

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

66TH STREET AND YORK AVENUE
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Dear Joshua:

Congratulations and best wishes on your award. No one deserved it more. Had I known I really would have made some effort to come to the meetings.

My part of the bacterial genetics course went very well and the data we obtained on the Lac, V¹ segregations agreed beautifully with the published data. The teaching of the coli story forced me to sit down and seriously review ~~the~~ it. In doing so the appeal of the Hayes' hypothesis became apparent. In the simplest of ways it encompasses almost all of the complexity of coli recombination. Without it things tend to be somewhat chaotic and explanations of the findings not at all concrete. One of the difficulties I found was that I'd been equating mating frequency with recombination frequency within a small factor. Is it possible that matings occur as Hfr or better in all cases, and that ~~but~~ it is in the region of producing successful and viable recombinants that the strain differences lie.? That is F- X F- produces no matings, F- X F+ many matings but only a few viable recombinants, etc. This would be one way of explaining Hayes' peculiar Hfr strain. Are there any data other than from the heterozygotes (which as you may know are not too kindly looked upon in some quarters) that bear on the timing of the elimination. I was aware of the het data and had to sit on my hands at CSH to keep from presenting them but the het itself is an anomaly and to explain one anomaly with another can oftentimes be a compounding of errors. Enough of coli and let me tell you of some of the new things in transduction.

Before I go any further I suppose that you've seen the paper by Baron in the Proc Soc. I wish he had consulted either of us prior to its submission. His misquote on the preparation of FA and that uncomprehensible ~~data~~ dose-response curve has created some confusion. From ~~that~~ paper alone one could quite easily draw the conclusion ~~that~~ the phage had nothing to do with the transduction.

While at CSH, Garen, as an able tutor, and I played with the ~~transduc~~ PLT-22V and the following information is now available. High titer 22V can be obtained by growing LT-2 to a little better than 10^8 /ml, centrifuging and resuspending in fresh broth giving a burst size of some 500 and about 10^{11} phage. Shaking with chloroform removes most of the bacterial debris and also makes the solution sterile for further manipulation. Three alternating cycles ~~of the Spinco~~ of centrifugation yields very pure phage (22,000 g in the Spinco sufficient).

The phage was hydrolysed and its NA composition determined. Unfortunatley it has the usual bases in equimolar composition, about 1/3 the amount of T2 which is about right for its size. A priori, it seems unlikely that a temperate phage would have different base composition from its bacterial host.

We also determined the transfer of phosphorus from the bacterium to the phage progeny and found it to be on the order of 20% which is too high for ~~that~~ there to be any specificity involved and just about kills any hopes of being able to identify the transducing part. Another experiment with labeled material bears ^{on} this point. The transducing material is turned over in each phage generation while with T2 about 30 % of the phosphorus label is transferred to the progeny phage.

Phage was labeled in two ways 1) hot bacteria with cold phage and cold medium 2) cold bacteria and phage and hot medium. The former should have the label in large molecule DNA prior to its incorporation into the phage progeny, the latter comes direct from the medium. The transfer from these batches of phage to their progeny was measured on the hope that one would turn over and the other wouldn't. Within the limits of the sensitivity of the method, which is quite poor, both transferred 30 % to their offspring. Obviously the bacterial contribution to the phage is too ~~ix~~ large and non-specific and overshadows the probably small amount of transducing material.

There are of course several further things that might be done to aid in the localization and characterization of the transducing part. The most ~~xx~~ promising being some experiments whereby sufficient hot phosphorus is incorporated such that the disintegrations lead to a measurable loss of viability of transduction and plaque formation depending upon the method of incorporation. This, if it works, may not provide any more information than the U.V. data but one can never be sure in biology.

All in all there is almost as much information about PLT-22 as there is for T2. It certainly is a lovely phage to work with and in some respects even more interesting than T2 considering the diversity of systems that one can study with it. I have enough mutants now to begin a recombination analysis and test the randomness of FA distribution in both growing and recombining (detectably that is) phage populations. I have been kicking around the notion that ~~their~~ there may not be an ~~identity~~ identity of the particles that produce transduction and those that produce plaques. By incorporating ~~enough~~ enough anti-phage serum in the selective ~~agar~~ agar it may be possible to show that transduced cells from sensitive populations become lysogenic for the mostpart by plate infection.

I hope this finds you all well. Busy I'm sure you are. Wish you'd come East one of these days and give us a chance to repay your hospitality. I was terribly shocked to hear of the death of Dr. Huskins. I guess he died of a broken heart. If you should see DTB tell him to drop us a line about his his new offspring etc. We're expecting another one next spring. A bit more planned than the first.

Best regards to everybody,

Sincerely,

