Characterization of Pleuropneumonia-Like Organisms by Deoxyribonucleic Acid Composition. (29893)

HAROLD C. NEIMARK AND JACQUES J. PÈNE (Introduced by Thomas P. Magill)
Department of Microbiology and Immunology, College of Medicine, State University of New York, Brooklyn, and Department of Biochemistry, Einstein College of Medicine, New York City

The relationship between the microorganisms of the pleuropneumonia group (PPLO, Mycoplasmatales) and the true bacteria is uncertain. The various organisms isolated from nature that comprise the Mycoplasmatales share singular and distinctive properties that have been utilized to draw them together into a group. The most fundamental of these properties is the lack of a complete cell wall. Indeed it appears that a number of the interesting characteristics of the group are a result of this absence (*e.g.*, the unique colonial and microscopic morphology, the growth requirement satisfied by horse serum, insensitivity to certain antibiotics and inhibitory agents, inhibition by antibody in the absence of complement, and their notable filterability).

Two general hypotheses have been advanced to explain the nature of this group and its relation to other microorganisms(1,2, 3): one holds that pleuropneumonia organisms are a natural biological class; the other that PPLO are an assemblage of the stable L forms of various bacteria.

The basic biochemical heterogeneity of the organisms making up the group has been emphasized previously(4). It was proposed that primarily these organisms could be divided into sets consisting of nonfermentative and fermentative strains. These fermentative strains were characterized as catalase negative, cytochrome negative organisms that accumulate lactic acid(4). This description is highly reminiscent of the lactic acid bacteria, since they lack heme enzymes and are virtually the only such bacteria capable of growth in the presence of free oxygen.

The resemblance between this set of pleuropneumonia-like organisms and the lactic acid bacteria occurs either because these are homologous groups that have arisen coincidentally in nature, or these PPLO are related in some manner, such as stable L forms or ancestral forms, to certain lactic acid bacteria. The relationships of pleuropneumonia-like organisms to possible bacterial parental forms can be examined directly by a determination of DNA base ratios. Lee, Wahl, and Barbu(5) first recognized that phylogenetic relationships of microorganisms may be reflected in the mean base composition of DNA. Although some unrelated organisms have the same average base composition, similar DNA base compositions would be expected where a taxonomic relationship exists.

Materials and methods. Deoxyribonucleic acid was isolated from 3 fermentative PPLO: Sewage A (*Mycoplasma laidlawii*, A), California Calf, and the kid strain of goat arthritis PPLO. Isolations were also made from the Eaton agent, *M. pneumoniae*.*

Organisms were grown at 37°C in trypticase soy broth (Baltimore Biological Laboratory, Baltimore, Md.) adjusted to pH 7.8-8.0 and (except Sewage A) supplemented with 10 or 20% horse serum and (Eaton agent) 2.5% yeast extract(6). Cells were collected at 24 to 40 hours with a Servall continuous flow centrifuge operating at 12,000 $\times$ *g* with a flow rate of 100 ml per minute. Organisms were resuspended and washed once in saline-EDTA (0.15 M NaCl and 0.1 M disodium ethylenediamine tetraacetate, pH 8.0); these conditions suffice to inhibit the DNase activity present in these cells(7). The glutinous mass of washed cells was dispersed in saline-EDTA with a rubber policeman, lysed with 3% sodium dodecyl sulfate for 10 minutes, and the deoxyribonucleic acid isolated by the method of Marmur(8).

Deoxyribonucleic acid base compositions were determined by 2 independent methods: CsCl density gradient centrifugation(9) and thermal denaturation temperatures(10). Buoyant density of DNA in CsCl and the

* Kindly supplied by Dr. N. L. Somerson and Dr. M. F. Barile, N.I.H., Bethesda, Md.

TABLE I. Physico-Chemical Characterization of DNA from Fermentative Pleuropneumonia-Like Organisms.

DNA source	Absorption 260/280 mμ	$S_{20,w}$	Mol wt	Density* (g/cc)	T_m† (°C)	Mole % G + C from density	Mole % G + C from T_m
Eaton agent (*M. pneumoniae*)	2.03	15.0	3.1×10^6	1.700	85.3	40.8	39.0
Sewage A (*M. laidlawii*, A)	2.0	22.8	11.4×10^6	1.695	82.0	35.7	30.9
Calf	1.74	31.7	31×10^6	1.686	79.0	26.5	23.6
Goat, kid strain	1.72	31.7	31×10^6	1.685	79.2	25.5	24.1

* Densities were calculated with *Escherichia coli* DNA as reference (1.710 g/cc).
† Determined in saline-citrate (.15 M NaCl and .015 M sodium citrate, pH 7.0).

thermal denaturation temperature (T_m) are each linearly related to the mean mole per cent guanine plus cytosine (G+C) content of DNA. Sedimentation coefficients of DNA dissolved in saline-citrate (0.15 M NaCl and 0.015 M trisodium citrate, pH 7.0) were determined in a Spinco analytical ultracentrifuge at 35,600 rpm using ultraviolet optics. Molecular weights were calculated by the equation of Eigner and Doty: $S_{20,w} = 0.116\ M^{0.325}$ (personal communication). These molecular weight values do not have biological significance in themselves, but are reported to describe the DNA used in the study.

Results and discussion. Deoxyribonucleic acid from these pleuropneumonia-like organisms undergoes sharp cooperative melting with a characteristic melting temperature indicative of double stranded molecules. Centrifugation in CsCl did not reveal the presence of any satellite bands. The data in Table I show there is agreement between the guanine plus cytosine contents calculated from the density and T_m values.

There is a marked heterogeneity of base composition, even within this selected set of PPLO, with the organisms falling into 3 ranges or groups of G+C content. The G+C contents of the Calf and Goat organisms are among the lowest reported for any microorganism, and coincide with a value recently reported for the agent of caprine pleuropneumonia, *M. mycoides* var. *capri* (11). No eubacteria have been shown to have such low G+C contents. Indeed these values are among the lowest known for any organism; other organisms known to have very low G+C contents are the ciliate protozoan, *Tetrahymena,* and the slime mold, *Dictyostelium discoideum*(12). A plausible conclusion is that the values establish these organisms as unique, and consequently they cannot be stable L forms of any known bacteria(11). Theoretically it is conceivable also that these forms might be mutants with altered base compositions; mutants with altered G plus C ratios have been described (13,14).

The base composition of one other pleuropneumonia-like organism has been determined(15). The deoxyribonucleic acid of *M. gallisepticum* (agent of an avian respiratory disease) was reported to have a density of 1.693 g/cc; the G+C content calculated from this density is 30.6%, very close to that of the Sewage A saprophyte.

Only the Eaton agent DNA has a G+C content near that of members of the Lactobacillaceae (39%). Certain other bacteria, notably *Hemophilus* species, possess the same average base composition(9,10). In this respect it is interesting to note that Shope(16) has recently described a new species, *Hemophilus pleuropneumoniae,* that produces a pleuropneumonia in swine strikingly similar in pathology to bovine pleuropneumonia, the disease caused by the type organism of the pleuropneumonia group, *Mycoplasma mycoides.*

Summary. Characterization of the DNA of 4 fermentative PPLO divides these organisms into 3 distinct groups, and demonstrates a fundamental heterogeneity within the class. Two of the organisms have guanine plus cytosine contents lower than any known

for eubacteria. DNA from the Eaton agent possesses the same average base composition as certain Lactobacillaceae and *Hemophilus* species; this finding is of special interest because both PPLO and *Hemophilus* species are specific agents of interstitial pneumonias, and characteristic pleuropneumonias.

1. Dienes, L., Recent Prog. Microbiol., 1963, v8, 511.
2. Edward, D. G. FF., Ann. N.Y. Acad. Sci., 1960, v79, 309, 481.
3. Klieneberger-Nobel, E., *ibid.*, 1960, v79, 483.
4. Neimark, H. C., Pickett, M. J., *ibid.*, 1960, v79, 531.
5. Lee, K. Y., Wahl, R., Barbu, E., Ann. Inst. Pasteur, 1956, v91, 212.
6. Chanock, R. M., Hayflick, L., Barile, M. F., Proc. Nat. Acad. Sci. U.S., 1962, v48, 41.
7. Neimark, H., Nature, 1964, v203, 549.
8. Marmur, J., J. Mol. Biol., 1961, v3, 208.
9. Schildkraut, C. L., Marmur, J., Doty, P., *ibid.*, 1962, v4, 430.
10. Marmur, J., Doty, P., *ibid.*, 1962, v5, 109.
11. Jones, A. S., Walker, R. T., Nature, 1963, v198, 588.
12. Schildkraut, C. L., Mandel, M., Levisohn, S., Smith-Sonneborn, J. E., Marmur, J., *ibid.*, 1962, v196, 795.
13. Weed, L. L., J. Bacteriol., 1963, v85, 1003.
14. Gause, G. G., Loshkareva, N. P., Zbarski, I. B., Gause, G. F., Nature, 1964, v203, 598.
15. Morowitz, H. J., Tourtellotte, M. E., Guild, W. R., Castro, E., Woese, C., Cleverdon, R. C., J. Mol. Biol., 1962, v4, 93.
16. Shope, R. E., J. Exp. Med., 1964, v119, 357.

Received October 26, 1964. P.S.E.B.M., 1965, v118.

Comparative Metabolism of ^{14}C-Estrone in the Rat and Guinea Pig.* (29894)

P. H. JELLINCK AND CATHERINE LAZIER (Introduced by R. L. Noble)
Cancer Research Centre, University of British Columbia, Vancouver, Canada

It is well established(1,2,3) that estrogens are excreted mainly as sulfate or glucosiduronate conjugates and that the formation of the latter requires uridine diphosphate glucuronic acid (UDPGA) present in the soluble fraction of liver cells and a microsomal glucuronyl transferase. Previous studies(4,5), however, have indicated that the water-soluble estrogen metabolites formed by rat liver may differ from the normal conjugates produced by other mammalian species and it was therefore decided to verify this by carrying out parallel incubations with rat and guinea pig liver preparations. In addition, the comparative *in vivo* metabolism of estrone-16-^{14}C was investigated in these two species.

Materials and methods. In vivo studies. Estrone-16-^{14}C (0.98 μc in 25 μg) dissolved in 0.25 ml of ethanol was added to 5 ml of isotonic saline and given intraperitoneally to 2 young female albino guinea pigs (470-490 g). The same amount of radioactive estrogen was administered to 2 female Wistar rats (155-160 g) and the first 24-hour collection of urine was pooled for each species. After adjusting the pH to 6.8, aliquots (0.2 ml) of the urine were counted in a Packard Tri-Carb Scintillation Spectrometer before and after extraction with ether. In addition, 3 ml samples of the rat and guinea pig urine were incubated for 48 hours at 37°C with 2.5 mg (187 units) of bacterial β-glucuronidase in the presence of one drop of chloroform (to retard bacterial growth) and extracted with ether. Other samples (8 ml) were subjected to acid hydrolysis by refluxing with 2 N HCl for 2 hours and the amount of ^{14}C removed by subsequent ether extraction was determined. The radioactive estrone was purchased from Amersham (England) and was shown by chromatography and autoradiography(4,6) to be virtually free of radioactive impurities.

In vitro studies. Subcellular fractions derived from 100 mg of rat and guinea pig liver

* This work was supported by the Nat. Cancer Inst. of Canada.