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The "Source" of the Lytic Principle.

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Our experiments involve attempts to generate lytic principle by enforcing dissociation through the use of (1) commercial pancreatin extracts unheated, (2) similar extracts heated at 60° C., or sterilized by heat, (3) peptone solutions sterilized by heat, (4) sterile distilled water, and (5) bacterial cultures unmodified by any foreign substance. Three brands of pancreatin were employed, Squibb's, Difco and Armour's. The peptone was the Parke Davis Co. "bacteriologic peptone." The cultures used comprised a stock strain of the Shiga dysentery bacillus and a strain of *B. coli* (Jordan). In both species, fully sensitive, S type cultures relatively free from O and R forms were employed.

Pancreatin extracts were prepared in sterile broth having a pH of 7.8. First, varying amounts, heated at 60° C. or unheated, filtered (Berkefeld N) or unfiltered, were placed in contact with young Shiga and coli broth culture on sterile 20 cm. agar plates. Such plates were found to tolerate loading with even 1.0 to 1.5 cc. of the extract, spread evenly over the slightly dried surface. No sign of lytic areas was observed after incubation. The same extracts, however, heated at 60° C. for 30 minutes and filtered through a Berkefeld N candle, when added in much smaller amounts to tubes of broth seeded with young Shiga culture, resulted in the generation of active lytic principle within two to six serial filtrations. Minute amounts of the principle so generated always registered directly by the production of lytic areas when the plate method was employed. The Squibb pancreatin extracts were the most effective, yielding active lytic principle in the Shiga cultures after the first filtration. This was followed by Difco pancreatin (fourth filtrate) and by Armour's pancreatin (sixth filtrate). Only the Squibb pancreatin caused the generation of lytic principle in cultures of *B. coli* (fifth filtrate). Thus, although the ability to generate quickly an anti-Shiga lytic principle was manifested by all the samples, no evidence was gained that any of the original extracts contained lytic principle, as such, in a "preformed" state.

In consequence of these findings, the suggestion of Hoder and Suzuki,[1] that the lytic units might be present in great number in pancreatin, but intimately "bound" to the organic constituents, was

taken into consideration. If such a binding were possible it might be expected that, unless the pancreatin were already "saturated" with lytic corpuscles, this substance should be able to bind more of the principle which had already been produced by the use of other samples of the same pancreatin (Squibb). Accordingly, to such dry or moistened pancreatin in tubes, was added small amounts of the homologous lytic filtrate previously obtained, and the samples allowed to stand for 24 hours at incubator, room or ice box temperatures, respectively. The samples were then dissolved or suspended in sterile broth and this was employed for tests by the direct plate method. As a result, no reduction in the amount of the lytic principle could be detected; there was no evidence of absorption by the pancreatin, nor any evidence of the destruction of the lytic principle by the pancreatin, within the time allowed for the tests.

The question then arose as to the specific ingredient of commercial pancreatin that might be responsible for the influence. The enzymes were naturally considered. Accordingly, there were prepared new extracts which were then heated in the autoclave at 110° C. for 30 minutes, or in some cases at 120° C. for 20 minutes, and finally cleared by Berkefeld filtration. Here, at least, there was no question of the presence or influence of active enzymes. Small amounts of such filtered extract were added to broth tubes which were then inoculated with a few drops of Shiga broth culture. The customary rotation of procedure, incubation, filtration, addition of fresh broth, feeding, was carried out successively. No indication of lytic action of the successive filtrates was observed until the seventh filtration had occurred. The eighth serial filtrate, when tested by the plate method, gave distinct lytic areas accompanied by broad lysis. From this eighth filtrate a strong lytic agent was subsequently built up. It thus appeared that the active enzymes contained in the Squibb pancreatin could not be responsible for the power of the Squibb extracts to generate lytic activity in the bacterial cultures.

The elimination of the active enzymes of pancreatin as a factor in the generation of lytic principle suggested the use of simpler organic preparations than pancreatin. Among these, peptone was next tried. The dissociative influence of peptone solutions on bacterial cultures has several times been noted. The solutions were prepared and sterilized at 110° C. for 30 minutes. They were then seeded with a small amount of young broth culture of the Shiga bacillus and incubated. Next followed alternate feeding and filtration in series. As a result, distinct lytic action, accompanied by the production of large and typical lytic areas, first appeared in the tests of the ninth serial filtrate. From this a strong lytic agent was eventually built up.

The success attained in the foregoing tests suggested simplifying still further the conditions of the experiment; and, in the following tests, use was made of sterile distilled water added to the culture tubes. Here, the method departed somewhat from that used with the peptone and will be reported in detail in a later publication. Suffice it to say, for the present, that a battery of broth tubes received varying amounts of sterile, distilled water and were then seeded with young culture of the Shiga bacillus; or, in another experiment, with *B. coli*. After growth occurred certain of these tubes were combined, filtered and re-distributed in 10 cc. portions, after which the procedure was repeated in series. As a result, lytic action appeared in the fifth serial filtrate for *B. coli* and in the eighth for Shiga. In another test it appeared in the ninth Shiga filtrate. From this stage a strong lytic agent was built up for each species.

Since it could scarcely be expected that a bacteriophage endowed with the power of life could be present in sterilized pancreatin extracts, sterilized peptone solution or in sterilized distilled water, it seemed possible that the experimental conditions might be even further simplified and still yield positive results. Accordingly, an attempt was made to generate the lytic agent in broth cultures of Shiga organisms without the addition of any foreign substance whatever. Three tubes of Shiga broth culture, aged, respectively, 24, 36, and 80 hours, were combined in a single tube. A small volume of fresh sterile broth was added, also a few drops of young Shiga culture. The tube was then incubated overnight. In the morning it was filtered and fresh broth, together with more culture, was added. After this, the same procedures were carried through twelve series, but without any distinct manifestation of lytic action on plate tests. The fourteenth serial filtrate, however, manifested clear lytic activity on plates, and from this filtrate a strong lytic agent was evolved by further passage.

From these experiments it appears that either fresh or sterilized pancreatin extracts, or sterilized peptone solution, in none of which can any bacteriophage, as such, be demonstrated at the beginning, can serve as the incentive for the generation, or "liberation", of abundant lytic principle in broth cultures of the Shiga bacillus or of *B. coli*. It appears, furthermore, that exactly the same result can be obtained with both cultures by the employment of sterile, distilled water only. And finally, it is also demonstrated that the same result appears, but after a longer time, in cultures where no foreign substance whatever has been brought to bear on the growing organisms.

For us, the results of these and related tests, taken in conjunction

with reports of a somewhat similar nature by other investigators, carry the definite implication that the bacteriophage is generated *de novo* by the bacteria themselves, under the "liberating" influence of a specific environment. We already have evidence that this "liberating" influence is closely associated with the phenomenon of microbic dissociation. What the specific environment may involve, remains for further study.

Finally, and most important, it must be added that the strains of *B. coli* and *B. dysenteriae* Shiga employed for the preceding experiments were "normal", sensitive, type S cultures, maintained uninterruptedly for many years in the laboratory collection. They are non-lysogenic, in the common meaning of this term, and are not open to the imputation of "contamination" with the bacteriophage in the d'Herelle sense.

[1] Hoder, F., and Suzuki, K., *Centralbl. f. Bakt.*, Abt. I, Orig., 1926, xcviii, 433.

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Advanced Development of Some Echinoid Plutei.

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Employing a modification of the method of Allen and Nelson[1] for rearing echinoderm larvae, I have been able to observe the development of three Pacific coast forms. These were the sea urchins, *Strongylocentrotus purpuratus* and *S. franciscanus,* which developed to advanced pluteus stage, and the sand urchin, *Dendraster eccentricus,* which readily underwent metamorphosis.

About 20 early plutei were put into a sterilized finger bowl containing 75 cc. of sterilized San Miquel sea water, to which a drop of the culture of *Nitschia closterium* was added. The dish was then covered with a glass plate and set in a shaded place, where the temperature during the entire summer did not vary beyond the limits 15° to 17° C. Every 5 or 6 days the larvae had to be transferred to a fresh dish of modified sea water, in order to prevent their being overwhelmed with the growth of diatoms.

After 3 to 4 weeks the plutei of *S. purpuratus* and *S. franciscanus* developed a third pair of arms, into each of which a supporting skeletal spicule grew. All the arms became very long, and then deteriorated, without the animals giving indication of any steps toward metamorphosis. These larvae always rested on the bottom.