$$\chi_0 = 2\alpha_1, \quad \psi_0 = \frac{\lambda_3}{\omega_2^2 - \omega^2}\chi_0, \quad \dot{\chi}_0 = \dot{\psi}_0 = 0. \tag{18a}$$

Formula (18a) give the initial conditions required for the solution to be of "principal modes" type.

Appendix.—If in the elements of the sequence $\{\alpha_n\}$, $n = 0, \pm 1, \pm 2, \ldots$, where $\alpha_{-n} = \bar{\alpha}_n$, the element α_1, and then α_{-1}, is dominant, it is easily seen that the dominant sum of the double series of (3) is given by

$$3\alpha_1^2\alpha_{n-2} + 6|\alpha_1|^2\alpha_n + 3\bar{\alpha}_1^2\alpha_{n+2}, \tag{A_1}$$

where n is any integer except $n = \pm 1, \pm 3$. For these exceptions, the dominant terms of the double series are

$$3\bar{\alpha}_1\alpha_1^2 \text{ for } n = 1, \quad 3\alpha_1\bar{\alpha}_1^2 \text{ for } n = -1, \quad \alpha_1^3 \text{ for } n = 3, \bar{\alpha}_1^3 \text{ for } n = -3. \tag{A_2}$$

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RESPIRATORY DISEASE IN VOLUNTEERS INFECTED WITH EATON AGENT; A PRELIMINARY REPORT

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Recent epidemiologic studies have provided evidence that the filterable agent first described by Eaton in 1944 was associated with human respiratory disease[1-3] A controlled field study among Marine recruits suggested that the Eaton agent caused a spectrum of disease including febrile respiratory illness and atypical pneumonia.[3] In the same Marine recruit population a double blind controlled study indicated that demethylchlortetracycline was effective in the therapy of pneumonia associated with Eaton infection.[4] During the course of these studies 14 strains of the agent were recovered in monkey kidney tissue culture.[5]

As a further step in assessing the etiologic role of Eaton agent in respiratory disease, tissue culture grown material was administered to volunteers in an attempt to reproduce the natural disease. This approach was made possible by the availability of an effective therapeutic drug and freshly isolated strains of the agent. This communication will present a preliminary analysis of the Eaton volunteer study while subsequent reports will present the findings in a more detailed manner.

Materials and Methods.—Inoculum: The 898 strain of Eaton agent was recovered in monkey kidney tissue culture from a patient with nonbacterial pneumonia.[5] The tenth- and fourteenth-day fluid harvests from the second monkey kidney tissue culture passage were pooled and distributed in aliquots which were stored in glass sealed ampoules at −60°C. The pooled material contained 640 egg infectious doses per ml as determined by amniotic inoculation of 13-day embryonated eggs and immunofluorescent examination of surviving embryos after 6 days' incubation at 35°C. The inoculum was safety tested as previously described in monkey kidney, HEp-2, and rabbit kidney tissue culture as well as in rabbits, guinea pigs, and suckling and weanling mice.[6]

The inoculum was found to contain a small quantity (10TCID50 per ml) of SV40 virus, a common contaminant of rhesus monkey kidney tissue culture.[7] This virus was neutralized by specific antiserum when the inoculum was administered to the volunteers.

Recovery of Eaton agent: Attempts to recover the agent from the volunteers were made using monkey and human kidney tissue culture as described previously.[5] The tissue culture medium consisted of Eagle's basal medium and 5% inactivated chicken serum with penicillin (1,000 units/ml) as the only antibiotic. Fourteen- and 21-day culture fluids were inoculated into 13-day eggs which were incubated for 6 days at 35°C and then tested for the presence of specific antigen in the bronchial epithelium by the indirect fluorescent antibody method.

Serologic techniques: The fluorescent antibody technique was performed as described previously.[3] Cold agglutinins and Streptococcus MG agglutinins were measured by the method of Hilleman and Feller.[8]

Volunteers: An approximately equal number of men with or without detectable fluorescent stainable antibody were selected for study. These healthy adults (21–36 years of age) from the Federal prison system were isolated for 1 week before they were given 2 ml of a 1:2 dilution of tissue culture fluid containing strain 898 by coarse spray and instillation into the nose and mouth. Simultaneous titration of the final inoculum in eggs indicated that the men received approximately 640 egg infectious doses of Eaton agent. The technique whereby the men were isolated and observed for illness has been described previously.[6] Clinical examinations were performed twice daily by physicians who were unaware of the volunteers' pre-inoculation Eaton antibody status.

Results.—A total of 52 men received infectious tissue culture fluid. As seen in Table 1, all volunteers who did not possess fluorescent stainable antibody prior to

TABLE 1

INFECTION OF VOLUNTEERS WITH EATON AGENT*

Fluorescent antibody titer prior to challenge	Type of illness	No. of volunteers	Fourfold or Greater Rise in Antibody** Eaton (fluorescent)	Cold agglutinin	Strep. MG agglutinin
less than 1:10	Pneumonia†	3	3	3	1
	Otitis media‡	11	11	7	2
	Febrile upper respir. illness	2	2	1	0
	Afebrile upper respir. illness	4	4	1	0
	None	7	7	0	0
	Total	27	27	12	3
1:10 or greater	Otitis media	1	1	0	0
	Afebrile upper respir. illness	6	4	0	0
	None	18	12	0	0
	Total	25	17	0	0

* A febrile upper respiratory illness preceded the occurrence of pneumonia, otitis media, or febrile upper respiratory illness.
† One patient with pneumonia also had otitis media.
‡ Two patients with otitis media developed fever (38°C or >).
** Post challenge sera were collected on the 23rd or 24th day.

challenge developed that antibody 23 to 24 days after the agent was administered. Similarly, the majority of volunteers who had detectable antibody prior to challenge developed a rise in antibody. These findings suggest that the second tissue culture passage Eaton agent infected all seronegative volunteers and a majority of those individuals who possessed antibody prior to challenge. Isolation studies, although incomplete at present, also indicated a high rate of infection. The agent was not recovered from 21 men on the day after instillation, whereas it was recovered from 15 of these volunteers at some time between the 3rd and 13th day following inoculation.

Twenty of the seronegative volunteers and 7 of the men who had antibody prior to challenge developed mild upper respiratory illness after 4 to 9 days. Three individuals in the former group subsequently developed signs and symptoms of

pneumonia on the 9th to 13th day; fine moist rales were detected in each instance, whereas only 2 of the men became febrile (38°C or greater) and developed X-ray changes indicative of pulmonary infiltration. Four additional volunteers developed a febrile illness without evidence of pneumonia. An unanticipated finding was the occurrence of a moderately severe hemorrhagic otitis media which was characterized by the occurrence of bullous lesions of the tympanic membrane and which followed the onset of minor upper respiratory symptoms by several days. These illnesses will be described in greater detail in subsequent publications.

All but one of the 17 men who developed moderate to severe disease (pneumonia, otitis, or febrile respiratory illness) were free of detectable antibody at the time of challenge. The inverse relation between this degree of illness and pre-existing antibody suggests that the Eaton agent contained in the inoculum was responsible for the observed illnesses.

A cold agglutinin response was confined to those volunteers who were Eaton seronegative prior to challenge. Further, cold agglutinins developed predominantly in those individuals who had moderate to severe illness. Previous studies have shown that cold agglutinins and fluorescent stainable antibody are distinct and that the former correlates well with severity of illness.[1,9] As shown previously, cold agglutinins were less efficient for serodiagnosis than was the specific Eaton antibody.[3] Streptococcus MG agglutinins developed infrequently; this response was associated with one pneumonia illness and with two cases of otitis.

Discussion and Conclusions.—The findings in this study suggest that a recently isolated tissue culture strain of Eaton agent possessed the capacity to infect humans and to stimulate the production of antibody. All individuals who were free of fluorescent stainable antibody became infected and a majority developed pneumonia, otitis, or a febrile respiratory illness. Although a majority of individuals who possessed Eaton antibody at the time of inoculation became infected, the presence of such antibody appeared to protect against the occurrence of moderate to severe illness. This antibody-illness relationship strongly suggests that the Eaton agent itself was responsible for initiating the sequence of events which led to pneumonia, otitis, or febrile respiratory disease. Further, these data, together with the observations made during previous epidemiologic studies in which the agent was recovered from serologically positive individuals, support the specificity of the fluorescent staining reaction in patients with atypical pneumonia.[3]

Approximately 10 per cent of seronegative volunteers developed pneumonia. This proportion is somewhat greater than the estimate made for the occurrence of pneumonia in natural infection. During a study of Marine recruits, 83 per cent of whom were free of detectable antibody when they entered training, it was estimated that 30 infections occurred for each pneumonia which was recognized.[3] The present findings and the previous observations are consistent, however, when one considers that pneumonia is more apt to be recognized in a longitudinal study of volunteers than in men undergoing recruit training.

It is of some interest that mild upper respiratory illness occurred in a proportion of the volunteers who possessed prior Eaton antibody. It is probable that the illnesses in this group resulted from reinfection. Mild illness associated with reinfection has been observed with other respiratory agents (para influenza types 1 and 3 and respiratory syncytial virus) and appears to be a not uncommon oc-

currence.[10-12] Although antibody did not prevent mild upper respiratory illness associated with Eaton infection, it was effective in protecting against the more serious consequences of infection.

The frequency of otitis in experimental Eaton infection suggests the need for evaluation of this agent in naturally occurring ear disease. At present it is not possible to evaluate otitis in naturally occurring Eaton infection, since previous studies were oriented primarily towards pneumonia. The present findings suggest that otitis may be a more common manifestation of infection than pneumonia. Before this association can be established, however, it must be determined whether the ear localization observed in this study reflects the inoculation procedure or represents an inherent property of the agent.

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