

Differences between Oral and Genital Strains of Human Pleuropneumonia-like Organisms.*† (20015)

L. DIENES AND SARABELLE MADOFF.

From the Department of Bacteriology, Massachusetts General Hospital and the Robert W. Lovett Memorial, Harvard Medical School, Boston, Mass.

Pleuropneumonia-like organisms have been isolated from various locations of the human body: from the genitourinary tract(1), buccal cavity(2), intestinal tract(3), blood, spinal fluid(4) and from suppurative lesions(1). In our experience they grow abundantly from the debris scraped from between the teeth and from material expressed from the crypts of the tonsils. These organisms present the characteristic morphology of the pleuropneumonia group, but they vary considerably in their properties. Three types isolated from mice differed in serological and pathological properties(5). Recently Edward distinguished 2 different types among strains isolated from the vagina of cows(6) and 3 different types among the strains isolated from dogs(7). The observations presented here suggest that at least 2 different types should

be distinguished in human strains.

Cultures of pleuropneumonia-like organisms obtained from specimens taken from the genitourinary tract grow in two forms. That most often observed grows to fairly large colonies (0.1-0.5 mm) and grows abundantly on ascitic agar plates. The appearance of the colonies is variable on the original plates as a consequence of different development and vacuolization of the large bodies on the surface. These differences disappear in subcultures. Cultures obtained from certain *specimens* are *markedly different*. In these the colonies are very small (0.01-0.05 mm) both in the original plates and in transplants and the growth is very poor. Whether these colonies represent a variant of the usual strains or a different species cannot be decided until a better method for their cultivation is devised. The cul-

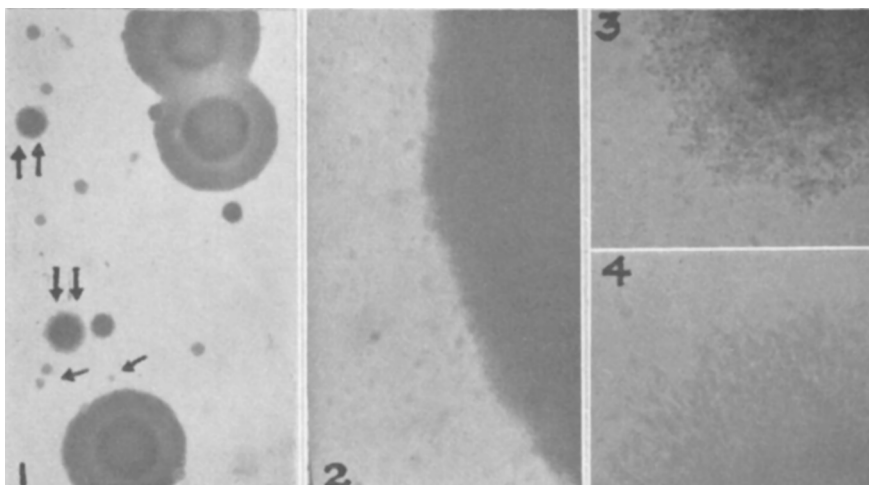


FIG. 1. "Smooth" and "rough" pleuropneumonia-like colonies on a horse-serum agar plate containing penicillin, inoculated from tooth scrapings. The culture was stained with methylene blue. 1. The large "smooth" and small "rough" colonies with low magnification ($\times 100$). Tiny "rough" colonies are marked with a single arrow and fully developed "rough" colonies are marked with two arrows. 2. Edge of a "smooth" colony. Oil immersion. ($\times 900$.) No structure is visible in the colony. 3, 4. Edges of two "rough" colonies. Oil immersion. ($\times 900$.)

* The expenses of this investigation have been defrayed in part by grants from the Commonwealth Fund and from the U. S. Public Health Service.

† This is publication No. 135 of the Robert W. Lovett Memorial Foundation for the study of crippling disease.

tures obtained from the *buccal cavity* also grow in 2 morphological types both of which are different from the genital strains. Under appropriate conditions the colonies usually isolated grow to a larger size than the colonies of genital strains and differ from the latter by their peculiar "smooth" appearance. The surface growth appears with the low power of the microscope as a homogeneous mass. The uniform small granules which produce the colonies can be observed easily in impression preparations. Large bodies are produced on the surface but they are not vacuolized and are filled with uniform granules.

These relatively "*smooth*" colonies can be seen fairly easily between the bacterial colonies if the plates are inoculated with high dilutions of tooth scrapings. They grow abundantly if the plates are heavily inoculated and bacterial growth is suppressed by penicillin. In addition, sometimes in large numbers, much smaller pleuropneumonia-like colonies develop. These tiny colonies differ in appearance both from the other oral strains and from genital strains. They are surrounded after 2-3 days with a peripheral surface growth and although they are very small they present the characteristic appearance of colonies of the pleuropneumonia group. The peripheral growth consists of large bodies but they are soon vacuolized giving a rough appearance to the colonies. It is difficult to induce growth of these colonies in transplant. After repeated transfers their growth becomes more abundant but the colonies retain their "rough" appearance.

The characteristic *morphological differences* between the genital and oral strains remain unchanged during cultivation. There is also some difference in their nutritional requirements. The "smooth" oral strains grow well on the usual horse blood agar plates while most genital strains grow poorly or not at all. There is also a marked difference in the serological properties of the strains. We found the easily cultivatable genital strains to be serologically similar, as reported by Norman, Saslow and Kuhn(8). There are some irregularities in the reactions of the strains with different antisera but it is uncertain whether these really indicate serological differences. On the other hand,

TABLE I. Agglutination Reactions of Genital and Oral Strains of Human Pleuropneumonia-Like Organisms.

TABLE I. Agglutination Reactions of Genital and Oral Strains of Human <i>Trichomonas vaginalis</i> O Antigen																				
	Antigens																			
	Genital strain "50"					Genital strain "Campo"					Oral strain "smooth"					Oral strain "rough"				
	1:20	40	80	160	320	1:20	40	80	160	320	1:20	40	80	160	320	1:20	40	80	160	320
Rabbit antisera																				
Genital strain "50"	—	3+	3+	3+	3+	—	—	+	2+	2+	+	0	0	0	0	0	0	0	0	0
Oral strain "smooth"	0	0	0	0	0	—	—	0	0	0	0	3+	3+	3+	2+	2+	2+	2+	2+	2+

0 indicates negative reaction; — indicates not done.
 The rabbits were treated with cultures grown in broth or on agar plates containing rabbit serum. For the agglutination test, the sediment of broth cultures or washings from agar cultures were used. The test was performed in .4 cc volume in tubes measuring 10 X 75 mm. The tubes were incubated overnight in a water bath at 37°C.

three oral strains, two "smooth" and one "rough", gave no reaction with agglutinating sera produced by 4 different genital strains and the latter were not agglutinated by the sera produced with the oral strains. Two of these strains, "Campo" and "50" were studied by Norman, Saslow and Kuhn. A fifth genital strain was an exception, giving a slight cross reaction with the oral strains. An example of the reactions observed is presented in Table I.

Serological examination of the "rough" oral strains remained inconclusive because in several trials we were unable to produce agglutinating sera with them. However, emulsions of a "rough" strain gave strong agglutination with the sera of the "smooth" strain. This would suggest that the "rough" colonies are probably a variant of the "smooth" ones, although transformation of one strain into the other has not been observed.

In addition to the pleuropneumonia-like colonies, L colonies similar in appearance to those developing from various bacteria develop occasionally on the plates if penicillin is added to the media. These colonies usually grow to a larger size and their morphology also distinguishes them from pleuropneumonia-like colonies. In one case we isolated a small Gram negative bacillus from L colonies developing on the plates. The bacillus produced similar L colonies in the presence of penicillin. In another case a *Clostridium* was present in the specimen and produced L colonies. It is necessary to bear in mind that colonies of various types, some of which may be quite similar to pleuropneumonia-like colonies, may develop on the plates.

One strain of pleuropneumonia-like organ-

isms isolated from spinal fluid(4) and several strains isolated from pathological processes associated with the buccal cavity corresponded in the appearance of the colonies to the "smooth" oral types. The strains isolated from suppurative lesions of the female genital tract corresponded to the genital type.

Conclusion and summary. Pleuropneumonia-like strains isolated from the human genito-urinary tract and from the buccal cavity, respectively, differ in the appearance of the colonies, in serological properties and, to a certain extent, in nutritional requirements. It is likely that they represent different species. In addition to the easily cultivatable strains, colonies of much smaller size develop occasionally on the plates inoculated from either source. These small colonies retain their identity in transfer. It is uncertain whether they are variants of the usually cultivated strains.

1. Dienes, L., Ropes, M. W., Smith, W. E., Madoff, S., and Bauer, W., *New Eng. J. Medicine*, 1948, v238, 509, 563.

2. Morton, H. E., Smith, P. F., Williams, N. B., and Eickenberg, C. F., *J. Dental Research*, 1951, v30, 415.

3. Dienes, L., unpublished observations.

4. Paine, T. F., Murray, R., Perlmutter, I., and Finland, M., *Ann. of Int. Med.*, 1950, v32, 554.

5. Sabin, A. B., *Bact. Rev.*, 1941, v5, 1.

6. Edward, D. G. ff., *J. Gen. Microbiology*, 1951, v4, 4.

7. Edward, D. G., ff., and Fitzgerald, W. A., *J. Gen. Microbiology*, 1951, v5, 566.

8. Norman, M. C., Saslow, S., and Kuhn, L. R., *Proc. Soc. Exp. Biol. and Med.*, 1950, v75, 718.

Received December 14, 1952. P.S.E.B.M., 1952, v82.