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Some Biologic and Immunologic Characteristics of a Pleuropneumonia-Like Microörganism of Human Origin.

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The first unequivocal isolation from human beings of microörganisms morphologically and culturally similar to those of bovine pleuropneumonia was reported by Dienes in 1940.[1] Using an ascitic fluid enriched agar medium he was frequently able to obtain such microorganisms from the secretions of the uterine cervix of female patients. This work was subsequently corroborated by Smith,[2] who in addition succeeded in cultivating a similar strain from the urethral discharge of a man with urethritis and arthritis in whom gonococci were not recovered.

Dienes and Smith[3] have recently reported the isolation of pleuropneumonia-like microorganisms from the genital tracts of about 22% of 129 unselected male and female patients, for the most part from the secretions of the cervix uteri. They conclude that these organisms may dwell in the female genital tract without causing any obvious pathologic changes. However, striking evidence is also presented which suggests that they may also be the incitants, either alone or in combination with other bacteria, of a clinical picture that is similar to gonococcal infection in the female. In males they have thus far only been isolated from cases of chronic prostatitis, and mainly in patients with a history of previous gonococcal infection. Two instances are cited in which it appears that the microorganisms were transmitted from husband to wife by sexual intercourse.

The very scanty growth of these strains in fluid media was a barrier to a proper study of the biologic, pathogenic and serologic characteristics of this new group of microorganisms. Dr. Louis Dienes very kindly gave us one of his strains in order that we might attempt to obtain a satisfactory growth in broth. This strain, No. 4330, was originally isolated from the cervix uteri and was received by us in the form of scrapings from ascitic fluid agar cultures.

The medium we have employed consisted of an infusion broth, (prepared from fresh beef hearts), containing 1% of Pfanstiehl peptone and 0.5% glucose. The reaction of this medium after sterilization and before the addition of serum was pH = 8.0. This basic medium was enriched by the addition of either filtered ascitic fluid to a concentration of 30% or of rabbit serum to a concentration of 10%. The final reaction of the rabbit serum broth was about pH = 8.2. Transfers were made by pipette using 0.1 cc of culture into about 5.0 cc of the medium.

The original agar scrapings containing this strain were inoculated on the surface of ascitic agar plates where an abundant growth appeared within 48 hr. Blocks of agar containing these colonies were then removed from these plates and dropped into tubes of 30% ascitic fluid broth and into 10% bovine serum broth. No growth was observed in the latter medium and this series was discontinued. However, a faint, diffuse opalescence appeared in the ascitic broth after 5 days. This same culture was "passaged" blindly on the fourth day, *i.e.*, before any gross evidence of growth was apparent and a similar faint "growth" appeared in this second broth transfer at the end of 4 days. That this opalescence actually represented multiplication of the microorganisms was proven by transferring some of the fluid culture onto the surface of 30% ascitic fluid agar plates where numerous colonies appeared in 72 hr. After 6 passages in ascitic fluid broth it was noted that the

1 Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 468.

2 Smith, W. E., *J. Bact.*, 1942, **43**, 83 (Abstract).

3 Dienes, L., and Smith, W. E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 99.

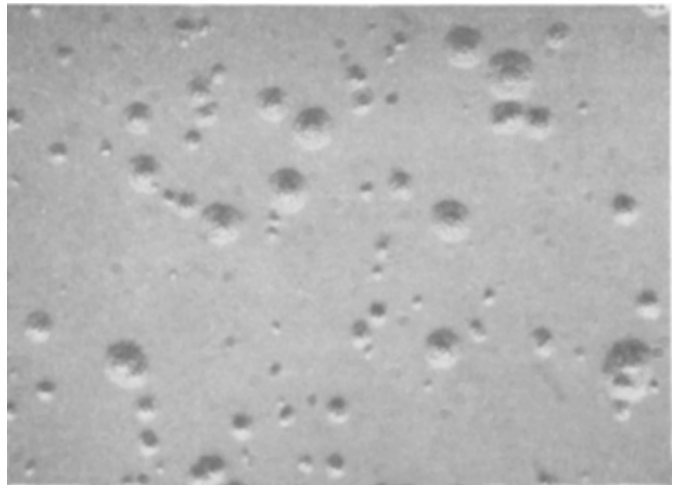

FIG. 1.
Microörganism of pleuropneumonia group of human origin, strain No. 4330 (Dienes). 72-hr culture; colonies on nutrient agar containing 30% ascitic fluid. Oblique lighting. × 95.

growth was heavier and was regularly noticeable within 72 hr. The eighth passage was transferred to 10% rabbit serum broth. Adaptation to this medium was rapid and after the first 4 passages growth regularly appeared in 48 hr. At best it was never very heavy although when 0.1 cc of such a broth culture was plated on the surface of enriched agar innumerable colonies developed. (Fig. 1).

Broth cultures retained their viability in the refrigerator for at least 2 weeks. Unlike certain of the rodent types of organisms of this group, this human strain remained viable when kept in the glucose serum broth at 37°C for as long as 7 days.

The human strain was tested for pathogenicity in mice using 24 hr (17th passage), 48 hr (15th passage), and 72 hr (6th passage) rabbit serum broth cultures. One-half cc was inoculated intravenously into groups of 4-week-old mice. The numbers of mice were 5, 8, and 10 for the 3 ages of culture respectively. All animals were observed daily for one month during which period they remained well and no gross evidence of arthritis was apparent.

When this human strain of the pleuropneumonia group was well adapted to the rabbit serum fluid medium, serological studies were carried out.

Antiserum was prepared in rabbits by the repeated intravenous injection of the resuspended sediments of broth cultures. The cultures used as sources of antigen had been passaged in rabbit serum broth for 34 generations. One hundred cc of a 10% rabbit serum broth culture, 4 days old, was spun on the angle centrifuge at 3000 rpm for one hr. The supernatant fluid was discarded and the sediment, resuspended in about 5.0 cc of physiological saline, was inoculated. The schedule of immunization consisted of 4 injections of 2.5 cc of the suspension of sedimented culture intravenously every other day, and 4.5 cc for the last injection. Blood was obtained from the heart 3 weeks after the last injection. Two rabbits were thus immunized. One animal was found dead 24 hr after the last injection. Necropsy revealed no gross changes. The second animal remained well throughout the course of inoculations.

Antigens for agglutination were prepared in the same manner as for injection except that the sediments were resuspended in 0.45% saline which appears to yield a smoother suspension.

TABLE I.
Reaction of an Antiserum to the Human Strain No. 4330 with the Homologous Antigen and Types A, B, and C of the Mouse Pleuropneumonia Group.

Final dilution of serum for human strain No. 4330	Antigen			
	Human No. 4330	Mouse		
		Type A	Type B	Type C
1:2	+++	0	0	0
1:10	++++	0	0	0
1:100	++++	0	0	0
1:500	++++	0	0	0
1:1000	++++	0	0	0
1:2000	0	—	—	—

The immune serum prepared against strain No. 4330 agglutinated this organism to a final serum dilution of 1:1000. However, this serum did not react to any degree with types A, B, and C microörganisms of the mouse pleuropneumonia group (Table I) (*Cf.* Sabin[4]). The type A, B, and C antigens were prepared in the same manner as the human strain using 48 hr 10% rabbit serum broth cultures. Conversely, antisera against types A, B, C, D, E of the mouse pleuropneumonia group and L3, and L4 of rats, did not agglutinate suspensions of the human strain. Thus, no serologic relation was found between the pleuropneumonia-like microörganism of human origin and representative types from the mouse and rat.

Summary. By means of serial passage in 10% rabbit serum broth it has been possible to secure sufficient growth in fluid media of a strain of pleuropneumonia-like microorganism derived from the human cervix uteri to permit serologic and pathogenic studies.

This strain was found not to be pathogenic for mice when cultures were injected intravenously, and no evidence of a toxin was observed.

Immune serum was prepared by the repeated inoculation of rabbits with saline-resuspended sediments of rabbit serum broth cultures. A satisfactory agglutinating antigen was prepared in the same manner. This serum agglutinated the homologous human antigen to a dilution of 1:1000, but failed to agglutinate antigens prepared similarly from Types A, B, and C of the mouse pleuropneumonia group.

Antisera to Types A, B, C, D, E from mice and L3 and L4 from rats, even when used undiluted, failed to agglutinate the human strain. From what is known of the pleuropneumonia group, microörganisms native to one host are rarely pathogenic for another and usually are serologically distinct. With a strain that is native to man one would expect neither significant serological cross-reactions with rodent strains nor would one expect pathogenicity in rodents. As a matter of fact it may not be possible to establish whether or not the human strains are pathogens or saprophytes by animal inoculation. The development of antibodies in human beings carrying such strains and tests in man may be necessary to elucidate this question.

[4] Sabin, A. B., *Bact. Rev.*, 1941, **5**, 1.