is no residual cavitation at one year and combined chemotherapy if there is; and (*c*) chemoprophylaxis of high-risk groups.

During treatment bacteriological examinations are more informative than radiography, and progress can adequately be assessed by smear examinations, which are very cheap. Initially, cultures should be reserved for diagnostic purposes and sensitivity tests for the measurement of the prevalence of drug resistance in the community.

A consideration of the results of controlled comparisons of sanatorium and clinic treatment, of rest in bed and ambulation in sanatorium, of the relapse rates in patients, and of the risk to contacts, as well as the economics of sanatorium and clinic construction and treatment, leads to the conclusion that developing countries should concentrate on ambulatory domiciliary chemotherapy.

Expenditure on tuberculosis must be related to the overall health priorities of the community.

Although the views expressed in this lecture are my own, they have crystallized as a result of a period of several years of work as a W.H.O. field-staff member and from many conversations with Indian, W.H.O., and M.R.C. colleagues.

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Studies on Eaton PPLO Pneumonia*

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American investigators, using the fluorescent antibody (F.A.) technique, have found that Eaton infection is common and occurs in all age-groups throughout the year. Thus Chanock *et al.* (1960) established it as the causative agent in 16% of the pneumonia and bronchitis observed in 110 infants and children. In a further one-year study of an adult military population (Chanock *et al.*, 1961a; Chanock, 1962) Eaton infection accounted for half of the 530 pneumonia cases which were studied, and it was estimated that this agent causes an average of 10% of acute illnesses of the lower respiratory tract. The university student material of Evans and Brobst (1961) had an Eaton infection incidence of 24%. Finally, in their study of adult subjects with primary atypical pneumonia Cook *et al.* (1960) established a rise in Eaton F.A. titre in 85% of 26 patients with cold and/or *Streptococcus MG* agglutinins and in 26% of 69 patients without cold agglutinins.

When Chanock *et al.* (1962a) showed that Eaton agent could be grown on Difco PPLO culture medium and identified it as a member of the PPLO group (pleuropneumonia-like organisms), it became possible to study Eaton infection by the complement-fixation (C.F.) technique. According to Chanock *et al.* (1962a) the Eaton C.F. antigen is a specific and moderately sensitive reagent for serodiagnosis of Eaton infection. It was estimated to be 80% as sensitive as the earlier F.A. technique (Chanock *et al.*, 1962b).

This paper is a report on the results of our studies of Eaton C.F. antibodies in patients with pneumonia who were treated at Aurora Hospital, Helsinki, from September 1962 to April 1963.

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Material and Methods

Patients Diagnosed as Cases of Pneumonia.—The series comprised those patients admitted to the Aurora Hospital between mid-September 1962 and April 1963. This hospital receives the majority of the adult and child patients in Helsinki diagnosed as suffering from pneumonia and requiring hospital treatment. Specimens of blood were submitted from all such patients who were 3 years of age or over. (Samples were, in fact, taken from a few children under this age.) The first sample was taken as soon as possible after admission, and further samples were taken at roughly weekly intervals during the time in hospital. When the serum of a patient showed a C.F. titre ⩾ 32 against PPLO antigen he was asked to return at the end of the study in April 1963 so that we might obtain a final sample of blood. The total number of patients with a clinical diagnosis of pneumonia was 246.

Controls.—Sera from 484 blood donors were collected during April–May 1963 and submitted to the same test. In addition, samples of blood were examined from 118 children between the ages of 7 months and 15 years who had undergone treatment in the surgical wards of the Aurora Hospital during the period January–August 1962.

Serum Specimens.—In order to retain the cold agglutinins blood samples were not initially refrigerated. Immediately after separation the serum was divided into two aliquots, one of which was inactivated at 56° C. for 30 minutes. Both specimens were thereafter stored at 4° C. until the tests were applied. All sera taken from the same patient (apart from the final sample taken in April at the end of the study) were tested at the same time. The method used in preparing the Eaton PPLO C.F. antigen will be described elsewhere (Jansson, to be published).

Complement-fixation Technique.—The macro-complement-fixation technique reported in detail in our earlier paper was used for examination of C.F. antibodies against Eaton PPLO, adenovirus, and ornithosis virus. Two units of haemolysin, complement, and antigen were used in the main test. The fixation was allowed to occur by incubating overnight at 4° C. Differing from this earlier technique, the sera were diluted by twofold steps from 1:8 to 1:64. If antibodies were found in the titre ⩾ 32, the serum was retested, diluting it up to 1:512. The highest serum dilution (before addition of reagents) that showed moderate or no haemolysis was recorded as the titre.

Cold Agglutinins.—These were studied according to the method of Feller and Hilleman (1956).

Serological Criteria.—Those patients whose sera contained an Eaton C.F. titre ⩾ 8 were designated "Eaton positive." The designation "Eaton pneumonia" was applied to those patients who serologically during the acute illness fulfilled one or other of the following criteria: group 1, those with a fourfold or greater rise to a titre ⩾ 64; group 2, those with a fourfold or greater rise to a titre of 32; group 3, those with a titre in any sample ⩾ 64; group 4, those with a fourfold fall in titre from 32 whose first blood sample was taken more than two weeks after the onset of the illness. None of the patients in these four groups showed a significant rise of antibody to adenovirus or ornithosis. A further condition for inclusion in the Eaton-positive and Eaton-pneumonia groups was a negative reaction in the examination of the patient's serum with control antigen.

Patients were regarded as "cold-agglutinin-positive" who showed a titre ⩾ 32.

Results

The age distribution of all the persons involved in the study is shown in Table I. So far as the series of pneumonia patients was concerned, it will be seen that 103 (42%) were Eaton-positive by definition. The rate was slightly higher (52%) in those under 16 years than in the adults (31%). The distribution of titres was: 8 (35 cases), 16 (24 cases), ⩾ 32 (44 cases).

So far as the control subjects were concerned, it will be observed that 26 (5.4%) of the 484 blood donors were Eaton-positive—a low rate compared with that found in the adult pneumonia patients (31%); the titres were usually low, and in 24 fixation occurred only at 1:8. Among the 118 surgical patients there were 38 (32%) Eaton-positive, a rate which may be compared with that encountered in the children with pneumonia—namely, 52%. Here again the titres were rather low, however, being 1:8 in 29 and 1:32 in only 2 patients.

TABLE I.—*Age Distribution and Eaton Serology*

Age (Yrs)	Patients with Pneumonia					Surgical Patients			Blood Donors		
	Total	Eaton Pneumonia		Eaton-positive		Total	Eaton-positive		Total	Eaton-positive	
		No.	%	No.	%		No.	%		No.	%
<3	20	1	5	6	30	3	—	—			
3–5	36	1	3	11	31	14	5	36			
6–10	42	12	29	26	62	48	14	29			
11–15	29	11	38	23	79	53	19	36			
16–20	17	4	24	8	47				9	1	9·0
21–30	15	4	27	6	40				146	7	4·8
31–40	11	—	—	1	9				116	5	4·3
41–50	24	3	13	7	29				103	7	6·8
51–60	21	3	14	10	48				83	3	3·6
61–70	14	—	—	2	14				26	3	11·5
>70	17	1	6	3	18				1	—	—
	246	40	16	103	42	118	38	32	484	26	5·4

Eaton-positive = Eaton PPLO CF titre ⩾ 8.

It must of course be borne in mind that the sera from these control subjects were not taken contemporaneously with sera from the patients suffering from pneumonia. Although the differences observed were formally significant it is appreciated that a seasonal effect may have been a contributory factor.

Eaton Pneumonia Cases

From Table I it will be seen that 40 patients were so designated. Their subdivision into the four defined groups was as follows: group 1, 10 cases; group 2, 17 cases; group 3, 10 cases; group 4, 3 cases.

The relation of the peak antibody titre to the duration of illness is shown in Table II and the distribution of the patterns of antibody change in Table III. The peak antibody was usually observed in the second or third week after the onset of illness. Antibody titres thereafter fell rather slowly; for of 10 patients examined two to five months after onset seven were still positive; while of 17 examined five months or more afterwards 12 remained positive.

TABLE II.—*Peak Antibody Titre in Relation to Duration of Illness*

Antibody	No. of Patients with Peak Titre at (Days)						
	0–7	–14	–21	–28	–35	–42	Over 42
PPLO	1	14	16	7	1	1	0
Cold agglutinins*	0	14	16	3	0	0	1

* 6 patients showed no cold agglutinins (titre <4).

TABLE III.—*Pattern of Antibody Change in 40 Cases regarded as "Eaton Pneumonia"*

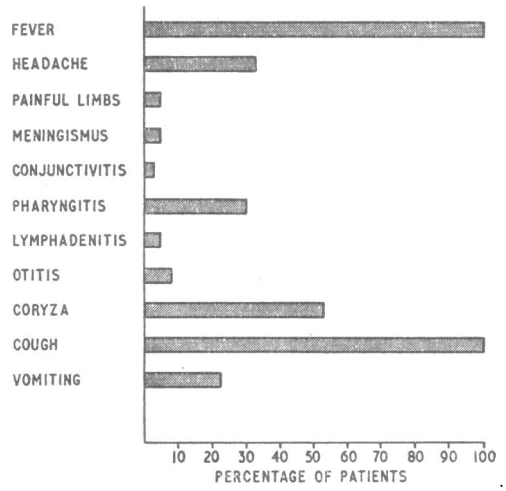
Pattern of Antibody Titre		No. of Patients
Acute Phase	Convalescent Phase	
<8	32	12
<8	64	3
<8	128	2
8	32	5
8	64	2
8	128	1
16	64	2
256	512	1
512	512	1
128	128	1
128	64	3
64	64	1
64	32	3
32	8	2
32	<8	1

From Table I it will be seen that these patients were observed in almost all age-groups. However, in those under 5 years the rate was 3.6% ; between 6 and 30 years the equivalent rate was 30% ; and in those over 30 years the rate was 8%. The peak incidence, therefore, was in older children and young adults.

Cold agglutinins in a titre of ≥ 32 were found in 27 (68%) of these patients. Among the 206 cases not classified as Eaton pneumonia cold agglutinins were present in 75 (36%). In all there were 102 patients who showed cold agglutinins, and of these 27 (26%) were classified as having Eaton pneumonia.

There was some indication that the cases were not evenly distributed throughout the study period. Thus 29 of the Eaton-pneumonia cases occurred in the first four months whereas there were only 11 in the second four months, representing rates of 21 and 10% respectively. This difference was reflected in the distribution of cases designated as Eaton-positive for which the equivalent rates were 55 and 25%.

The main *signs and symptoms* are shown in the Chart. There is nothing unusual in this clinical picture which would enable



Clinical symptoms of patients with Eaton pneumonia.

a diagnosis to be established without laboratory assistance. The erythrocyte sedimentation rate was usually rapid, being over 50 mm. in 25 patients and over 80 mm. in 6 patients. The pattern of white-blood-cell changes was not characteristic. Usually the total count was normal or low, and only two patients showed a count over 10,000/c.mm.

The distribution of *radiological changes* was as follows:

	Children	Adults	Total
Right lung	5	8	13
Left „	9	6	15
Both lungs	9	3	12

The changes noted were very variable. Thus in 17 patients the pattern was similar to that usually described as "atypical pneumonia." In a further 17, however, the picture was that of ordinary bronchopneumonia, and in the remainder a segmental lobar pneumonia was present. Atelectasis was a fairly common finding, and was seen in 27 patients. Lung collapse seemed rather commoner in the children. Enlargement of hilar glands was noted in 25 patients, and again this observation was more often made in children. The radiological changes resolved moderately rapidly and had disappeared in under three weeks in 31 of the patients.

So far as *chemotherapy* was concerned, the antibiotics used were penicillin (15 cases), chloramphenicol (9 cases), and tetracycline (19 cases). The duration of fever averaged between two and three days, and it was not possible to observe any difference between the effects of the three antibiotics. On the whole clinical recovery was slowly established and the average duration of the illness was about four weeks.

Family Outbreaks

Two instances of intrafamilial spread were established during the investigation. One member of each family belongs to the series. In one example both of the parents and their five daughters developed acute infection of the respiratory tract. One of the daughters was admitted to hospital with bilateral pneumonia and developed C.F. antibody to a final titre of 128. Eaton PPLO C.F. antibodies were demonstrated in six members of the family, the peak titres being 128 in one, 64 in two, 32 in one, and 16 in two.

The other example involved both parents and three sons. The children were all admitted to hospital. Two of them fulfilled the serological criteria for Eaton infection, attaining final antibody titres of 128 and 64. The former is included in the series, but the latter is excluded, since the final diagnosis was acute respiratory infection without pneumonia.

Discussion

The present study suggests that Eaton pneumonia occurs in this northernmost part of Europe. The results reported here are in general agreement with the American investigations as regards the incidence, age, and seasonal distribution. The clinical and radiological findings are also similar to those already reported from America (Mufson *et al.*, 1961). The relation of Eaton pneumonia to the presence of cold agglutinins is interesting. Seven of 17 patients with Eaton pneumonia did not develop any detectable cold agglutinin (Liu *et al.*, 1959). Kingston *et al.* (1961) found in a military population a rise in cold agglutinins in 47% of the cases with Eaton pneumonia and in 7% of those with non-Eaton pneumonia. Chanock *et al.* (1961b) came to the conclusion in their studies of volunteers that cases in which no Eaton antibodies were demonstrable prior to reinfection would seem to be cold-agglutinin-positive. In the present series only 26% of the cold-agglutinin-positive pneumonias established during the investigation period fulfilled the serological criteria for Eaton pneumonia. On the other hand, in 32% of the Eaton pneumonias no cold agglutinins were demonstrated. A detailed analysis of the occurrence of cold agglutinins in the present series will be published separately.

The finding of PPLO antibody in a high proportion of the pneumonia cases, in a considerable number of children with surgical illnesses, and even in a small proportion of adult blood donors would combine to suggest that Eaton infection is fairly common in Finland. The communicability of the infection is reflected in our two family outbreaks. No doubt a high proportion—possibly the majority—of the infections are mild or inapparent.

The present study emphasizes the significance of Eaton PPLO as a cause of pneumonia in Helsinki. Investigations carried out in the same hospital during 1958–60 showed that the ornithosis group of viruses were responsible for 5.6% of 539 cases of pneumonia ; while in a study of 517 cases 12% were established as adenovirus infections (Jansson, 1960 ; Jansson and Wager, 1961). The rate of 16% found for infection by Eaton's agent places it as a perhaps commoner agent—at least in the past year.

Summary

During the period September 1962 to April 1963 246 paired sera from hospitalized patients with pneumonia were studied with Eaton PPLO C.F. antigen. Forty cases were classified as Eaton pneumonia on the basis of their serological pattern. The incidence was 20% in children under 16 years of age and 13% in adults, with an average of 16%. The highest incidence was in the age-group 6–30 years. Most of the cases occurred during September–December 1962.

The peak Eaton PPLO C.F. antibody titre was generally recorded in the second or third week of illness, an average of

17 days from its onset. Twelve out of the 17 patients studied still had Eaton C.F. antibodies five months later.

The incidence of Eaton pneumonia in 102 cases with cold agglutinins in titre ≥ 32 was 26% and in 144 pneumonias without cold agglutinins 9%. Conversely, 68% of the Eaton pneumonias and 36% of non-Eaton pneumonias were cold-agglutinin-positive.

In two control groups, comprising 484 blood donors and 118 surgical patients aged under 16 years, Eaton antibodies in titre ≥ 8 were found in 5.4 and 32%. The corresponding figures for the same age-groups in the pneumonia series were 31 and 52%. These differences found between the pneumonia group and the control series were highly significant. None of the blood donors and only two of the surgical patients had an Eaton PPLO C.F. titre of 32.

It is to be hoped that the results obtained by studying the Eaton PPLO C.F. antibodies in patients with pneumonia will stimulate the investigation of the role of PPLO in other human diseases. The C.F. technique provides a simple method.

We wish to express our gratitude to Professor Thomas Anderson, Glasgow, for his criticism and valuable advice and for correcting the manuscript. We wish to thank Dr. Harri Nevanlinna, head of the Finnish Red Cross Blood Bank, for the blood donors' samples. We are also grateful to Dr. G. R. Wallgren, assistant head of the Children's Surgical Department of Aurora Hospital, for blood samples from surgical cases.

One of us (E. J.) had the opportunity in October 1962 in London of meeting Dr. E. Klieneberger-Nobel and Dr. R. Lemcke (Lister Institute) and discussing their PPLO methods. Dr. L. Hayflick (Wistar Institute, Philadelphia) was kind enough to send us the Eaton PPLO strain. We wish to express our sincere thanks to these three doctors.

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Sensitivities of Colonies and Suspensions of Actinomyces Israelii to Penicillins, Tetracyclines, and Erythromycin

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The usual method of assessing antibiotic sensitivity of *Actinomyces israelii* is the inoculation of serial dilutions of the antibiotic in liquid or semi-solid media with suspensions of *A. israelii* prepared from a pure culture. The inoculated tubes are incubated at 37° C., if necessary in an anaerobic atmosphere, until a no-growth level can be determined. Because of the slow growth of the organism, a minimum period of five days' incubation is usually required.

During the period of culture the activity of the antibiotic in the media may fall considerably. In one experiment with benzylpenicillin in a medium of brain–heart infusion in sloppy agar, incubated at 37° C., the penicillin activity (assessed by inhibition of a penicillin-sensitive staphylococcus) fell by more than 75% in five days (Fig. 1). With antibiotics which are labile in solution, therefore, organisms whose growth may have been inhibited during the first 24 hours of culture, but which remain viable, may begin to grow as the antibiotic activity falls, so that growth is eventually observed in tubes in which the true antibiotic concentrations are much below the original values. Minimum inhibitory concentrations found in this way are likely to be too high and may in some cases approach bactericidal levels.

A further difficulty in antibiotic testing of *A. israelii* arises from the apparent resistance to antibiotics of colonial masses of the organism (Holm, 1948). Because of this resistance the particle size of the suspension may affect the minimum inhibitory concentration (M.I.C.) found, and it is necessary to prepare fine homogeneous suspensions of the organism if accurate and constantly reproducible results are to be obtained.

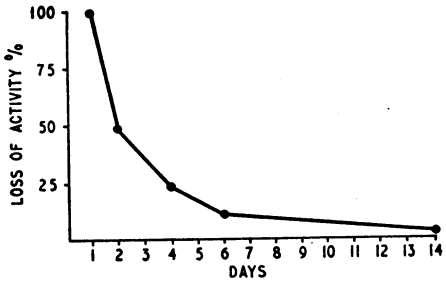

FIG. 1.—Loss of activity of benzylpenicillin in brain–heart infusion sloppy agar medium at 37° C. The assessment was calculated from minimum inhibitory concentrations against a sensitive staphylococcus, after 24 hours' incubation in each sample. The first reading, 24 hours after the beginning of the experiment, was 0.06 μg./ml. and is taken as 100% on the graph.

Different methods of preparing the suspensions have been employed by various workers, and the methods used may affect the result of the experiment. When suspensions of *A. israelii* (National Collection of Type Cultures (N.C.T.C.) No. 10236) were prepared by crushing the mycelial masses with a glass rod,

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