assessment of all relevant facts which might lead to the formulation of relative or absolute survival rates. Furthermore, our knowledge of such things as average life expectancy and life expectancy of untreated patients with particular forms of cancer is so incomplete that such elegant studies are impossible at this time.

It is hoped that efforts to operate a minimal follow-up system will enable us to complete this study along these lines.

**Summary**

A review of 110 cases of oral cancer treated at the radiotherapy department of the Port Moresby General Hospital was made. The incidence of oral cancer in patients reaching hospital in Papua and New Guinea is higher than is found in European communities, but the overall incidence may be lower than that in countries of South-east Asia, for instance, where tobacco is chewed with betel nut.

The age and sex incidence of the condition in our patients differs from those found in European communities, but there is a similarity with figures from the countries of South-east Asia.

The relatively high incidence of oral cancer compared with other cancers is thought to be due partly to easy detectability of the cancer in an accessible site. Properly conducted clinical studies might throw light on environmental and racial factors in the development of oral tumours.

This paper is published with the permission of Dr. R. F. R. Scragg, Director of Public Health, Territory of Papua and New Guinea.

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# INFECTION WITH EATON'S PRIMARY ATYPICAL PNEUMONIA AGENT IN ENGLAND

BY

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Studies during the last ten years have done much to reinforce the conclusions of Eaton and his colleagues (Eaton *et al.*, 1944 ; Eaton and van Herick, 1947 ; Eaton, 1950) that an organism isolated from a patient with pneumonia in California in 1944 is a cause of various forms of respiratory illness ranging in severity from febrile bronchitis to that form of primary atypical pneumonia in which a proportion of patients develop agglutinins for *Streptococcus MG* or cold agglutinins against human erythrocytes (Liu *et al.*, 1956, 1959 ; Cook *et al.*, 1960 ; Chanock *et al.*, 1961a, 1961b ; Clyde *et al.*, 1961 ; Evans and Brobst, 1961).

Eaton and Liu (1957) expressed no final opinion about the nature of their organism, but in recent years it has frequently been referred to as a virus. However, a reconsideration of its properties—namely, its size, sensitivity to broad-spectrum antibiotics and an organic gold compound, the type of pneumonia produced in the hamster lung, and the presence in infected chick-embryo lungs or tissue cultures of coccobacillary bodies stained by an intensified Giemsa method—led to the view that the organism is not a virus but is related to the pleuropneumonia-like (*Mycoplasma*) group of organisms (Marmion and Goodburn, 1961 ; Clyde, 1961 ; Goodburn and Marmion, 1962a, 1962b). Substantiation of this view has been provided recently by Chanock *et al.* (1962a), when they cultured on agar a pleuropneumonia-like organism from one of the standard strains of Eaton's agent which had been maintained in eggs and tissue culture and showed that the colonies reacted specifically by immunofluorescence with convalescent-phase, but not with acute-phase, sera from cases of atypical pneumonia or with antisera against various other pleuropneumonia organisms, including some from chickens and contaminated tissue cultures.

The purpose of this paper is to show that infection with Eaton's agent occurs in Britain by describing the clinical and serological findings in some representative sporadic cases and in those of a small institutional outbreak. In passing, evidence is also provided which supports the claim (Chanock *et al.*, 1962b) that the organisms propagated in chick embryos and in cell-free media (P.P.L.O. agar) are antigenically related.

## Materials and Methods

Specimens of sera were taken from those submitted for routine virus diagnostic tests. They were selected either because of a history suggesting atypical or "virus" pneumonia or because of a positive cold haem-agglutinin or *Streptococcus MG* agglutinin reaction in the absence of serological evidence of infection with myxoviruses, psittacosis, Q fever, or adenoviruses. In addition, there were sera from seven cases of infection of the lower respiratory tract in a boy's boarding-school. A description of this school, consisting of about 370 boys

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between the ages of 12 and 18 years, and the methods of study of respiratory illness among the pupils has been given elsewhere (Kendall *et al.*, 1962).

Indirect fluorescent antibody staining followed the method of Liu (1957), and has been described in detail elsewhere (Goodburn and Marmion, 1962b). Briefly, serum titrations were done by covering cold microtome sections of infected chick-embryo lung (Hetter or Mac strains) with each of a series of serum dilutions (in 1/10 guinea-pig complement) for 60 minutes at 37° C., then washing, covering with fluorescein-conjugated anti-human globulin for 30 minutes at 37° C., washing again, and finally mounting in buffered glycerol, and examining in the ultra-violet microscope. Fig. 1 illustrates a typical result by this method. All the sera from one patient were titrated at the same time on sections from the same chick embryo, and a control titration with a standard serum of known staining titre was always included as part of the test.

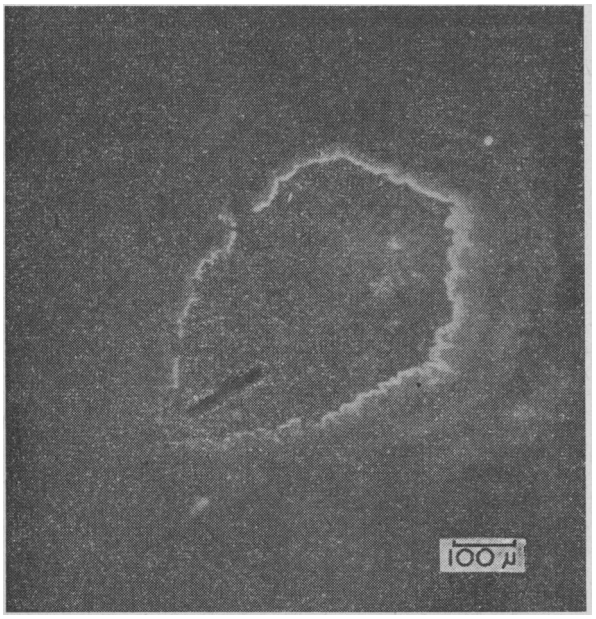


FIG. 1.—Mesobronchus in chick-embryo lung section infected with the "Hetter" strain of Eaton's agent and stained by the indirect fluorescent antibody method with a pool of convalescent sera from English cases of atypical pneumonia. The bright line at the surface of the epithelial cells marks the site of absorption of antibody and fluorescein-conjugated antihuman globulin. (Figure reproduced by permission of E. and S. Livingstone, Edinburgh.)

*Streptococcus MG* agglutination tests, when done at Leeds, were performed by the method of Feller and Hilleman (1956), using suspensions and control serum from the Standards Laboratory, Colindale. Cold haem-agglutinins at Leeds were tested by a rapid method. One drop of serum was mixed with one drop of a 1% suspension of human group O erythrocytes in plastic trays and the presence or absence of agglutination determined after one hour at 4° C. When positive the sera were titrated out, doubling dilutions of serum being mixed with equal volumes of 1% erythrocyte suspension and placed at 4° C. for one hour. Reversibility of the agglutination was confirmed by subsequent incubation of the tubes at 37° C.

Complement-fixing (C.F.) antigen was prepared at the Lister Institute, London, by Dr. R. M. Lemcke by the method of Card (1959) and Lemcke (1961) from the agar-adapted line of the Hetter strain of Eaton's agent (Chanock *et al.*, 1962b), kindly provided by Dr. L. Hayflick (Wistar Institute, Philadelphia). The washed and concentrated suspension of organisms was titrated at Leeds for C.F. antigen in "chessboard" fashion against a convalescent-phase serum from a case of atypical pneumonia previously shown by immuno-fluorescence to have a high titre of antibody to Eaton's agent in chick-embryo lung sections, and also against a serum without such antibody. The C.F. system was that described by Stoker *et al.* (1955) for Q fever diagnosis. Two exact units of complement, determined in the absence of antigen, and overnight fixation at 4° C. were used. That dilution of antigen which gave the highest serum titre with the convalescent serum, but which was not anticomplementary, and did not react with the negative serum, was used to titrate the sera from the patients in the series. Antigens were in general somewhat anticomplementary, and the range of dilutions over which they showed specific activity was rather narrow. Accordingly the complement dose was also determined in the presence of a low dilution of antigen, and many of the sera were retested with this combination of strong antigen and complement. The results obtained were similar by both methods. Certain controls for specificity are described below as part of the results.

## Results

The serological findings suggesting infection with Eaton's agent in 16 representative sporadic cases occurring in the general population are summarized in Table I. together with the clinical diagnoses. With the exception of Case D, who apparently had a double infection with influenza virus A and Eaton's agent, all tests for infection with influenza and parainfluenza viruses, psittacosis, Q fever, adenoviruses, and for infectious mononucleosis were negative and so are not given in detail.

In general the clinical features of this selection of cases are similar to those in numerous reports in the literature and illustrate the variety of ways in which the condition may present. Thus there were some cases of undiagnosed fever, some with febrile bronchitis without pneumonia, and one (Case H) with severe pneumonia and haemolytic anaemia. Cases C, P, and V were part of household outbreaks of pneumonia or acute bronchitis, and antibody was demonstrated by immuno-fluorescence in some of the family contacts. All cases were diagnosed on rising antibody titres to Eaton's agent by the immunofluorescence method, and later tests with the Hetter C.F. antigen showed either high or rising antibody titres (some serum specimens, particularly the acute-phase samples, were by then exhausted, and unfortunately it was not possible to test all patients by both methods).

The specificity of the C.F. reactions was explored in various ways. A batch of 50 sera, made up of 41 samples from blood donors and nine sera showing true or false-positive Wassermann reactions, were tested with the Hetter antigen. Only one of the 50 samples had a titre as high as 1/128 and there was no consistent cross-reaction with the W.R.-positive sera. It is known that convalescent sera from some patients with this particular form of atypical pneumonia may contain "antibodies" which fix complement with a wide variety of dissimilar antigens (Eaton and Corey, 1942; Thomas *et al.*, 1943; Eaton, 1950). A number of the sera in the present series were tested, therefore, with a physically similar antigen made from an avian mycoplasma, *Mycoplasma*

*gallisepticum*, which was diluted to the same opacity as that prepared from the agar-line of the Hetter strain of Eaton's agent; but no fixation was observed with this unrelated antigen (see Table I). Lastly, a few high-titre sera were absorbed with concentrated *Streptococcus MG* suspension or with chick embryo or mouse-tissue powder. The former did not lower the C.F. antibody titre; the latter caused a slight reduction.

It was also of interest to find that infection with Eaton's agent has been present in England for at least 10 years, as a pool of convalescent sera from patients with positive *Streptococcus MG* reactions collected in 1950–1 contained antibody demonstrable both by immunofluorescence and by complement fixation. Lastly, the antigenic similarity of the agent causing illness in the United States and Holland is suggested by the findings with the sera from the American and Dutch cases (see Table I) kindly given to us by Dr. R. M. Chanock (National Institutes of Health, Bethesda) and by Dr. P. J. Hers (University of Leyden, Holland) respectively.

TABLE I.—*Serological Findings in 16 Cases of Infection with Eaton's P.A.P. Agent Occurring in the General Population in England*

| Case | Clinical Description | Duration of Illness | Reciprocal of Titre in Test for: Influenza A | Cold Agglutinins | *Str. M.G.* Agglutinins | Eaton's Agent, Fluor.* | Eaton's Agent, C.F.A.† |
|---|---|---|---|---|---|---|---|
| A | Persistent fever and cough | 9 days | — | <16 | 10 | 4 | × |
| | | 20 ,, | — | <16 | 80 | 32 | × |
| | | 27 ,, | <32 | <16 | ⩾80 | 64 | ⩾1,024 |
| B | Aspiration pneumonia | 10 ,, | — | — | 40 | 10 | <8 |
| | | 20 ,, | <16 | — | 40 | 30 | 64 |
| C | Virus pneumonia | 10 ,, | 16 | — | ⩾80 | 30 | × |
| | | 34 ,, | — | — | ⩾80 | 100 | × |
| | | 42 ,, | — | — | — | 100 | × |
| D | Pneumonia | 6 ,, | <8 | 16 | 10 | <5 | × |
| | | 19 ,, | — | 16 | 80 | — | — |
| | | 2 mths | 32 | 16 | 40 | 100 | 512 |
| E | Pneumonia with small effusion | 10 days | — | <16 | 20 | 10 | 512 |
| | | 25 ,, | — | <16 | 20 | 100 | 256 |
| | | 4 mths | <16 | <16 | 20 | 30 | 256 |
| F | Haemoptysis, tracheitis, fever and malaise | 4 days | — | <16 | <10 | 10 | 64 |
| | | 49 ,, | <32 | <16 | <10 | 50 | 256 |
| G | Febrile bronchitis then broncho-pneumonia | 3 ,, | 16 | 2 | <16 | 4 | 16‡ |
| | | 17 ,, | 16 | 128 | <16 | 16 | 32‡ |
| | | 34 ,, | 32 | 32 | <16 | 32 | 64‡ |
| H | Atypical pneumonia with haemolytic anaemia | 20 ,, | — | — | 40 | 8 | 2,048‡ |
| | | 27 ,, | <32 | — | 40 | ⩾64 | 8,192‡ |
| | | 2½ mths | <32 | — | 10 | 64 | 4,096 |
| O | Influenza-like fever, then pneumonia | 2 days | 16 | — | 40 | 5 | × |
| | | 14 ,, | 16 | — | 320 | 300 | × |
| | | 30 ,, | 16 | — | 320 | 300 | × |
| P | Atypical pneumonia | Pre-illness | 32 | — | 16 | 10 | × |
| | | 13 days | 32 | — | 16 | 300 | × |
| Q | Virus pneumonia | 6 ,, | <32 | <4 | <16 | 64 | × |
| | | 20 ,, | <32 | 128 | 128 | 512 | 4,096 |
| | | 39 ,, | — | — | — | — | 1,024 |
| R | ,, | 20 ,, | 32 | 32 | 64 | 256 | × |
| | | 26 ,, | 32 | 4 | 128 | 1,024 | ⩾4,096‡ |
| S | Atypical pneumonia | 6 ,, | 32 | 32 | 32 | <8 | 64 |
| | | 28 ,, | 32 | <16 | 16 | 64 | 16 |
| T | ,, | 15 ,, | 16 | 64 | 16 | 256 | 512‡ |
| | | 21 ,, | 32 | 512 | 64 | 1,024 | 4,096‡ |
| U | Undiagnosed fever | 3 ,, | — | — | <16 | 8 | × |
| | | 13 ,, | <32 | — | 32 | 8 | 32 |
| | | 43 ,, | — | — | 32 | 32 | 32 |
| V | Atypical pneumonia | 6 ,, | 16 | 4 | 16 | 4 | 32‡ |
| | | 13 ,, | 16 | 32 | 32 | ⩾64 | 512‡ |
| | | 20 ,, | 16 | 16 | 64 | ⩾64 | 512 |
| American case (Bethesda) | | Acute | — | — | — | <8 | <16‡ |
| | | Conv. | — | — | — | 128 | ⩾1,024‡ |
| Dutch case L.P.35 (Leyden) | | Conv. | — | — | — | 1,024 | ⩾1,024 |
| Pooled sera from *Str. M.G.* positive pneumonia (London, 1950–1) | | Conv. | — | — | ⩾80 | 512 | 640‡ |

* Antibody titration by immunofluorescence on sections of C.E. lung infected with Hetter or Mac strains of Eaton's agent.
† Complement-fixing antibody tests with antigen from agar-line of Hetter strain.
‡ Serum negative at 1/8 with *Myco. gallisepticum* antigen.
— Not tested.
× Serum specimen exhausted: not tested.

## Patients with Illness of Lower Respiratory Tract in the School

The first case of primary atypical pneumonia occurred on January 22, 1961, and was followed by three cases of infection of the lower respiratory tract about 17 days later. Three further cases occurred about three weeks after the latter group. Common colds and sore throats were fairly frequent throughout the term, which lasted from January 18 to April 4. There were also a few cases of otitis media, tracheitis, and bronchitis, but neither these infections nor those of the upper respiratory tract showed any particular grouping in time about the lower-respiratory-tract cases (see also Kendall *et al.*, 1962).

Table II shows the ages, clinical diagnoses, and serological findings in six cases with acute and convalescent sera. The seventh case, suffering from bronchiolitis, had a convalescent serum only; this had a titre of 1/64 to Eaton's agent by immunofluorescence. The principal symptoms and signs of these six cases are set out in Table III. Their illnesses were moderately severe, usually starting slowly, with development of symptoms such as headache, sweating, and malaise during the first week of illness. The temperature gradually rose to a maximum of 100–103° F. (37.8–39.4° C.) over this period, and then declined by lysis to normal levels by about the tenth day. Fine crepitations were heard in the chest in most cases at the height of the fever but remained localized and were never prominent. Rhonchi were also heard in some cases either alone or with crepitations. Chest *x*-ray films taken at this time showed a "pneumonitis" type of consolidation in four cases. Total white-cell counts ranged from 3,800 to 5,300/c.mm. Convalescence was slow, with persistent headache and cough, and patients were not fit to return to school in less than three to four weeks.

TABLE II.—*Serological Findings in Cases in School Outbreak of Lower-respiratory-tract Illness*

| Case | Age | Clinical Diagnosis | Duration of Illness | Reciprocal of Titre in Test for: Cold Agglutinins | *Str. M.G.* Agglutinins | Eaton's Agent, Fluor.* | Eaton's Agent, C.F.A.† |
|---|---|---|---|---|---|---|---|
| I | 14 | Atypical pneumonia. Erythema nodosum | 4 days | — | <5 | <8 | × |
| | | | 18 ,, | 320 | <5 | 16 | 256 |
| | | | 5 mths | — | — | 32 | 128 |
| J | 14 | Atypical pneumonia | 3 days | — | <5 | <8 | 8 |
| | | | 21 ,, | 640 | <5 | 16 | 128 |
| | | | 4 mths | — | — | 64 | 512 |
| K | 14 | Atypical pneumonia | 7 days | <8 | 10 | 8 | × |
| | | | 22 ,, | <8 | 60 | 64 | 256 |
| | | | 3½ mths | — | — | 64 | 512 |
| L | 13 | Acute bronchiolitis | 5 days | <8 | <5 | >8 | × |
| | | | 20 ,, | 64 | <5 | ⩾8 | × |
| | | | 28 ,, | — | — | 64 | 128 |
| M | 14 | Atypical pneumonia | 6 days | — | <5 | 16 | × |
| | | | 29 ,, | — | <5 | 256 | 256 |
| | | | 3 mths | — | — | 64 | 128 |
| N | 15 | Acute bronchitis | 13 days | — | 5 | 8 | 512 |
| | | | 27 ,, | 256 | 5 | 8 | 1,024 |
| | | | 3 mths | — | — | 64 | 512 |

* Antibody titration by immunofluorescence on sections of C.E. lung infected with Hetter or Mac strains of Eaton's agent.
† Complement-fixing antibody tests with antigen from agar-line of Hetter strain.
— Not tested.
× Serum specimen exhausted: not tested.

TABLE III.—*Clinical Features of Six Cases of Infection of the Lower Respiratory Tract Caused by Eaton's Agent*

| Type of Onset: | Case I | Case J | Case K | Case L | Case M | Case N |
|---|---|---|---|---|---|---|
| | Sudden | Gradual | Gradual | Gradual | Sudden | Gradual |
| Headache | + | + | + | 0 | + | + |
| Muscle aches | + | + | 0 | 0 | + | + |
| Shivering | + | + | 0 | + | + | 0 |
| Sweating | + | + | + | + | + | + |
| Nasal | + | + | + | + | + | + |
| Sore throat | + | + | + | + | + | + |
| Hoarseness | + | 0 | 0 | + | 0 | 0 |
| Cough | + | + | + | + | + | + |
| Gastro-intestinal symptoms | + | + | 0 | 0 | + | + |
| Throat changes (moderate) | + | 0 | 0 | 0 | + | + |
| Rhonchi | 0 | 0 | + | 0 | 0 | + |
| Crepitations | + | + | + | + | + | 0 |
| Fever max. (°F.) | 103 | 102·5 | 100·5 | 102 | 100·2 | 100 |
| ,, duration (days) | 9 | 11 | 6 | 10 | 9 | 8 |
| Evidence of consolidation *x*-ray of chest | RLZ | RLZ | RLZ | 0 | LLZ | 0 |

+ = Symptom or sign present. 0 = Absent. RLZ or LLZ = Pneumonitis in right or left lower zone.

## Case I

The patient was a boy aged 14 with primary atypical pneumonia and erythema nodosum. The onset was sudden, with fever, insomnia, and abdominal discomfort. On the second day cough developed and the abdominal pain persisted with some diarrhoea. On the third day crepitations were heard at the right base, but the upper respiratory tract was only slightly affected. Fever gradually increased to a maximum of 103° F. (39.4° C.) on the fifth day, when the cough became worse, being accompanied by pain in the right anterior chest. His nose was then blocked and the conjunctivae were flushed. Thereafter the temperature fell gradually, reaching normal on the ninth day of illness. During this period he improved slowly, but cough and signs in the chest did not disappear until he had been ill for two and a half weeks.

He was treated with sulphadimidine 4 g. daily from the second to the fifth day, followed by chlortetracycline 1 g. daily until the tenth day.

The total white-cell count was 3,800/c.mm. (neutrophils 44%, lymphocytes 53%) on the fifth day; a chest *x*-ray film taken on the eighth day showed an ill-defined area of consolidation at the right cardio-phrenic angle (Fig. 2); two weeks later the chest film was clear. Bacterial pathogens were not grown from nose and throat swabs taken on the third and fifth days of illness.

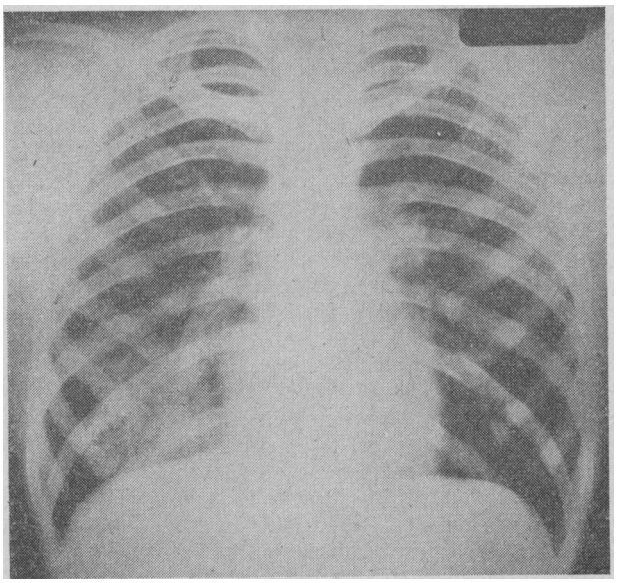

FIG. 2.—Photograph of chest *x*-ray film of Case 1 eight days after onset of illness, showing consolidation in right lower zone of lung

Six weeks after the beginning of his illness he developed a slight fever. The lesions of erythema nodosum appeared on the right shin. Fresh nodules continued to appear on both shins over a period of one week, during which time he felt tired and had a headache. Fourteen days after this episode of the illness the Mantoux test was negative at 1/1,000; the total white cells 6,700/c.mm. (neutrophils 46%, lymphocytes 53%, monocytes 1%); and the E.S.R. (Westergren) 10 mm. in the first hour. The chest remained clear radiologically.

## Case L

The patient was a boy aged 13 with acute bronchiolitis. His illness developed slowly with a slightly sore throat and a running nose. On the third day he felt feverish but had no further symptoms, though his temperature rose to 102° F. (38.9° C.). By the fourth day he had a slightly hoarse voice, a sore throat, and a cough. Both nose and throat were red, but no abnormal signs were present in the chest. On the fifth day his temperature had begun to fall, but he felt listless and appeared pale; crepitations were now audible at the right base. From this time he gradually improved, and his temperature was normal after the tenth day. Cough with sputum continued, however, for nearly three weeks from the onset of the illness. He was treated with chlortetracycline 1 g. daily from the fifth to the tenth day. Cultures of nose and throat swabs made on the fifth day grew no pathogenic bacteria. On the seventh day the white-cell count was 4,400/c.mm. (neutrophils 71%, lymphocytes 26%). On the same day an *x*-ray film of the chest showed no sign of consolidation, but there was some increase in the vascular shadowing at the right base. A second *x*-ray film made two and a half weeks from the onset of the illness showed clear lung fields.

## Comment

The laboratory finding in these 16 cases of infection with Eaton's P.A.P. agent in the general population and the six or seven cases in the small school outbreak serve to demonstrate the presence of the organism in this country, where it has been since 1951 at least.

The clinical features of these cases in Britain closely resemble those previously described in America. Cook *et al.* (1960), in a serological survey of American patients for infection with Eaton's agent, also mentioned some findings with a few patients in England.

In the present series the laboratory diagnosis was made principally by titrating sera on the Hetter or Mac strains in infected chick-embryo lung by immunofluorescence. Rising antibody titres were shown in all instances.

Complement-fixation tests with an antigen prepared from a pleuropneumonia-like organism (mycoplasma) isolated by Chanock *et al.* (1962a) from the Hetter strain of Eaton's organism showed reasonable agreement with the serological tests by immunofluorescence. The specificity of the C.F. method and its sensitivity compared with immunofluorescence remain to be assessed in detail. Since the investigation of the cases reported here routine testing of 112 patients with infection of the lower respiratory tract has yielded 12 (~10%) positive by complement-fixation (Marmion and Davies, unpublished). Chanock *et al.* (1962b) have reported favourably on their experience with a C.F. antigen.

Some other aspects of the recent American work with the organism have been confirmed, in collaboration with Dr. Hers and his colleagues (Leyden, Holland), by the isolation of mycoplasmas (P.P.L.O.) with the specific antigen from our sublines of the Hetter and Mac strains

of Eaton agent and from several recent Dutch isolates of the organism from patients with atypical pneumonia (Marmion and Hers, 1963).


### Summary

Sixteen sporadic cases in the general population and six cases in a small school outbreak of infections due to Eaton's primary atypical pneumonia agent are described with two characteristic clinical histories. The clinical features of these cases resemble those described in similar investigations in the United States. Thus patients had pneumonia, an acute febrile bronchitis or bronchiolitis, or fever of uncertain origin.

Diagnosis was made by serological methods, serum antibody being detected principally by immunofluorescent methods, using the indirect staining technique. The development of a complement-fixation test for the laboratory diagnosis of infections with Eaton's agent is described, the results of which appear to be in reasonable agreement with those obtained by immunofluorescent methods.

The antigenic similarity of agents causing similar illnesses in the United States, Holland, and England is shown, and evidence is presented that infection has been present in this country for at least twelve years.


We are grateful to many persons who sent serum specimens and clinical information about the sporadic cases. In particular to Dr. J. Boissard (Cambridge); Dr. G. K. H. Hodgkin (Redcar); Drs. E. C. Benn, Tattersall, T. Marmion, K. Zinnemann, J. V. Shone, all in Leeds; Dr. D. A. J. Tyrrell (Salisbury); Dr. J. E. M. Whitehead (Coventry); Dr. Denton Guest (Huddersfield); Dr. D. H. Garrow (London); and Dr. M. S. Pereira (Colindale, London). We are much indebted to Dr. Ruth M. Lemcke (Lister Institute) for preparing and passing on to us the antigen from the Hayflick strain of Eaton's agent.

In the investigation of the cases at Epsom College we were much helped by Dr. I. McKelvie, consultant radiologist, Epsom District Hospital, who took films of the chests and reported upon them for us; by Dr. G. T. Cook, Director, Public Health Laboratory, Guildford, who carried out the routine serological tests; by Dr. N. Richardson, Director, Department of Pathology, Epsom District Hospital, for the white-cell counts and cold agglutination tests; and also by Dr. D. Stone, late Director, Public Health Laboratory, Epsom, who carried out bacteriological tests.

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# TREATMENT OF LEAD-POISONING WITH ORAL PENICILLAMINE


BY

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Lead-poisoning is still a disease of importance in industry and an occasional hazard in childhood. Sodium calciumedetate (calcium disodium ethylenediaminetetraacetic acid; $CaNa_2E.D.T.A.$) was first used 10 years ago for the treatment of lead-poisoning; when given intravenously it induces a marked urinary excretion of lead. There are obvious disadvantages in the use of a parenteral preparation, especially in children, and renal damage has been reported after excessive dosage (Dudley *et al.*, 1955; Foreman *et al.*, 1956). An effective oral non-toxic lead chelator would be of considerable value in the treatment and possibly in the prophylaxis (Seignette *et al.*, 1959) of lead-poisoning. The success of penicillamine ($\beta\beta$-dimethylcysteine) as a copper chelator in Wilson's disease (Walshe, 1956) suggested that this drug might be used in lead-poisoning.

Boulding and Baker (1957) reported an increased urinary excretion of lead in two patients with lead-poisoning after oral penicillamine. Ohlsson (1962) reported the intravenous use of penicillamine in five patients with lead-poisoning. Weight for weight penicillamine given intravenously appeared to effect an excretion of urinary lead similar to intravenous sodium calciumedetate. In three cases penicillamine, when given orally, caused a moderate increase in urinary lead excretion. The present studies were carried out on nine patients with chronic or subacute lead intoxication to determine the effect of oral D-penicillamine, in varying dosage, on the excretion of lead, urinary coproporphyrin, and $\delta$-aminolaevulic acid, as well as on the clinical manifestations of the disease. The urinary excretion of coproporphyrin (Chisholm and Harrison, 1956) and of $\delta$-aminolaevulic acid (Haeger-Aronsen, 1960) provides sensitive biochemical indices of "metabolically active" and presumably toxic lead in tissues.

A further study was carried out for periods of 9 to 16 weeks on four oxyacetylene metal-burners working