this was easily accomplished, but below and above these ages only a proportion of the numbers aimed at was achieved. In addition to the samples from the general community, specimens of sera were collected from nursing staff whose histories regarding Asian influenza were known and from cases of chronic bronchitis, many of whom had been under medical supervision during the previous year.

Treatment.—The sera were stored at -30° C. until required. To prevent bacterial contamination, penicillin and streptomycin were added to them (1,000 units of each per ml. of serum approximately). Before use the sera were treated with potassium metaperiodate (KIO_4) to remove non-specific inhibitors (Svedmyr, 1949; Lim *et al.*, 1957).

Virus.—Two strains of influenza virus were used—namely, Asian influenza A/England/341/57 isolated in our own laboratory, and influenza B (Lee). The antigens were prepared from the allantoic fluid of 11-day-old chick embryos.

Haemagglutination inhibition tests were carried out by a modification of the World Health Organization's plastic plate technique (Lépine, 1954). 0.2 ml. of virus containing four haemagglutinating doses was added to 0.2 ml. of serum, and to this was added 0.2 ml. of a 0.25% suspension in saline of fowl R.B.C.s. The titres of the sera were expressed as the reciprocals of the highest dilution of serum giving *complete inhibition* of haemagglutination. The lowest dilution of serum used was 1:8. The usual controls were set up with each batch of sera tested.

Results

The results are given (1) as the percentage experience of infection as gauged by the proportion with positive sera in a dilution of 1:8 or over; and (2) as the arithmetic means of the reciprocals of the antibody titres for each age group (Tables I and II, Figs. 1 to 4). The graphs for the percentages with demonstrable antibody in each age group fit reasonably well into those showing the average titre distribution. Results were calculated separately for men and for women, as it was thought that there might be a difference in experience of infection due to sex or environment. No significant difference between the two sexes was found.

Apart from the special groups of nurses and chronic bronchitics, 1,187 sera were examined for antibodies to Asian influenza virus A, and positive haemagglutination inhibition tests were obtained in 413, or 34.8%, with a range of from 14 to 69% in the different age groups.

The findings (Table I; Figs. 1 and 2) of the percentage experience and of antibody titre to Asian influenza virus show a high level in the younger age groups (0–15 years), although the total number of children in these age groups is small. However, the data correspond with the evidence that the incidence of influenza is at all times highest in the younger age groups (Hennessy, Davenport, and Francis, 1955). Thereafter the antibody titre falls to almost its lowest level between 25 and 40