

February 28, 1953

Dr. P. R. Edwards  
Box 185  
Chamblee, Ga.

Dear Dr. Edwards:

In a couple of days, I expect to be sending the cultures to be mentioned. I am writing first to warn you about them, and because I have just a free minute right now.

The kunzendorf story seems to be straightening out clearly. By one passage through an appropriate serum (minnesota-240; morehead, etc), one easily recovers from 6145-52 *S. cholerae-suis* kunzendorf a c-phase (SW-958). The same type can be isolated from the very rough, slow-growing blobs that eventually spread out in kunzendorf serum SS. As I noted at Chamblee, the SW-958 type migrates readily through morehead, etc., but not through kunzendorf serum. These observations have been repeated here several times now with proper attention to verifying purity of cultures, etc.

At Chamblee, I was disturbed not to be able to demonstrate the agglutinability of SW-958 type in kunzendorf serum. The same thing happened here, but it seemed there had to be a reaction judging from the inhibition of motility. It turns out that the unique fraction (let me call this c' for now) of SW-958 is rendered inagglutinable by formalin. c' is also at least as labile as c to heat: nearly inactivated at 60°, 30 mins., complete by boiling, 10 mins. With overnight, living cultures tested directly in Penassay broth, c' agglutinated to a titre of 1:200 in kunzendorf, 1:500 (trace at 1:1000) in berlin serum. No reaction was observed at 1:50 in lw; d; enr; 1,2 (#157); 1,2,3 (Colindale: puerto-rico?); or 1,5 serums from minnesota 240; new mexico 20; paratyphi A 228, or morehead. It was still present in 5-serum (berlin absorbed with #157). SW-958 also agglutinated to the titer (ca. 50,000) of c serum (Blindale), whether formalinized or not.

c' is also present in your #153, though somewhat less well developed (living cells react with ~~kunzendorf serum~~ berlin serum at 1:100, but not 1:500). Unfortunately, I do not have your c serum, but would suspect that it contains anti-c' (judging from the agglutinability of #153). It would be useful to test for the presence of c' in kunzendorf 1,5... (predicted from the c'-agglutinin in the serum).

This seems to be nearly all that I am equipped to do with this problem. Am I missing something very trivial in wondering about a formalin- and heat-labile antigen, or have the likes of this already been described? The behavior of kunzendorf seems to be explicable on the hypothesis that its phases are actually c, c': 1,5, c'... SW-958 seems to be quite stable in c-serum SS, presumably free of c'-agglutinin, so that the stability of this strain is not solely a matter of the c' factor. The agglutinogenicity of c' must be more stable to formalin than its agglutinability. Nevertheless, the best approach to a proper purified reagent

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for c' might be to immunize properly prepared rabbits with living cultures of SW-958 (or even # 153). It might be easier to remove c from such a serum, using SW958C (958 selected through kunzendorf serum for loss of c'), SW-902 (c:- from altendorf -x b:-), or other c, non-c', phases than it would be to remove all of the 1,5... complex from the berlin serum. Could it be that the fairly low titer for c', on the one hand, and the failure to recognize it (?) previously, on the other, are both due to the inactivation by formalin? Until a clean c' reagent is available, it will be difficult to study the distribution and possible identity with other factors.

The evidence that c'  $\neq$  c is : 1) Colindale<sup>c</sup>-serum does not agglutinate the kunzendorf strain SW961, although it does, of course, SW-958, 958C, and SW-902 (c:-). On the other hand, berlin serum does not agglutinate either 958C or 902, but does 958. 2) Formalin destroys the agglutinability of 958 with berlin serum, but does not impair its reaction with c-serum. There is no proof that c' is a flagellar antigen, but it is heat-labile, and anti-c' inhibits motility at remarkably high dilution. Some of the group C nonmotiles, if they can be made to revert (as I know some can) may be helpful in this respect.

There are just a few odds and ends I can still do with this— check on the remaining serums, e.g., old and new para A 228, and on the stability of the c phases. Do you think the problem important enough to warrant an active interest on your own part?

Other experiments are in progress, some possibly disappointing. There are three --1,2 stocks I have been testing as recipients x- zega d: z<sub>6</sub> in an ~~effort~~ effort to obtain a --:z<sub>6</sub>. They are your #19k; Kauffmann's 5594-51; and "Hines V.A.H." Do you have the history of 191 and of Hines? I am rather disturbed that all three seem so far to be behaving like #157, i.e., as phase 1 homologues. At least, the first trials x- zega have given d phases in each case, rather than the otherwise expected z<sub>6</sub>. These are being tested further. #157 -x miami a:1,5 is swarming out now, should give us 1,2:1,5.

For the attempt at securing --:1,7, the stock bredeney strain is unhelpful as it is resistant to PLT-22. If you have a number of other bredeney's, or other B-D group stocks with authentic 1,7... phases, they would be worth trying. I am just hoping something will turn up that will clarify the monophasic phase-2 situation: there are still some angles worth trying.

Stock S. napoli, lz<sub>13</sub>, is also unsusceptible. Are there any others anywhere that could be tried? Finally, the two S. salmatis are both unsusceptible, so I think this problem had better be deferred for the moment. Please let me know if any other useful combination within the B-D groups should occur to you; it is a useful test of the measure of our experimental control to have problems that are posed on other grounds than a priori possibility.

Sincerely,

Joshua Lederberg

3/1/53 Resume:

Sent: SW-958 = 961 selected through 1,5 (~~morehead~~) serum. (c, c')

SW-958C = 958 selected through 1,5,c' (kuzendorf) serum. (c)

SW-961 = S. cholerae suis, kuzendorf, your 6145-52 1,5...

Requested:

- S. bredeney to test PLT-22 susceptibility, especially non-XXVII forms.
- S. abortus equi (if any come to hand) for same purpose. I already have #26.

Information: ?

—:1,2 Hines V.A.D., and #191 Origin? Can these be classified as paratyphi B?

JL