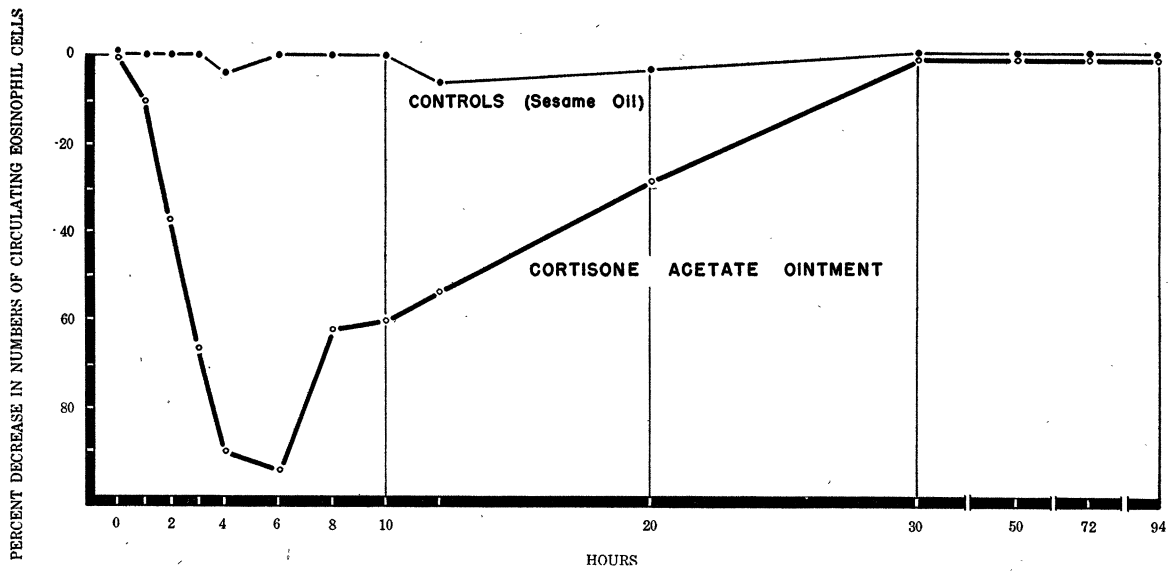

FIG. 1. Percentage decrease in number of circulating eosinophil cells following a cutaneous application of 100 μg of cortisone acetate.

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Isolation of Pleuropneumonialike Organisms from the Throats of Humans

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Because of the frequent association of pleuropneumonialike organisms (PPLO), or L organisms, with a polyarthritis in rats and mice, earlier investigators quite logically looked for these microorganisms in humans exhibiting arthritic symptoms. In mice the conjunctiva, the mucosa of the nose, and occasionally of the trachea, and the brain serve as the habitat of the organisms (*1, 2*). When Sabin and Johnson (*2*) cultured the nose, throat, and conjunctiva of 100 individuals, they failed to find PPLO. In another attempt the above authors examined tonsils removed from 58 children for various reasons. A piece of tissue from each of the 116 specimens of tonsils was minced and then streaked over the surface of 30% ascitic fluid agar plates. In 3 of the 58 cases colonies 20–40 μ in diameter were observed among the bacterial colonies. These small colonies, designated *X* colonies, resembled those of PPLO. Impression smears of these small colonies were unsatisfactory and the *X* colonies never grew in subcultures. The nature of the *X* colonies remains unknown. It is possible that they were PPLO, as it has been observed recently that PPLO will grow in symbiosis with bacterial colonies (*3*).

At the time of their review in 1948, Dienes *et al.* (*4*) stated that PPLO had not been isolated from the human body from areas other than the genitourinary tract except when the individuals had received penicillin therapeutically or when penicillin was added to the culture medium to suppress the growth of bacteria. Dienes *et al.* stated that the PPLO in these cases represented a variant growth form of other bacteria produced under the influence of penicillin.

Smith *et al.* (*5*) discovered PPLO were not inhibited by crystal violet in concentrations that usually inhibit most gram-positive bacteria, nor by potassium tellurite in concentrations that usually inhibit most gram-negative bacteria. By adding both chemicals to an appropriate culture medium both gram-positive and gram-negative bacteria were inhibited, whereas PPLO grew readily. A suitable selective medium for PPLO was described by Morton, Smith, Williams, and Eickenberg (*6*) to consist of the infusion of 50 g Bacto-beef heart for infusion in 1,000 ml distilled water, to which are added 5 g NaCl and 10 g Bacto-peptone. After adjusting the pH to 7.8, 0.013 g crystal violet is added. Following sterilization in the autoclave at 121° C for 15 min, 0.53 ml Bacto-Chapman tellurite solution and ascitic fluid to a concentration of 25% of the final volume is added. This enrichment broth was employed with some of the throat cultures in these studies, but with a majority of the specimens a 1% concentration of a recently characterized serum fraction (*7*) was substituted for

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the ascitic fluid, with appropriate diminution of the inhibitors to give concentrations of crystal violet of 1:100,000 and of potassium tellurite of 1:35,000. As PPLO produce little or no visible growth in liquid media, growth in the enrichment broth has to be ascertained by inoculating the enrichment broth cultures onto the surface of solid medium in Petri dishes. The medium employed for this purpose was that described by Morton, Smith, and Leberman (*8*). A few cultures in which some bacterial growth occurred were subcultured onto the solid medium containing 0.006 g crystal violet and 0.53 ml Bacto-Chapman tellurite solution per liter.

In the case of 11 individuals a sterile cotton swab was rubbed over the tonsillar areas and the posterior portion of the throat. The swab was then placed directly into a tube of 5 ml of enrichment broth and incubated at 37° C for 2 days. Growths in the tubes were inoculated onto Petri plates of Bacto-PPLO Agar, Experimental (*8*), enriched with ascitic fluid and incubated aerobically for 2 days at 37° C. In the case of 103 medical students, the throat swabs were placed in test tubes containing 1 ml of extract broth. After a suspension of the material on the swabs was made in the broth, the swab was discarded, and 0.5 ml of the suspension in each case was transferred to a tube of the enrichment broth. These were then treated as described above.

The Petri dish cultures were examined under the low power of the microscope (100×) for characteristic colonies. Verification of the colonies of PPLO may be made by staining according to the method of Dienes, which has been described fully elsewhere (*6*).

The throat cultures of 6 of the 11 individuals were positive for pleuropneumonialike organisms. In addition, the throat cultures of 32 of the 103 normal, healthy, medical students were positive for PPLO. A total of 38, or 33.6%, of 114 throat cultures yielded pleuropneumonialike organisms. This incidence is lower than that of PPLO in human saliva, PPLO having been detected in the saliva of 46 of 100 individuals (*6*). The colonies of pleuropneumonialike organisms isolated from throat cultures are similar to those isolated by us from the genitourinary tract of humans (*9*) and to a known culture isolated from a human by Dienes and to cultures of human and rat origins furnished by I. G. Schaub, of Johns Hopkins Hospital.

By means of improvements in the cultivation of PPLO, these organisms have been detected in saliva and throat cultures of individuals and in a frequency exceeding that reported for the normal female (*10, 11*) and male (*12*) genitourinary tracts. However, if some pathology exists in the genitourinary tract, the incidence of isolation of PPLO is greater (*10–13*).

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Atmospheric Nitrous Oxide and the Nitrogen Cycle

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The author's discovery of nitrous oxide (*1*) in the earth's atmosphere has received numerous confirmations (*2–4*), both in this country and abroad. The amount present is comparable with that of atmospheric ozone, about 3 mm N.T.P. (*5*).

It was subsequently discovered[1] that nitrous oxide is one of the most abundant constituents of soil air (*7*), whereupon it was suggested by the present author that escaping soil air might well be a principal source of the atmospheric nitrous oxide (*8*).

It is the purpose of this note to summarize several lines of evidence, recently adduced, which lend strong support to this early hypothesis regarding the gas's origin, which make it seem probable that the nitrous oxide "layer" is "adjacent" to the earth's surface, and which strongly suggest the idea that atmospheric nitrous oxide is an important phase in the nitrogen cycle.

a) The effective radiation temperature of atmospheric nitrous oxide is about 10° C, as deduced from observations of the atmosphere's infrared emission spectrum (*9*).

b) A British Admiralty group, investigating atmospheric transmission in the infrared, has found large concentrations of nitrous oxide in air paths parallel and close to the surface of the land and sea (*4*).

c) An inspection of solar spectra recorded aboard high-flying aircraft reveals a greatly diminished absorption by nitrous oxide in the 7.8-μ region (*10*).

d) Finally, a recent mass spectrographic analysis has revealed that in the atmosphere near ground level the concentration of nitrous oxide is about 5×10^{-5}% by volume (*11*).

Apropos of the foregoing evidence, it appears very likely that nitrous oxide escapes from the soil into the atmosphere, and the conclusion cannot be avoided that this escape constitutes an important phase of the nitrogen cycle. It thus becomes clear, for the first

[1] The discovery of nitrous oxide in soil air has been confirmed by Taylor *et al.* (*6*). They find it the most abundant constituent of soil air, constituting more than one fifth, by volume.