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Correspondance — Briefwisseling

COMPARATIVE ANTIGENIC ANALYSIS OF *NAEGLERIA* SPECIES

The ubiquity and multiplicity of amoebas encountered in water and moist soil is a subject worth-while studying, not only from the morphological but also from the immunological point of view. It was therefore considered of interest to study the antigenic constitution of amoebas, which might enable us to establish a valid classification.

Carter (1970), first isolated in 1966, from a case of Primary Amoebic Meningo Encephalitis (P. A. M. E.), an amoeba closely resembling *N. gruberi*, which he called *N. fowleri*. Singh (1970) studied the HB-1 strain isolated by Butt (1966) and, considering it also a new species, called it *N. aerobia*. Nevertheless, the identification of non pathogenic and pathogenic amoebas of the *Naegleria* genus is complicated by their morphological resemblance of both the vegetative and cystic forms.

Hydrosoluble antigens were prepared from human-virulent strains of *Naegleria sp.* 0 359, 0 360 (Jadin *et al.* 1971), Vitek (Cerva 1969), HB-1 (Butt 1966); from a weakly mouse-virulent *Naegleria sp.* 0 400 and from a non virulent *N. gruberi* 1518 (C. C. A. P., Cambridge, U. K.).

The techniques used for antigen preparation (Willaert 1971), immunisation and antigenic analysis are described by Biguet and Capron (1965).

An hyperimmun antiserum was prepared against *Naegleria sp.* 0 359 objectivating 27 antigenic fractions through immunoelectrophoresis (IEP) when opposed to its homologous antigen.

The hyperimmunserum anti-*Naegleria sp.* 0 359 opposed to the hydrosoluble extracts of *Naegleria sp.* 0 359, Vitek, 0 400, *N. gruberi, E. histolytica* and *H. castellanii,* apparently showed an isologous structure between *Naegleria sp.* 0 359 and *Naegleria sp.* Vitek, an average isologous antigenic structure with *Naegleria sp.* 0 400 and a less isologous structure with *N. gruberi*. No relationship was observed with *E. histolytica* and *H. castellanii* (figure 1).

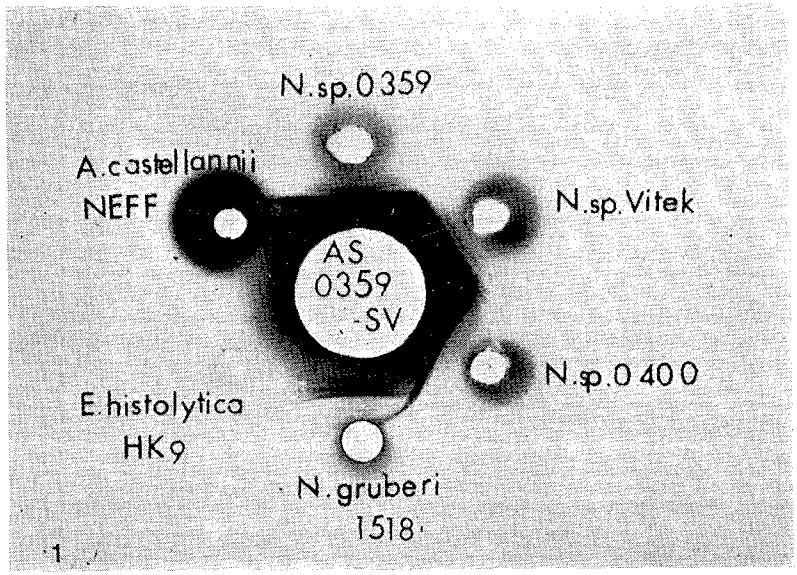

Figure 1

Ouchterlony plate. The central well contains the hyperimmunserum anti-*Naegleria sp.* 0 359, surrounded by the smaller wells containing the hydrosoluble antigens of *Naegleria sp.* 0 359, Vitek, 0 400, *N. gruberi* 1518, *E. histolytica* and *H. castellanii* Neff.

The antigenic extracts of the four human isolates revealed an identical immunoelectrophoretic pattern when opposed to the *Naegleria sp.* 0 359 antiserum (figure 2).

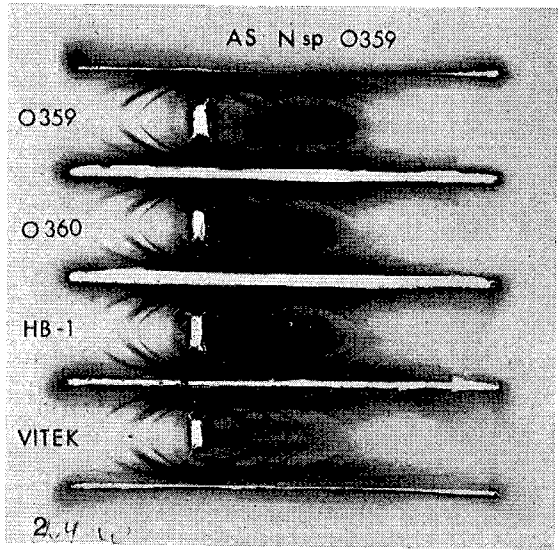

Figure 2
Immunoelectrophoretic pattern of four human-virulent strain extracts of *Naegleria sp.* 0 359, 0 360, Vitek and HB-1 opposed to the hyperimmunserum anti-*Naegleria sp.* 0 359, distributed in the five antiserum-ruts.

The hyperimmunserum anti-*Naegleria sp.* 0 359 opposed to the antigens of *Naegleria sp.* 0 400 and *N. gruberi* 1518 showed after IEP respectively 14 and 8 antigenic fractions in common (figure 3).

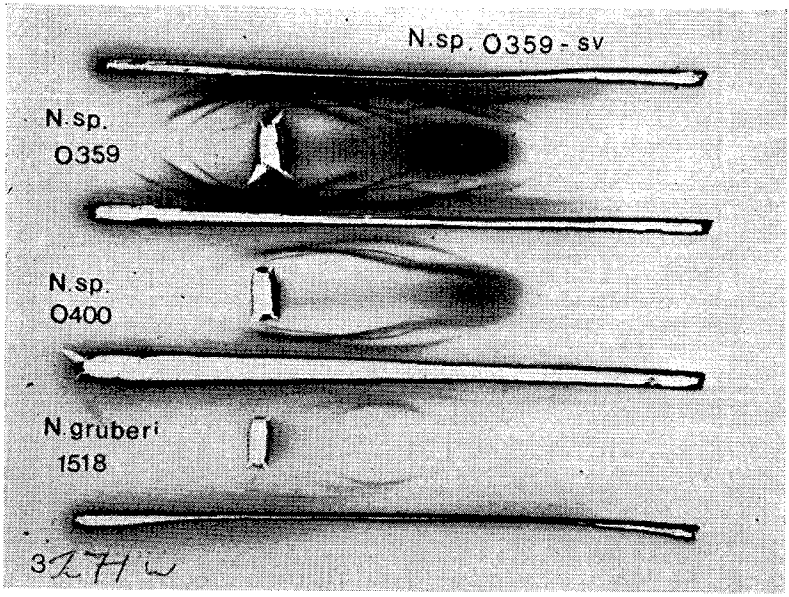

Figure 3
Immunoelectrophoretic pattern of the antigens of *Naegleria sp.* 0 359, 0 400 and *N. gruberi* 1518 opposed to the hyperimmunserum anti-*Naegleria sp.* 0 359.

The present results clearly indicate the absence of any antigenic relationship between the Vahlkampfiidae *Naegleria,* the Hartmannellidae *H. castellanii* and the Entamoebidae *E. histolytica.*

Among the genus *Naegleria,* an antigenic difference between the three species studied is observed; in particular the very important immuno-structural difference between *Naegleria sp.* isolated from human P. A. M. E. cases and the type species *N. gruberi* seems to confirm the genetic personality, characteristic to the species designed as the human pathogen.

The immunostructural identity of four human strains originated from Belgium, Czecoslovakia and the U. S. A. allows us to consider the geographical antigenic homogeneity of this species.

Moreover, *N. fowleri* is found free-living in water. Recently, Anderson and Jamieson (1972) isolated, in South Australia from tap water, on several occasions, *Naegleria* strains potentially pathogenic and serologically identical to *N. fowleri.* Singh (1972) also isolated in Lucknow (India) from sewage sludge a strain of *N. aerobia.*

About the *Naegleria sp.* 0 400 strain, the results show that it takes an intermediate position between *Naegleria sp.* 0 359 and *N. gruberi.* Must we consider it a distinct species or an evolutive form to the human pathogen ? Will an antigenic comparison of several strains of the *Naegleria* species prove us the existence of a few antigenically distinct types, or will it reveal a serial progressing of poor antigenic differentiating types.

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