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The Liquefaction of Inspissated Serum by the "Lamb Dysentery Bacillus."

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DALLING (1928) and later Mason, Ross and Dalling (1931) reported that the lamb dysentery bacillus liquefied inspissated horse serum, and emphasized this point when comparing it with classical *B. welchii*. Recently the present writer (1933) examined original and single cell cultures of the lamb dysentery bacillus, *B. paludis* (McEwen) and of *B. welchii*, and found that in no instance was solid serum liquefied. As possible explanations of this apparent anomaly, it was suggested that either the original lamb dysentery culture was contaminated with a proteolytic organism at the time when the first reports were published, or that Löffler's inspissated serum had, in the past, been used instead of solid serum.

While both these possibilities existed and were, at the time, the only ones that occurred as likely explanations, the writer was by no means convinced that they were the true reasons, nor did he feel at ease in suggesting them, for the following two reasons:—

1. The original lamb dysentery culture, upon which most of the work was done, was under constant observation for six years * and was subjected to some manipulation (plating, shaking, etc.), almost daily. With the exception of single cell isolation, every standard method of purifying anaerobes was adopted in the attempt to isolate a proteolytic contaminant. At no time was there any indication that the culture was impure. Further, cultures submitted to and returned from other workers, all expert in anaerobic technique, behaved like the original, both toxico-serologically and culturally.

In addition to the original culture, some 10 other strains were isolated from lambs affected with lamb dysentery. Whilst these were not submitted to the same detailed treatment as the original, still, the platings, shakings, and rapid subcultures that were conducted should have resulted, in all cases, in pure cultures. If the lamb dysentery bacillus had been, at the time, an accepted entity, the resultant cultures would have been accepted as pure.

The main difficulty in the acceptance of the purity of these cultures was the fact that they all rapidly liquefied solid serum. The time elapsing between inoculation and commencement of liquefaction varied, depending on the strain (and no doubt on the amount of inoculum, medium and anaerobiosis), but within one week every strain had to some definite extent caused liquefaction, and usually not more than 48 hours was required to see softening of the medium.

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Whilst the failure to demonstrate a proteolytic contaminant did not rule out the possibility of its presence, there was definite reason to consider that the cultures were pure.

2. That Löffler's serum medium was, on occasion, used instead of coagulated serum, is a possibility, but that it was used on all occasions is extremely unlikely.

The author's attention was again focussed on the subject by statements of Tunnicliff (1933) and of Dalling (personal communication). Tunnicliff, working on a lamb dysentery-like disease in the United States of America, stated that Dalling's lamb dysentery bacillus liquefied solid serum. Dalling, discussing with the writer the toxin-producing power of lamb dysentery and lamb dysentery-like organisms, noted that the toxin produced by his original organism, sealed off since 1922, differed from that produced by a serial subculture of that organism, maintained in the laboratory by short interval subcultures in meat broth. It was such a subculture that was brought to South Africa in 1931 by the author and on which the work already noted (1933) was carried out. Since this investigation was commenced Glenny, Barr, Jones, Dalling and Ross (1933) have published an article in which they state that the lamb dysentery bacillus has, since 1930, undergone a change in its toxin-producing power.*

Being given these facts, it occurred to the writer that there was the possibility that, in addition to an alteration in toxin production, the 1930 subculture of the lamb dysentery bacillus had also undergone another change, viz. it had lost the power of liquefying solid serum. The author had at his disposal a number of *B. welchii*-like anaerobes, including, through the courtesy of Dalling, a subculture of the lamb dysentery bacillus " 1922 " and of another recently isolated lamb dysentery strain " U 3 ". The cultures, other than lamb dysentery " 1922 " and " U 3 ", had all been " single celled " many times and no original culture had been retained. All strains were sown from young meat broth cultures on to inspissated horse serum and Löffler's serum medium and incubated in a McIntosh and Fildes' jar at 37° C. No tube (unless liquefaction was definite at an earlier date) was discarded until a month had elapsed.

Löffler's medium was liquefied rapidly by all cultures, being soft and liquid at the bottom of the tube in 5-7 days and nearly completely liquefied in 10-14 days. Charts 1 and 2 give the history of the lamb dysentery " 1922 " and " U 3 " strains respectively on solid serum and Table I of the other organisms on solid serum. It may be stated that when solid serum was liquefied by either lamb dysentery " 1922 " or " U 3 " this was evident in a few days and nearly complete in 10-12 days.

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* As this change in the toxin-producing power of the lamb dysentery bacillus will form the subject of a communication by Dalling and his colleagues, the writer is only at liberty to state that such a change has occurred, without indicating the nature of the change.

CHART 1.

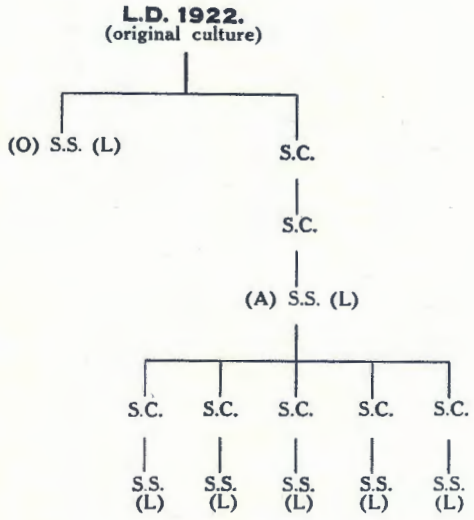

CHART 2.

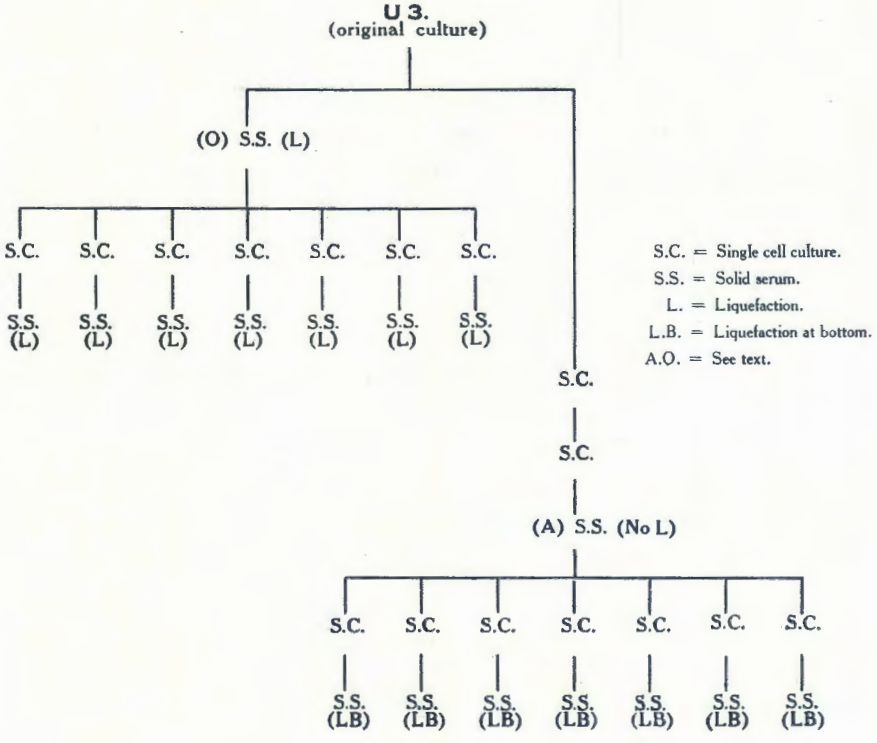

TABLE I.

Organism.	*Effect on Solid Serum* (one month).
L.D. 1930 (s.c.)	No effect.
Ovitoxicus (Bennetts) (s.c.)	,, ,,
Paludis (McEwen) (s.c.) ...	,, ,,
Welchii (S.R. 12) (s.c.) ...	Soft at bottom.

TABLE II. (See Charts 1 and 2.)

Organism.	*Type of L.D. Toxin Produced.*
L.D. 1922 O	1922
L.D. 1922 A	1930
L.D. 1930 (s.c.)	1930
U 3 O	1922
U 3 A	1922

s.c. = single cell culture.
O and A = See Text and Charts 1 and 2.

DISCUSSION.

Reference to Charts 1 and 2 and to Table I explains the apparent discrepancies in the statements of Dalling and Mason respectively on the serum liquefactive properties of the lamb dysentery bacillus. The orginal and single cell cultures of both lamb dysentery " 1922 " and " U 3 " produce rapid liquefaction, whilst, as previously reported, the 1930 subculture fails to do so. It is remarkable that, quite fortuitously, the first single cell (A) isolated from the " U 3 " strain was non-liquefactive: 6 daughter cells, obtained from it, produced after 3 weeks incubation, a softening and partial liquefaction of the bottom portion of the medium. On the other hand, 7 other single organisms separated from the original (O) serum culture, behaved as did their parent, viz. they produced rapid liquefaction.

Table 2 records the type of toxin produced by the lamb dysentery and " U 3 " strains. It will be noted that the toxin of lamb dysentery " 1922 " (original) and " U 3 " (original and single cell) was of the " 1922 " variety, whilst that of lamb dysentery " 1922 " (single cell) and lamb dysentery " 1930 " (single cell) was of the 1930 type. On each of three separate tests put up with the two last-mentioned strains the same result was obtained.

None of the other three *B. welchii*-like anaerobes produced true liquefaction within one month. *B. welchii*, itself, definitely softened the inspissated serum after 3 weeks' incubation, but this was confined to the bottom portion of the medium and was in no way comparable with the almost complete liquefaction by lamb dysentery " 1922 " in less than one fortnight.

The " U 3 " (single cell) A culture, whilst failing to liquefy solid serum, produced the " 1922 " type of toxin. How often such a variant may be obtained is unknown, but the fact that such a one has been demonstrated shows that the absence of liquefactive power is no proof that a strain cannot produce the " 1922 " type of toxin.

The relative unimportance of liquefactive power in its connection with toxin production is further exemplified by the fact that lamb dysentery (single cell) A brought about rapid liquefaction but yet produced the 1930 type of toxin.

Conclusions.

1. The original statement of Dalling, that the lamb dysentery bacillus rapidly liquefies inspissated horse serum has been confirmed.

2. A serial subculture of Dalling's original strain has lost its liquefactive power.

3. The power of liquefying solid serum (or the lack of this power) should not be applied as a "major" test in classifying a lamb dysentery-like micro-organism.

Acknowledgement.

I have much pleasure in thanking my former colleague, Major T. Dalling, M.R.C.V.S., for supplying cultures of the lamb dysentery bacillus (L.D. "1922" and "U 3") and for the suggestion which initiated this short investigation.

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