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The Identity of C. Oedematoides and B. Sordellii.

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In 1927, we, with Karp,[1] reported the isolation from a fatal human case of "gas gangrene" of a pathogenic anaerobic organism which did not correspond either culturally or immunologically to any of the hitherto well recognized clostridia; and, shortly after, we were able to isolate the same organism from specimens of imperfectly sterilized cat-gut.[2] This organism somewhat resembled Weinberg's *B. oedematiens* culturally. When injected into mice subcutaneously it produced much the same type of lesion, *i. e.*, the white gelatinous oedema so characteristic of the latter organism.

It differed from *B. oedematiens,* however, in failing to ferment glycerol; it was more actively proteolytic; and, more especially, the toxin readily obtained by filtering cultures, was in no way neutralized by high-titre anti-oedematiens serum. On the other hand, anti-serum prepared by the immunization of a rabbit with this sterile filtrate afforded full protection against the homologous toxin, but not at all against the toxins of *B. welchii,* of *vibrion septique* or of *B. oedematiens.*

For these reasons we concluded that the organism should be recognized as a distinct species and suggested the name *Clostridium oedematoides.*

A short time later, Hall and Scott[3] published a restudy of 2 strains of organisms sent to them from South America by Sordellii, which the latter had described first in 1922[4] and had named *B. oedematis sporogenes.* Although Hall and Scott found one of these strains to be entirely non-pathogenic for laboratory animals, they found that the other was fully virulent and that it produced a potent exo-toxin which could be separated from cultures by filtration. Hall and Scott suggested that the original name, *B. oedematis sporogenes* should be replaced by the binomial *B. sordellii.* They also suggested that the cultural characteristics of Sordellii's organism were so similar to

[1] PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 675.

[2] *Surg. Gyn. and Obstet.,* 1927, 775.

[3] *J. Infect. Dis.,* xli, 329.

[4] *C. R. Soc. de Biol.,* lxxxvii, 838; lxxxix, 53; xci, 1033.

those of *C. oedematoides* as described by us, as to render it probable that the 2 species were identical. They did not, however, carry out any immunologic or specific-protection experiments.

It is our conviction that, in the case of the pathogenic clostridia at any rate, specific toxin-antitoxin experiments, where applicable, should be the final court of appeal in determining the identity or non-identity of two possibly different species.

Minor cultural characteristics, details of colony configuration and of individual morphology, etc., often show such variation even within the same strain under slightly different conditions of growth, that they afford unsafe criteria. Again, agglutination reactions in this group are notoriously unreliable owing on the one hand to the indefinite number of sub-groups liable to be encountered, and, on the other hand, to their frequent tendency to spontaneous agglutination.

It seemed necessary to settle the question: Does antitoxin prepared against the toxin of *B. sordellii* protect against the toxin of *C. oedematoides;* and, conversely, does the antitoxin prepared against the toxin of *C. oedematoides* protect against the toxin of *B. sordellii?*

Strains of *B. sordellii* were kindly furnished us by Professor Hall. Rather casual cultural comparison of the 2 organisms revealed no fundamental differences. The surface colonies on anaerobic blood agar plates were very similar, though *B. sordellii* was slightly more hemolytic than the other. Correlated with this, perhaps, it was found to be somewhat more powerfully proteolytic when growing on the surface of a Loeffler coagulated serum slant. No constant morphological differences were noted. Both organisms made gas and acid from dextrose but not from lactose, saccharose, salicin or glycerol.

A toxin of *B. sordellii* was prepared by heavily seeding a flask of cooked meat broth (1% Wittes peptone) to which 0.2% dextrose had been added. This was incubated for 40 hours in a modification of the McIntosh and Filds anaerobic jar, strained through glass wool, centrifugalized until clear, and finally sucked rapidly through a Berkefeld N filter. The resultant fluid was sterile, clear and slightly acid (pH 6.0). Injected subcutaneously into mice in doses of 0.02 mil, it caused death in 48 hours with slight gelatinous oedema. Preserved unsealed, even on ice, the titre fell off rapidly, 0.075 mils failing to kill 1 week later; when sealed with sterile vaseline, however, the titre was maintained fairly well.

Attempts were then made to immunize rabbits to the filtrate. Rabbits appear to be peculiarly susceptible to the toxin; and several

animals, apparently well on their way toward immunization were lost, owing either to too rapidly increasing dosage or to too short intervals between injections. But finally by starting with doses of 0.05 mils and injecting intravenously at weekly intervals, covering in all a period of 2 months, very gradually increasing the amounts, we obtained an animal that could withstand 1.5 mils without symptoms and whose serum was distinctly antitoxic. Eleven days after this last injection of 1.5 mils, the rabbit was bled to death.

Meanwhile a fresh filtrate from our *C. oedematoides* was prepared in exactly the same manner as described above. It resembled that obtained from *B. sordellii* in every way except that it was somewhat less acid (pH 7.0). The M.L.D. for mice was also 0.02 mil.

For oedematoides antitoxin we used some of the serum that had been made the previous year for our original experiments and which was still sufficiently potent.

Twenty gm. mice were injected intraperitoneally with mixtures of serum and filtrates as indicated below. The mixtures were incubated in the water-bath at 37° C. for one half hour before injecting.

Mouse No. 1—0.2 mil. *oedematoides* serum + 0.2 *oedematoides* filtrate. Result: No symptoms. Survived indefinitely.

Mouse No. 2—0.2 mil *B. sordellii* serum + 0.2 mil *oedematoides* filtrate. Result: No symptoms. Survived indefinitely.

Mouse No. 3—0.2 mil normal rabbit serum + 0.2 mil *oedematoides* filtrate. Result: Died within 24 hours.

Mouse No. 4—0.2 mil Saline + 0.2 mil *oedematoides* filtrate. Result: Died within 24 hours.

Mouse No. 5—0.2 mil *oedematoides* serum + 0.2 mil *B. sordellii* filtrate. Result: No symptoms. Survived indefinitely.

Mouse No. 6—0.2 mil *B. sordellii* serum + 0.2 mil *B. sordellii* filtrate. Result: No symptoms. Survived indefinitely.

Mouse No. 7—0.2 mil normal rabbit serum + 0.2 mil *B. sordellii* filtrate. Result: Died within 24 hours.

Mouse No. 8—0.2 mil Saline + 0.2 mil *B. sordellii* filtrate. Result: Died within 24 hours.

Thus it is shown that 0.2 mil of either *B. sordellii* or *C. oedematoides* antitoxin protects against 10 M.L.D. of the toxic filtrate of either organism, whereas normal serum has no such action.

Conclusion: Clostridium oedematoides as described by Meleney, Humphreys and Carp is identical to Sordelli's *B. oedematis sporogenes,* redescribed by Hall and Scott as *B. sordellii.*

To Sordellii is due the credit of priority, although our study of the organism was made independently and without knowledge of his work. The occurrence of Sordellii's strains and ours in such widely separated places is of interest.