THE ESSENTIAL CHARACTERISTICS OF THE SPECIES CLOSTRIDIUM HEMOLYTICUM

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Clostridium hemolyticum was first described by Vawter and Records (J. Am. Vet. Med. Assoc., **68**, 494, 1926) who found it to be the causative agent of bovine hemoglobinuria in Nevada, and who named it *Clostridium hemolyticum bovis*. Sordelli *et al.*, (Compt. rend. soc. biol., **106**, 142, 1931) in Chile, isolated from cattle afflicted with a similar disease a pathogenic *Clostridium* which they considered to be quite similar to the organism isolated by Vawter and Records. This organism was named *C. hemolyticum* var. *sordelli* by Hauduroy (Dict. d. Bact. Path., 1937, 125).

Several investigators have assumed that the organism isolated by Sordelli belongs to the species *C. hemolyticum*, whereas, considerable difference exists between this organism and *C. hemolyticum* as originally described. *C. hemolyticum* ferments only glucose, fructose, and glycerol, produces acid and slow coagulation in milk with no digestion of the casein, and possesses no somatic antigens in common with *Clostridium novyi*. The hemolysin, which is also the lethal toxin (Jasmin, Am. J. Vet. Research, **28**, 289, 1947), is a lecithinase serologically related to the beta toxin of type B *C. novyi* (MacFarlane, Biochem. J., **47**, 267, 1950). The organism isolated by Sordelli, on the other hand, ferments a number of carbohydrates in addition to glucose and fructose, including maltose, inositol, and mannitol, and digests milk without coagulation. It possesses a somatic antigen in common with *C. novyi* (Turner and Eales, Australian J. Exptl. Biol. Med. Sci., **21**, 79, 1943). Comparison of strains of *C. hemolyticum* isolated by Vawter and Records, as well as a number of strains isolated in this laboratory, with a subculture of Sordelli's organism received from Dr. A. R. Prévot of the Pasteur Institute, Paris, has provided further evidence that these organisms are not identical; for it was found that the organism isolated by Sordelli produces a hemolysin which was not serologically identical with the lecithinase of *C. hemolyticum* or the beta toxin of type B *C. novyi*.

Since *C. hemolyticum* is used for the active immunization of cattle against bacillary hemoglobinuria, and antitoxin for therapeutic treatment is prepared by immunization with *C. hemolyticum* toxin, it is essential that this organism not be confused with clostridia which are only superficially similar. It is suggested, therefore, that the species *C. hemolyticum* be restricted to those strains of pathogenic clostridia whose fermentative ability is restricted to the simple monosaccharides, whose principal toxin is a hemolytic lecithinase serologically related to that of the classical strains of *C. hemolyticum*, and which possess no somatic antigens in common with *C. novyi*.

INDUCED VARIATION IN THE g PHASES OF SOMATIC GROUP B OF THE GENUS SALMONELLA[1]

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In previously reported studies dealing with the g antigens of the Kauffmann-White schema it was demonstrated by Bruner (J. Bact., **57**, 387, 1949; **64**, 138, 1952) that *Salmonella oranienburg* could be transformed into *S. montevideo* and that *Salmonella blegdam* could be induced to form *S. enteritidis*, *S. moscow*, and *S. dublin*. These variations were accomplished by growth of

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S. oranienburg and *S. blegdam* in absorbed agglutinating serums.

Within somatic group B of the genus *Salmonella* the H antigens of the g-complex are represented by fg (*S. derby*), gm (*S. essen*), gmt (*S. california*), gst (*S. kingston*), gt (*S. budapest*), and mt (*S. banana*). In this study attempts were made to induce variation among these serological types in the hope of obtaining information concerning their origin. Agglutinating serums were absorbed to make five single factor (f, g, m, s, t), antiserums constituting the H antigens assigned to these types. They were prepared as follows:

Factor f—*S. derby* (fg) absorbed by *S. essen* (gm) and *S. budapest* (gt)

Factor g—*S. california* (gmt) absorbed by *S. banana* (mt)

Factor m—*S. california* (gmt) absorbed by *S. budapest* (gt)

Factor s—*S. kingston* (gst) absorbed by *S. budapest* (gt)

Factor t—*S. california* (gmt) absorbed by *S. essen* (gm)

Although these five absorbed antiserums are labeled single factor antiserums and agglutinate their respective antigens in the g-complex of the genus *Salmonella*, the methods used above are not recommended in preparing the diagnostic single factor serums regularly employed in routine *Salmonella* identification. The five single factor antiserums were added individually to semisolid agar and inoculated with growth from the six serological types mentioned above. That is, semisolid medium containing single factor f antiserum and the same medium containing single factor g antiserum were stabbed with growth from a culture of *S. derby* (fg). Likewise *S. essen* (gm), *S. california* (gmt), *S. kingston* (gst), *S. budapest* (gt), and *S. banana* (mt) were inoculated into semisolid medium that contained the individual factor antiserums representing their respective H antigens.

Although the g-types in somatic group B are listed as monophasic strains, they were induced readily to produce flagellar variants. In all cases the growth that spread from the point of inoculation was tested with the single factor antiserums (f, m, p, q, s, t, u) of the g-complex of the genus. These test serums were prepared according to the standard methods outlined by Edwards and Bruner (Kentucky Agr. Exptl. Sta. Cir. 54, 1942). Where necessary, absorption tests utilizing the appropriate standard antiserums were conducted.

S. derby split off at least two phases that apparently do not appear as such in nature. *S. essen* could not be induced to form recognizable variants. *S. california* (gmt) grown in semisolid medium that contained single factor g antiserum split off an mt phase. Upon absorbing *S. oranienburg* (mt) antiserum with this induced mt phase, it was found that the titer of the homologous antiserum was reduced from 1 to 20,000 to 1 to 500. *S. california* grown in semisolid medium plus m antiserum produced a gt phase indistinguishable from that of *S. budapest* (gt). In these studies *S. california* was not induced to spread when inoculated into semisolid medium containing antiserum. In a previously reported work Peso and Edwards (U. S. Pub. Health Repts., **66**, 1694, 1951) were able, however, to induce a gm phase, similar to that of *S. essen*, from *S. california*. When *S. kingston* (gst) was inoculated into semisolid mediums containing single factor antiserums for its respective H antigens, the only recognizable phase obtained was gs. It appeared in the medium impregnated with t antiserum. *S. budapest* (gt) did not produce any naturally occurring variants. *S. banana* (mt) was transformed to *S. budapest* (gt) by cultivation in semisolid agar plus m antiserum.

Although it was not possible to show that the g types of somatic group B originated from a common source, it does appear that *S. essen* (gm), *S. budapest* (gt), and *S. banana* (mt) descended from *S. california* (gmt), and that *S. banana* can be transformed in a type identical with *S. budapest*. Heretofore a gs form had not been described. In the present schema s is found in combination with g and m or t.