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Standfast says impossible to get price of reprints till paper set up, but he is sure there will then be time to consult you & get reply. (He sends proofs to US now by air he says)

30th June, 1953.

Dear Josh. (sending copy to Norton I.D.)

Thanks for your air letter of the 30th May, and for suggestions in it about paper. The paper went in in the middle of May, and was accepted after some weeks cogitation. I just managed to get the typescript back yesterday, the editors have made or suggested only a few minor alterations of wording. I have been over it again and have dealt with all the points you mention (except ones where the matter criticised has been cut out anyway). I enclose a few pages which I had retyped. I gather the stuff goes to the printers in a few days, but if you see anything that should be corrected in proof I hope you will point it out.

I saw Felix today, to let him look over, at his request, the bits in which he is quoted. All went well as to this. I have restored the "Dr." etc., he agreed that the new wording re B Vi was more suitable; he rather wanted me to put in a bit about how stable Glasgow O was as indicated by use in serodiagnosis, etc. but agreed it was non-essential. Actually he was quite affable, though I always approach these interviews with some trepidation. He was very disappointed to hear you are not going to be at Rome.

Kauffmann has toldme about his and your strains which acquired I at the same time as an H antigen, this is a surprising thing. It would be interesting to try and get a I - form of SL13, serial enrichment in broth with anti-I as worked out by Clive S out to work, one would then perhaps be able to get triple transfers (if the I is really a transduction, not selection of I+ mutant). I saw Clive today, seems well, not doing any transduction stuff as he is involved in locum duties.

(K. found no XII$_2$ in SL13)

I have done a few more micro-manipulation experiments. It seems that the majority, or half anyway of motile cells picked early (about 3 hours at 37° after mixing with phage) give rise, when incubated at room temperature to the equivalent of more than 1 trail. The maximum so far is the equivalent of 5

with λ and gal (but I doubt it): thank heaven the Salm. business is not so complex as K-12. I am amazed to hear that the AIBS organises its groups by sex of component persons rather than subject. How (2) clever of E to make a ♀ with the type-writer. Thanks for the de-strict correction. (tho. too late to correct.) BS.

trails from one cell. It is now clear that there is a discrepancy with results on semi-solid medium at 37° on which I am no longer satisfied that I can get even twin trails with the same combination (TM2 - X SW541). Have done a few experiments using semi-solid agar (no gelatin) incubated at 23°, but again no definite multiple trails. I suspect reason may be that cells concerned are insufficiently motile to move through soft agar, from a sort of dose effect. Possibly also the disintegration of clump of replicated chromosome fragment only occurs at low temperature. All this is on the assumption that there is either normal or no replication of the imported gene; it's hard to believe there is an intermediate cond-ition. Also so far as the results go there is no evidence yet of segregation of motility preceding clonalisation; I have separated products of first division after isolation of various cells, two or three of which gave all (or nearly all) motiles from both progeny, never motiles from one and non-motile from the other; some of these single-cell isolations were made using non-log-phase cultures, so that the population at the time the cells were captured was only about X2 that present when phage added. This is difficult to account for on a multi-strand theory. Maybe there is a master strand which can pair with chromosome and a lot of others which can't. A propos this have you seen any significant instances of swarm arising from a trail? I have seen it several times, but inretrospect I suspect that it may have been mutation, as the most clear-cut case was in SW573, where the trail was probably made of paralysed but H mutants.

Another odd thing is that either gene is more than 5-plicate at times, or else phenotypic lag can be up to 5 generations, for in one of the cases where I had 5 trail-equivalents I picked 18 motile cells from a drop and got only non-motile progeny from them all. Mortality still a nuisance, even in lysogenic recipient.

I don't understand what you say (May 30th) about triphasics, but the mimeo of your and Edwards paper has just arrived, perhaps I will grasp it after reading that.

On reading over the above after typing, it seems, like the situation described, somewhat obscure. The situation itself may be clarified when I get some more experiment done: it's one in the eye for me, for I felt pretty sure, ~~but~~ before I started, that everything would go as predicted on hypothesis of single gene either entering chromosome or floating free.

I have got out the NCTC S.tm strains you want, will post tomorrow. Have sent stocks of the gal +/- one to Clive S to test on Gal. EMB as I have no gal. medium made up.

I said I would go up to Meeting of Genetics Soc. next week, & read joint paper (as for Bellagio), I fear I did not get your NZ's permission first but I take it this is OK. I enclose abstract. ~~This~~ My apologies for not sending before. My love to Esther: of course I don't have any opinion ~~on~~ on idea elimination occurs. I hope I shall be able to understand the 2N XN crosses

so that motile strains spread through it as they grow; but because of its high gelatin content it solidifies at room temperature. To economise materials, this medium was used in 5 cm. Petri dishes, about 10 ml. per dish. Inoculated plates were incubated uninverted for 10 to 18 hrs. at 37°, allowed to solidify at room temperature and examined for swarms or other evidence of bacterial movement by inspection with a plate microscope and oblique transmitted light. In plates incubated for 10 hrs. swarms were usually only about 3 cm. in diameter and, if not numerous, were still discrete. But after 18 hr. incubation even a single swarm would generally have spread right across the plate.

BEHAVIOUR OF UNTREATED NON-MOTILE STRAINS GROWN ON SEMI-SOLID MEDIUM

The growth of most of the non-motile strains on semi-solid agar was strictly confined to the site of inoculation, even when incubation at 37° was continued for 48 hr. or longer (Pl. 1, fig. 1). Some strains, however, gave rise to spontaneous swarms which on sub-culture were found to consist of stable, motile, H-agglutinable mutants. In many such strains this mutation was a rare event, so that only a few plates showed swarms. In others the mutation was more frequent, so that most or all plates showed spontaneous swarms, but they usually appeared rather late, that is later than the 12th hour of incubation; this presumably indicates that only after several hours of incubation was the population of cells large enough, in relation to the mutation rate, for there to be more than a small probability of a mutation occurring. A few O strains were discarded because of their inconveniently high rate of mutation.

S. typhimurium O strain SW545 grew on the surface of the semi-solid medium at the site of inoculation and also produced large numbers of micro-colonies, singly or in small

groups, one mm. or less below the surface of the agar (Pl. 1, fig. 2). Sub-culture from these colonies yielded only stable O forms similar to the original strain, and indeed H mutants have never been obtained from strain SW545. This phenomenon is being further investigated, but it seems probable that it results from the spontaneous occurrence in this strain of a very small fraction of cells which are motile, and therefore migrate through the agar, but which produce only non-motile progeny. Micro-colonies separate from and deep below the site of inodulation have been seen in several other O strains, but in smaller numbers than in SW545.

The centre, and therefore presumed point of origin, of a swarm was frequently occupied by an area of a few mm. in diameter in which there was a dense crowd of micro-colonies, of diameters inversely related to their distance from the centre of the area. This appearance, which is illustrated in Pl. 1, fig. 3, will be termed a "flare". Numerous single-colony isolations from flares crowded with micro-colonies always gave stable <u>motile</u> cultures similar to those obtained from the periphery of the swarm, which indicates that the micro-colonies consist of cells which give rise to motile progeny. Flares have also been obtained by inoculating a few cells of certain partly rough motile strains onto semi-solid agar. The mechanism of the formation of micro-colonies near the point of origin of swarms is not clear; the trapping of motile cells amongst agar fibrils perhaps plays a part, but partial roughness or other physiological or genetic factors which hinder the progression of the initial motile cells may be involved.

The appearance of non-motile strains grown on semi-solid agar has remained constant from experiment to experiment. When a strain was treated with phage 22 propagated on that same strain, the behaviour of the phage-treated cells, as to

production of swarms or isolated deep colonies, was exactly the same as that of the untreated culture. This would be predicted if phage in itself has no effect on hereditary properties, such as motility, but can carry across genetic material from its last host; when the genetic material of the last host and the recipient is identical, its transfer from the one to the other will have no detectable effect.

Behaviour of non-motile strains treated with transducing lysates and incubated on semi-solid agar

In tests for transduction of motility a broth culture of a non-motile strain was incubated with an equal volume of a lysate for 30 min. to allow phage absorption. Three loopfuls of the mixture, or the whole of the deposit obtained by centrifuging 1 ml. of the mixture, were then placed on semi-solid agar and incubated at 37° for 10 to 18 hr. The appearances produced when transduction of motility occurred are illustrated in Pl. 1, fig. 4, which may be compared with Pl. 1, fig. 1, which shows the same recipient strain plated without phage treatment. With combinations such as that illustrated, the inoculated area was occupied by dense growth, and surrounded by a wide border of confluent swarming which extended throughout the depth of the agar; when a smaller number of phage-treated cells were inoculated, this continuous border was replaced by discrete swarms (Pl. 1, fig. 5). The centres of the swarms were sometimes marked by flares, as described in the previous section. Unlike spontaneous swarms, these induced swarms were for the most part well developed by the 12th hour of incubation. Sub-culture from the edge of a swarm always gave a pure growth of motile organisms, and motility was retained on further sub-culture. The growth obtained by sub-culture from the swarms differed from the parent non-motile strain by its motility and H agglutinability, and,

amounts detectable by agglutination tests) and carry phage B2 only. This evidence is not unequivocal for the isolation of both V-positive and V-negative strains from the same patient (Kauffmann, 1934) suggests that antigen V may appear or disappear by mutation; and strains may be made lysogenic for phage A2 in the laboratory, and presumably therefore this may also occur in nature. It is therefore uncertain whether this group of O strains are all derived from a common O ancestor, or whether they have arisen on two or more occasions by mutations perhaps affecting the same gene.

The high frequency with which lysates of O strains render motile other O strains might suggest that the production of H forms under these conditions is not dependent on the transfer of genetic material from the lysed strain, as has been argued above, but is a consequence of exposure of an O strain to phage 22 grown on <u>any</u> heterologous host. However phage grown on a spontaneous H mutant of O strain SW543 evoked numerous swarms from its ~~pres~~ parent O strain. Similarly a lysate of an induced H form obtained from O strain SL15 evoked swarms from its parent O strain. In each case a lysate of the O strain itself had no effect. Tests with other O strains and their spontaneous or induced H derivatives have given similar results. Each of these H derivatives, so far as we know, differed from its O parent only by the presence of flagella, and of the gene determining this; but phage grown on the H derivative could confer motility on the O parent. Thus motility may result from exposure of an O strain to phage grown on a strain which is not an unrelated strain, provided that it possesses the gene which is lacking in the recipient O strain.

Flagellar Antigenic Phase Latent in Cells of O Strains

Motile forms of S. typhimurium, obtained from O strains by transduction, underwent diphasic flagellar variation exactly as do normal H cultures of S. typhimurium. The first culture from any one swarm gave growth which was agglutinated strongly by antiserum for one of the two H antigens, weakly or not at all by antiserum for the other; that is, the swarm was (predominantly) in one phase. The phase of induced swarms was determined by the recipient O culture, and not by the inducing lysate. This was demonstrated by treating single colony broth cultures of O strains with lysates of each of a number of different strains. In each such experiment all or nearly all of the swarms resulting from transduction (or arising ~~fr~~ by spontaneous mutation), proved to be in one and the same flagellar phase, even when the lysate used was made from an H strain known to have been almost entirely in the opposite phase at the time of lysis. A single colony broth culture, as used in these experiments, would have been derived from one cell by a small number of generations, and would therefore be nearly homogeneous, even for a character which, like flagellar antigenic phase, mutates at a very high rate (Stocker, 1949). Cells of an O strain may then be considered to be in one flagellar antigenic phase or the other, though this character is unexpressed in the absence of flagella.

Experiments on Non-Motile Flagellated ("Paralysed") Strains

All motile Salmonella strains have flagella, but several non-motile flagellated strains have been reported (Colquhoun & Kirkpartick, 1932; Kauffmann, 1939; Edwards, Moran & Bruner, 1946; Hirsch, 1947; ~~Prew~~ Priewer & Leifson, 1952). Hirsch's strain of S. paratyphi B and Priewer & Leifson's strain of S. typhimurium