the second sample was drawn. The results of this series are shown in Table II.

Anesthesia usually produced a progressive rise in blood sugar values. Initial values in the two series ranged between 37 and 130 mg. per cc. In two instances (No. 8, Table I, No. 5, Table II) when the animals had not been fed for two days, the level remained practically constant throughout a period of 95 minutes of anesthesia. In all other cases the figures were distinctly higher within 5 to 10 minutes.

The initial figures are generally higher in the experimental series, for which no explanation is attempted, as the animals were selected at random from stock. Curves constructed from these tables, but not included in the paper, are so nearly identical that it can only be concluded in the basis of these experiments that irradiation with a special carbon arc emitting a high percentage of ultra-violet rays has no influence on blood sugar of etherized dogs.

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## The Bacteriophagic Relationships Between B. Coli, S. Fecalis and S. Lacticus.

PHILIP HADLEY AND EUGENIA DABNEY.

*From the Department of Bacteriology, University of Michigan, Ann Arbor, Mich.*

The systematic relationships existing between *S. fecalis* and recognized streptococci of the hemolytic and viridans group, or with acid-forming organisms common to the intestinal tract, such as *B. coli, S. lacticus (B. acidi lactici), M. ovalis* or the "Enterococcus" of Thiercelin and others, have long been a subject of controversy. Andrews and Horder[1] attempted to establish the

fecal streptococcus as one of their six streptococcus types. Holman[2] placed it among his various types of streptococcus. Brown,[3] in his comprehensive review of the blood-reactions of the streptococci, states: "Furthermore, strains of *S. fecalis* are green producers and therefore belong to the *S. viridans* group as originally proposed by Schottmüller." Gordon[4] established, on a basis of serological tests, three types—*S. pyogenes* (hemolytic), *S. salivarius* (viridans), and *S. fecalis* ("Enterococcus"). These he also distinguished on the basis of hemolysis, raffinose-fermentation and mannite-fermentation. He also regarded these groups as serologically distinct. Dible,[5] largely on the basis of fermentation tests, attempted to show that there existed an identity between *S. fecalis* and the Enterococcus of Thiercelin, often mentioned by French writers. Kendall,[6] in 1924, placed *S. fecalis* with the greening and hemolytic streptococci in tabular form, but avoided in the text a discussion of its actual position. He mentioned *M. ovalis* as probably identical with the Enterococcus but did not relate it to *S. fecalis*. Ayers and Johnston[7] stated that *S. fecalis* was identical with, or at least similar to, *S. lacticus*. They used as criteria (1) heat-resistance, (2) morphology (oval form), (3) cell-grouping (short chain) and (4) mannite-fermentation. Thus we have some diversity of view on the position of *S. fecalis*.

Without here taking occasion to enter into the consideration of merits or demerits of these various conceptions regarding the actual affiliation of *S. fecalis*, we wish to report briefly the results of certain experiments which seek to attack the problem of the position of this organism from a somewhat different point of view, namely, the bacteriophagic inter-relationships of some of these bacterial species. More explicitly, we desire to place on record the results of some cross-tests involving the use of the bacteriophage or lytic principle, active against *S. fecalis*, *B. coli*, *S. lacticus* and, incidentally, *B. typhosus*. It is, however, not within the scope of this paper to consider in any way the nature of the lytic agent or the mechanism of bacteriophagic action.

Quite recently the junior author and E. M. Brill isolated independently a bacteriophage active against a typical S type strain (No. 77, Dental College Collections) of *S. fecalis* obtained about one year ago by Rickert and Hadley[8] from the pulp chamber of an infected tooth. It agreed in morphological, fermentative

and serological characters with the typical fecal *S. fecalis,* probably the most common agent in root-canal infections. In addition, a fresh lytic principle was isolated for S type *B. coli* (Jordan strain). These two lytic agents, having been enhanced to a high, but probably not maximum, titer against their respective homologous substrata, were employed in preliminary cross-tests against the homologous and heterologous cultures, and the results several times confirmed. The method was as follows:

Six tubes filled with 10 cc. of sterile beef infusion broth (pH 7.8) were set up, containing

1. One lp. fecalis culture plus 5 gtts. fecalis lytic filtrate.
2. One lp. coli culture plus 5 gtts. fecalis lytic filtrate.
3. One lp. fecalis culture plus 5 gtts. coli lytic filtrate.
4. One lp. coli culture plus 5 gtts. coli lytic filtrate.
5. One lp. fecalis culture _____
6. One lp. coli culture _____

All tubes were incubated 12 hours at 37° C. and examined for inhibition of growth. Tubes 1, 2 and 4 were perfectly clear. Tubes 3, 5 and 6 showed strong (normal) clouding. The appearance of the tubes was essentially the same, except for further clouding of the control tubes, after 96 hours at room temperature. Thus, the fecalis lytic filtrate gave complete inhibition against *B. coli* as well as against the homologous substratum. The coli lytic filtrate, on the other hand, while completely inhibiting the homologous culture, had no influence on *S. fecalis.* These tests were repeated several times with similar results. The employment of one drop of lytic filtrate instead of five drops gave approximately the same effect. Indeed, a dilution of $10^{-4}$ of the fecalis lytic filtrate yielded complete inhibition of *B. coli,* while a dilution of 1:1,000 gave complete inhibition of *S. lacticus.*

In view of these findings it seemed desirable to ascertain the bacteriophagic relationship of *S. lacticus* to *S. fecalis* and *B. coli.* Accordingly a bacteriophage active on an old laboratory strain of *S. lacticus* was produced and the following tests performed. At the same time a comparison was made with the action of the fecalis lytic principle.

From Table 1 it is apparent that lytic filtrates active against both *S. lacticus* and *S. fecalis* give a complete inhibition of growth of *B. coli* as well as of the respective homologous culture. How-

TABLE I.

Showing action of lytic principle for *S. fecalis* and a lytic principle for *S. lacticus* against *B. coli, S. fecalis* and *S. lacticus.* (Results in terms of inhibition.)

| Substratum: One loop of culture in 10 cc. broth. | Amount of L. P. Lacticus | | Amount of L. P. Fecalis | | Culture control |
|---|---|---|---|---|---|
| | 5 gtts* | 1 gtt** | 5 gtts | 1 gtt | |
| *B. coli* | C | C | C | C | — |
| *S. fecalis* | — | — | C | C | — |
| *S. lacticus* | C | C | C | 2+ | — |

*Dilution of L. P. calculated at 1:50; ** as 1:250.
(C) complete inhibition of growth for 24 hours or more.
(3+), (2+), (1+) intermediate grades of inhibition.
(—) no inhibition observed.

ever, while the filtrate active on *S. lacticus* has no action on *S. fecalis,* the fecalis lytic filtrate is effective in inhibiting the growth of *S. lacticus.*

Having now ascertained the existence of a bacteriophagic relationship between *S. fecalis* and *B. coli,* also between *S. lacticus* and *B. coli,* as well as an asymetrical relation between *S. fecalis* and *S. lacticus,* it was of interest to observe the possible relationship between the streptococcus strain and *B. typhosus.* A bacteriophage for an old laboratory strain of the S type (Eberth) was therefore produced and used in the tests described in the following table, along with a repetition of the former tests. The method was the same as previously described.

TABLE II.

Showing the action of lytic principle derived for *B. typhosus, B. coli, S. lacticus* and *S. fecalis* respectively, against the homologous and heterologous substrata. (Results expressed in terms of inhibition.)

| Substratum: One loop of culture in 10 cc. broth. | Added one drop* of L. P. for | | | | Culture control |
|---|---|---|---|---|---|
| | Typh. | Coli | Fecalis | Lacticus | |
| *B. typhosus* | C | 3+ | 2+ | 2+ | — |
| *B. coli* | C | C | C | C | — |
| *S. fecalis* | — | — | C | — | — |
| *S. lacticus* | — | — | C | C | — |

Symbols the same as in the preceding table.
*Dilutions calculated as 1:250.

From the results presented in Table II, which serves as a summarizing statement of all the reactions previously considered, it

appears that the fecalis and lacticus lytic filtrates behave in the same manner when brought into action against *B. coli* and *B. typhosus* substrata. Moreover, that the fecalis and lacticus substrata comport themselves in an identical manner toward *B. coli* lytic filtrate and toward *B. typhosus* lytic filtrate.

It may be added that the lytic filtrates in each case were checked for their ability to produce typical lytic areas when streaked, in appropriate dilution, against the homologous substratum. The titer was such that one or more standard loops of (one standard loop of stock lytic filtrate plus 10 cc. of sterile broth) gave countable areas (*i. e.*, a few to 100) after incubation for 24 hours on slanted agar. The maximum observed inhibition-titers in broth for the various filtrates were as follows: *B. coli*, $10^{-7}$; *B. typhosus*, $10^{-8}$; *S. fecalis*, $10^{-9}$; *S. lacticus*, $10^{-7}$. That is to say, a $10^{-7}$ dil. of *B. coli* filtrate in 10 cc. of broth inhibited the growth of one standard loop of young coli broth culture.

We are of the opinion that the evidence suggests, though by no means demonstrates, the affiliation of the fecal streptococcus with members of the colon-typhoid group of bacteria rather than with the true streptococci as represented by the viridans and hemolytic types. Indeed, the bacteriophagic reactions of *S. fecalis* and *S. lacticus* show a considerable degree of uniformity. That the relation is close, however, is by no means necessarily implied, for we know that the reactions of any specific lytic principle often, and perhaps usually transcend the limits of the species and group. This has already been demonstrated by one of us[9] in an instance in which typhoid lytic principle caused lysis of *B. pullorum* and *B. gallinarum*, as well as of the homologous substratum. In this case the reaction was also in large measure reciprocal, and was in harmony with the corresponding serological tests. However, we know of no instance in which a specific lytic agent, already well adapted to a member of the colon-typhoid-dysentery group, has been found to act directly against such distantly related bacterial species as staphylococcus or streptococcus. At the same time we see that, within narrower limits, the bacteriophagic relationships between various species seems to exist on a fairly broad footing; and that these relationships are certainly more comprehensive than those demonstrated by the usual serological tests. Indeed, the bacteriophagic relationships seem to be more in harmony with those serological reactions ex-

isting between the R types of bacteria (from active microbic dissociation) represented by Schütze[10] in his so-called "serological cosmopolitanism" of the R forms; also by the commonly recognized serological heterogeneity of the pneumococcus types as indicated by the studies of Stryker,[11] Yoshioka,[12] and, more recently, Reimann[13]; also by the serological heterogeneity of the O and R types of *B. typhosus* and *B. enteritidis* as studied by Goyle.[14] To what extent serological reactions among R type cultures (as opposed to S type cultures, or cultures in any other cyclogenic state) parallel bacteriophagic reactions may prove an interesting study and one that may bear closely upon significant aspects of the problem of transmissible autolysis.*

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* Since this paper went to press the authors have tested the action of fecalis lytic principle against strains of *S. viridans* and hemolytic streptococcus. No inhibition was observed in any case.