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*THE INFECTION OF SUB-CELLULAR ESCHERICHIA COLI, STRAIN B, WITH A DNA PREPARATION FROM T2 BACTERIOPHAGE**

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The ability of deoxyribonucleic acid (DNA) to carry genetic information was first demonstrated for the pneumococcus-transforming principle.[1] Even after the facts of transformation had been well established for several years, it was regarded by many as an anomaly, or at best of very limited scope, until the demonstration by Hershey and Chase[2,3] that the DNA of T2 bacteriophage carries all the genetic information necessary for reproduction of this virus. It is obvious that a system whose genetics is as well developed as that of the T2 phage[3] would be ideal for chemical genetic studies and investigations of the mechanism of action of fundamental genetic material. This paper describes experiments which demonstrate infection of protoplasts of *Escherichia coli*, strain B, with a preparation of disrupted T2 bacteriophage. As will become apparent, we believe the infective agent to be some form of DNA; but for the purposes of this discussion and pending physical and chemical characterization, we shall designate it as "DNA preparation" or simply "DNA."

METHODS AND MATERIALS

In general, the bacteriophage techniques were those described by Adams.[4]

Protoplasts.—We have modified our previous procedure[5] by concentrating the *E. coli* from 2×10^8 to 5×10^9 cells per milliliter (instead of to 5×10^8) and adding more lysozyme (0.1 ml. of 1 mg/ml per milliliter of bacteria) and more Versene (0.2 ml. of 1 per cent per milliliter of bacteria). An excellent and convenient semiquantitative criterion for the degree of protoplasting is the lack of streaming birefringence of the spherical protoplasts compared to the starting rodlike *E. coli*.

DNA Preparation.—Three milliliters of concentrated, purified T2 bacteriophage[6] at 5×10^{11} viable T2/ml was diluted into 27 ml. of concentrated urea solution (8 *M* urea in 0.1 *M* saline adjusted to pH 8.2 with hydrochloric acid). The phage titer dropped from the starting 5×10^{10} to about 10^5/ml in 1 minute and to about 500 viable T2 in 3 minutes. After 1 hour at 37° C., the preparation was dialyzed overnight at 2° C. (Visking tubing) against 3 liters of 0.1 *M* saline, with stirring inside and outside the dialysis tube, and then against a second 3 liters of 0.1 *M* saline for about 4–6 hours. Micro-Kjeldahl analyses of the dialyzate showed that all diffusible nitrogenous material was removed by this procedure. All such preparations

of phage DNA were viscous and slightly opalescent and contained no viable phage when plated straight and after dilutions in the usual medium.

A Standard "DNA Infection" Experiment.—Virtually all the experiments were done by slight variants of the following technique (all solutions and media have been previously described[5]). The aerated *E. coli* were grown in 3XD to 2×10^8 cells/ml, centrifuged, and resuspended in D at 10^9 cells/ml (when the protocol required pre-infection with T2) or in 0.1 *M* tris. The cells were then washed (usually twice) with cold 0.1 *M* tris buffer. The culture was resuspended at 5×10^9 in protoplasting medium, and a part was protoplasted as described above. For DNA infection, 0.5 ml. of either this preparation or a 1 : 10 dilution of it in protoplasting medium was mixed at room temperature with 0.5 ml. of the above DNA preparation, either straight or diluted 1:10 in 0.1 *M* saline. (The cell controls consisted of a sample of the unprotoplasted suspension treated in a parallel fashion.) After 10 minutes, this adsorption mixture was plated and/or diluted further in B-BSA.

B-BSA.—The "broth" of previously reported experiments[5] has been modified in the present work by the addition of bovine serum albumin (BSA). Armour 30 per cent sterile solution of BSA was diluted, with sterile technique, 1 volume into 14 volumes of sterile broth. This medium (B-BSA) was used for dilutions throughout the present experiments except as noted.

EXPERIMENTS AND RESULTS

Table 1 shows a typical experiment illustrating the infection of protoplasts with T2 "DNA." The chief feature of this experiment, as of all similar ones, is the complete absence of plaque-forming units in controls consisting of either protoplasts or "DNA" compared with the large numbers found in the mixture immediately, and with the greater numbers (some hundred fold) found after incubation of the mixture at 37° C. These experiments are completely reproducible and have been done many times with several lots of T2 "DNA." Because of the importance of establishing the nature of the infective agent, the remainder of this paper describes experiments designed to eliminate sources of misinterpretation and artifact and to establish the phenomenon beyond reasonable doubt.

Ordinary Controls—In all the experiments presented, blank platings of the plating bacteria were negative. Blank platings of the "DNA" and of experimental bacteria and protoplasts were made routinely both at the beginning of an experiment and after dilution into the nutrient medium (B-BSA) and incubation for the full time of the experiment. No experiment was considered valid unless the possibility of accidental pre-infection was completely eliminated by such negative controls.

TABLE I*

INFECTION OF *E. Coli* PROTOPLASTS WITH T2 "DNA"

	Plaque Counts at Zero Time	Plaque Counts after 75 minutes at 37° C.
0.5 ml. T2 "DNA" + 0.5 ml. protoplasting medium	0	0
0.5 ml. T2 "DNA" + 0.5 ml. 1/10 protoplasts	6.5×10^5	6×10^7
0.5 ml. 0.1 *M* saline + 0.5 ml. 1/10 protoplasts	0	0

* In this and all similar experiments several dilutions in B-BSA were incubated and plated. The figures given are corrected for dilution to show plaque-forming units per ml. of original mixture.

"Masked" T2.—In no experiment was the total yield of recovered T2 more than a very small proportion (*ca.* 0.1 per cent) of the total T2 equivalents represented by the "DNA" used. In Table 1, for example, the input "DNA" was equivalent (in the adsorption tube) to 2.5×10^{10} T2/ml. The yield was 6×10^{7} T2/ml on the same basis. The following experiments were designed to eliminate the possibilities that (*a*) the DNA preparation contained T2 masked by the DNA, other degradation products, or traces of urea; (*b*) the "yield" represented initially inactive, but still intact, phage particles revivified in some way; or (*c*) the "yield" represented phage particles dissociated by the urea—i.e., into protein and DNA—and reconstituted by the action of protoplasts.

a) Possible inhibition of T2: Platings of several concentrations of T2 mixed with either 0.1 *M* or 0.01 *M* urea did not differ from control platings. Traces of urea in the preparation, therefore, are not inhibitory. An actual DNA preparation was also shown not to inhibit the plating of deliberately added T2.

b) Possible revivification of reversibly inhibited T2: Control platings of the DNA preparation diluted and incubated parallel to the experimental samples were consistently negative. The possibility of revivification by action of some agent leaking from the protoplasts—conceivably urease—was examined particularly because, by this hypothesis, protoplasts might be expected to show (as they did) much greater effects than intact cells. Logically, maximum effects would then be expected by exposure of the DNA preparation to a lysate. Tests were made with a lysate made by the identical treatment used for protoplasting, but with omission of sucrose from the protoplasting medium,[7] and also with sonically oscillated *E. coli*. Neither caused the appearance of T2 immediately or after incubation for 1 hour. The mixture of a standard experiment after adsorption of "DNA" and protoplasts showed 3,909 plaque-forming units/ml. When it was centrifuged cold for 12 minutes at 11,000 rpm in the Spinco No. 42 head, 100 per cent of the plaque-forming material sedimented, demonstrating that it was not free T2. Moreover, when the mixture of DNA and protoplasts was shocked into water at the end of the adsorption time, infective centers were completely eliminated. We have shown that identical treatment has no appreciable effect on ordinary T2.

Table 2 summarizes an experiment in which it was shown that the DNA preparation can infect protoplasts of T2-resistant B/2. The mechanism of "DNA infection" must, therefore, be quite different from the normal mechanism of cell inva-

TABLE 2*

INFECTION OF PROTOPLASTS OF *E. Coli* B/2 WITH T2 "DNA"

	Plaque Counts at Zero Time	Plaque Counts after 120 minutes at 37° C.
0.5 ml. B/2 protoplasts + 0.5 ml. 0.1 *M* saline	0	0
0.5 ml. B/2 protoplasts + 0.5 ml. T2 at 8.7×10^{5}/ml	8.6×10^{5}	9×10^{5}
Plated on B/2	0	0
0.5 ml. B/2 protoplasts + 0.5 ml. T2 "DNA"	800	2.7×10^{5}
Plated on B/2	0	0
Shocked 1/10 in water	0	0
0.5 ml. protoplasting medium + 0.5 ml. T2 "DNA"	0	0

* See footnote to Table 1.

sion by whole phage.[8] Since it is apparent that many of the possible complications of casual infection, etc., can be eliminated by the use of B/2, it might seem more sensible to have used this strain exclusively. This was not done for purely practical reasons, since B/2 grows much more slowly than B, is generally less convenient to handle, and, by our procedure, forms protoplasts somewhat less satisfactorily than B.

Any explanation of the present results which presupposes that the DNA preparation contained some form of T2 must assume further that the protoplast preparation contained some whole cells or else that protoplasts can be infected by ordinary T2. We have demonstrated repeatedly[5,7] that our preparations contain less than 0.1 per cent of viable cells by ordinary plating, and by direct microscopic observation we have found routinely less than 0.1 per cent (the limit of practical observation) of cell-like structures. We have failed completely in many attempts to infect protoplasts with intact T2 under conditions like those of the "DNA" experiments. Noninfection of protoplasts has, moreover, been the consistent finding of other workers in the field.[9]

c) Possible reconstitution of T2: The assumption might be made that DNA is being recoated with existing protein as in the reconstitution experiments of Fraenkel-Conrat[10] on tobacco mosaic virus. The protein might conceivably be present in a urea-denatured form re-natured by the action of protoplasts. Another assumption might be that DNA is entering the protoplast and synthesizing T2 protein coats with no net DNA synthesis. These assumptions are certainly possible in view of the low yield of phage compared to the total DNA input, but they require that the action occur either in the solution (a possibility which is eliminated by the centrifugation experiment above) or in the protoplasts themselves. In either case one would expect behavior quite different from that observed in the single-burst experiment of Table 3. By the former assumption, one would expect the action to be strongly cur-

TABLE 3*

SINGLE-BURST EXPERIMENT WITH PROTOPLASTS AND T2 "DNA"

	Plaque Counts at Zero Time	Plaque Counts after 3 Hours at 37° C.	Average Burst
1.5 ml. protoplasts + 1.5 ml. T2 "DNA"	6.9×10^4†	1.2×10^7†	180
1st 1/10 dilution	...	All samples *ca.* 1.2×10^7†	...
2nd 1/10 dilution‡	...	9, 181, 125, 31, 253, 127, 678 244, 399, 599, 366; 5 samples 0	247
3rd 1/10 dilution	...	110, 292, 227, 356; 12 samples 0	246

* The adsorption mixture was diluted 1/50 × 1/10 × 1/10 × 1/10 × 1/10, and one drop (0.069 ml.) of each of the 1/10 dilutions was diluted into each of 16 tubes (containing 1 ml. of B-BSA) which were incubated for 3 hours, mixed with 1 ml. of double-strength top agar containing bacteria, and poured on a plate.

† Corrected for dilution to original mixture.

‡ By Poisson distribution 3 tubes would be doubly infected.

tailed by dilution. If the reconstitution is occurring *within* certain protoplasts, we would predict from the fact that protoplasts are not limiting and from the one-hit nature of the data of Table 4 that, with rare exceptions, only a single phage could arise from each protoplast. As can be seen in Table 3, the average of the individual bursts which occur equals the burst size in mass culture.

Any assumption of co-operation between degradation products in a reconstitution requires a very strong dependence on the dilution of the "DNA." Table 4 shows

however, that the yield of infective centers is linear with dilution of the "DNA." This agrees with the experiments of Levinthal and Thomas,[11] which show that 40 per cent of T2 nucleic acid consists of a single "big piece" of DNA necessary for reproduction. The remaining 60 per cent is composed of smaller, apparently non-essential pieces. Since the "big piece" and the smaller pieces have been shown by star-counting to be separated by the urea treatment used in our experiments[12] and since the results of Table 4 preclude any co-operative action of more than one particle in infection, we concur in the belief that there is a single essential piece of DNA.

TABLE 4

DEMONSTRATION OF SINGLE-PARTICLE INFECTION BY T2 "DNA"

0.5 ml. Protoplasts + 0.5 ml. of:	Plaque Counts at Zero Time	Plating Dilution	Average Corrected for DNA Dilution
T2 "DNA" straight	32, 38	1/500	17,500
T2 "DNA" diluted 1/3	683, 466	1/10	17,250
T2 "DNA" diluted 1/9	259, 158	1/10	18,750
T2 "DNA" diluted 1/27	88, 85	1/10	23,600
T2 "DNA" diluted 1/81	32, 20	1/10	21,100
T2 "DNA" diluted 1/243	8, 7	1/10	18,200

The Infected Host.—In addition to the above experiments demonstrating that the early-eclipse infective centers are *not* T2, experiments have been done to show that they *are* all infected protoplasts. An odd feature of all the present "DNA infection" experiments was the immediate appearance of large numbers of infective centers. The experiments with pre-infected protoplasts indicated that they are unstable and that the eclipse period is characterized by essentially zero infective centers which survive plating.[7,13] This would seem to indicate that the DNA-infected host is not a protoplast.

The essence of the discrepancy between these and our previous results lies in the use of 2 per cent BSA in all dilutions of the present series of experiments. By direct microscopic observation and by turbidity measurements we have found, in accordance with Zinder and Arndt,[14] that protoplasts, either infected or uninfected, are tremendously more stable in a dilute BSA solution. We have now shown directly in experiments with pre-infected protoplasts (Fig. 1) that the BSA greatly enhances the apparent numbers of early infective centers. In the "DNA infection" we find quite similarly a few stable infected hosts but many more which survive plating after the addition of BSA. It is of interest to compare this finding with the parallel increase in the activity of pneumococcus-transforming DNA through the use of BSA.[15]

Figure 1 shows a single-step growth experiment in which we have compared the properties of pre-infected cells and pre-infected protoplasts with those of "DNA-infected" protoplasts. It is obvious that the infected host in the "DNA" experiment is like a protoplast in properties and not at all like an intact cell normally infected. It is interesting to note that in the two protoplast systems phage production is apparently not sharply terminated, i.e., the normal lysis mechanism is not operating.

Still further demonstration that the zero-time infective centers are actually infected protoplasts, as differentiated from stray infected cells and from stray T2, is the repeated observation that a 1-to-10 dilution through distilled water completely destroys the plaque-forming ability of the "DNA"-protoplast mixture (Table 2).

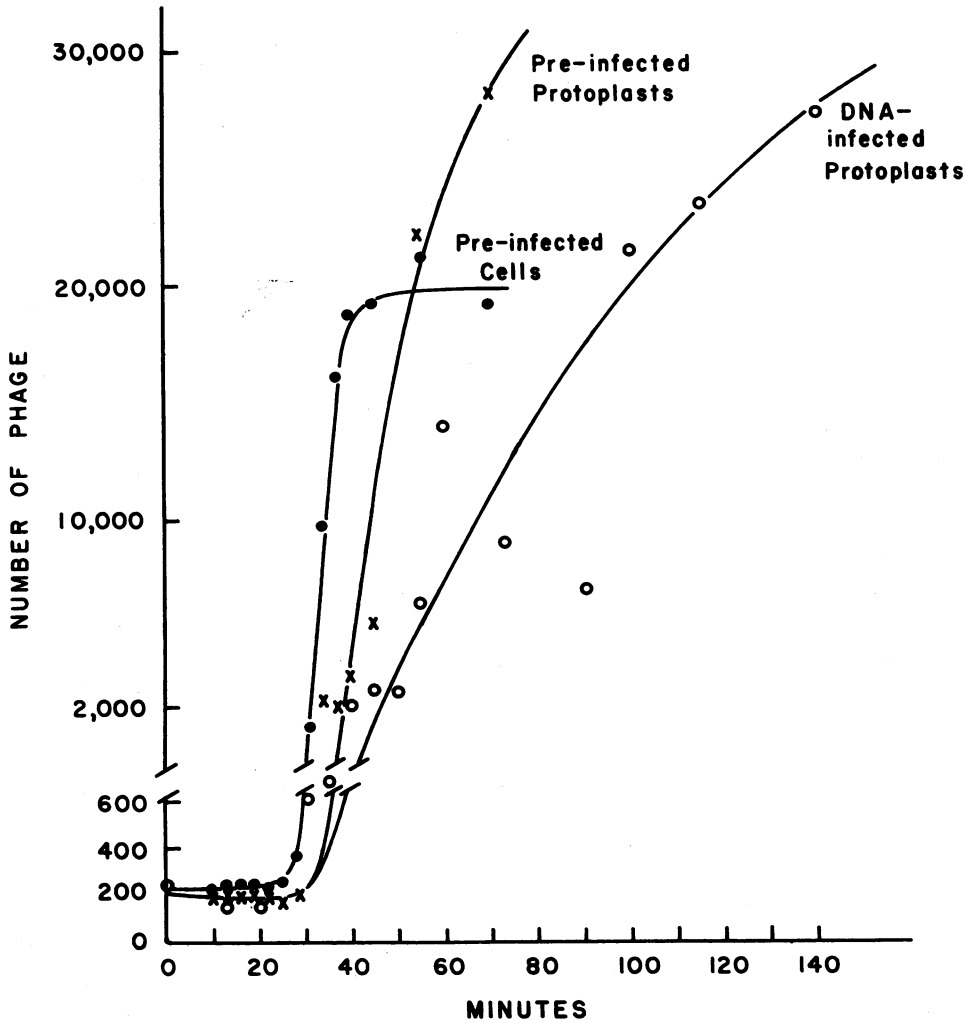

FIG. 1.—Comparison of single-step growth curves of pre-infected cells, pre-infected protoplasts, and "DNA-infected" protoplasts. Procedures described in the text. Latent-period plate counts arbitrarily adjusted to the same level. Considering dilution differences, this amounts to multiplying the pre-infected counts by 3, the "DNA-infected" counts by 2000.

The sum of our experience thus leads us to the belief that the host in "DNA infection" is certainly subcellular and probably the same protoplasts observed in our pre-infection experiments.[7] It is important to realize, however, that the objects of infection, at present efficiencies, might easily be a type far too rare to be observable.

The Nature of the Infecting Material.—Obviously it is essential to show not only that the infecting material cannot be T2 but that it *is* DNA. The most direct evidence at present is treatment of the urea preparation with DNase. One-half of a mixture of 3.1 ml. of T2 "DNA" and 3.1 ml. of water (total DNA estimated to be 150 μg.) was treated for 80 minutes at 37° C. with 0.1 ml. of 0.05 *M* $MgSO_4$ and 0.04 ml. of 1 mg./ml. DNase solution. The increase in optical density at 260 mμ in control experiments indicated essentially complete reaction in 5 minutes. Mixture

of such treated T2 "DNA" with protoplasts under standard conditions gave only some 3 per cent as many initial plaques as did the corresponding untreated half of the DNA material (3×10^4 versus 9.9×10^5). The remote possibility that the infecting material is T2 whose tail specificity has been altered by urea and whose DNA is now susceptible to DNase action cannot be discarded at present. The present results also do not exclude the possibility of protein bound to the DNA but show only that the T2 DNA is now accessible to DNase action, which was not true before urea treatment.

The Infection of "Whole Cells" with "DNA."—In virtually all the experiments on "DNA infection," controls have been run with non-protoplasted cells handled identically except for the addition of lysozyme and Versene. It was quite puzzling at first to find a small but persistent amount of infection in this control. This was, in fact, one of the main reasons for the great care which was taken to establish the absence of T2 in the preparation and the fact that the infected system behaves like protoplasts and not like cells. In the face of many completely negative control platings of cells and of the urea preparation alone and under experimental conditions identical except for the presence of the cells, we have been forced to the belief that it is indeed possible to infect non-protoplasted cells, particularly non-protoplasted B/2. The facts that this infection is reduced markedly by the prior action of DNase on the urea preparation and that it occurs with B/2 differentiate it sharply from normal T2 infection.

We invariably observed infection with the DNA preparation in cells which had been prepared for protoplasting, i.e., chilled, washed, and resuspended in the protoplasting medium before the addition of the DNA preparation. It is of interest to compare this with the enhanced cellular permeability effects observed by Jerne and Maaløe,[16] who found strong inhibitory action by RNase on the reproduction of T4 in *E. coli* suspended in distilled water. In previous experiments,[17] which will be reported separately, we have found that our washed cells always behave in part like protoplasts in that, although still rod-shaped, they are somewhat susceptible to osmotic shock, showing a loss of turbidity of about 25 per cent. Brief teatment of our pre-infected cells (i.e., ordinary whole cells) with RNase did not inhibit T2 growth,[5] but RNase treatment before "DNA infection" sharply inhibited phage reproduction, indicating to us that the particular "DNA" infectible cells are unusual and protoplast-like.

We suspect that this phenomenon has not been previously observed, primarily because it would not be detectable unless the T2 background were zero, as in our current preparation, and unless the cells had been made susceptible in some way. Because of the obvious importance of establishing whether such a DNA infection, possibly akin to pneumococcus transformation, might exist in nature, we intend to investigate this effect further.

DISCUSSION

Protoplasts, by our method and definition, are spherical bodies about the size of the original cell but much less stable to rapid changes in tonicity and incapable of forming colonies when plated on ordinary agar. We have also shown that under appropriate conditions our protoplasts are permeable to ribonuclease (RNase) and apparently to deoxyribonuclease (DNase), since both these enzymes lead to specific

removal of the homologous nucleic acid from protoplasts[12] and have strong effects on the reproduction of bacteriophage in protoplasts pre-infected with T2 and T3.[5] We have also found that specific antiphage serum can profoundly influence the course of phage reproduction in pre-infected subcellular *E. coli*.[18]

The infection with "DNA," then, is presumably another demonstration of the ready accessibility of the phage-replicating centers in protoplasts. In view of the tremendous size of the T2 DNA "big piece,"[11] one must question whether simple "permeability" is the answer. As alternatives, one might pose some specific entry mechanism or the hypothesis that the synthetic machinery is totally exposed.

The obvious items of next importance seem to us to include the separation, purification, and fractionation of the DNA and the improvement of the efficiency of infection to the point that genetic recombination experiments become possible. There would also seem little reason to suppose that the DNA from T2 is the only nucleic acid capable of instituting self-replication in *E. coli*. It will certainly be necessary to try using DNA from other viruses, DNA from genetically marked strains of bacteria, and even RNA with recognizable properties.

SUMMARY

T2 bacteriophage treated with 8 *M* urea is known to release much of its DNA. We have found that such a preparation was devoid of viable phage but, upon mixture with protoplasts of *E. coli*. strain B, and also the T2-resistant strain B/2, produced T2. Evidence has been presented to support the contention that actual infection of the protoplasts with DNA has occurred.

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Shortly after the preparation of this manuscript a paper appeared describing very similar observations (J. Spizizen, these PROCEEDINGS, **43**, 694, 1957). The methods of preparation of the protoplasts and of disrupted phage are quite different from those of our work, and the sensitivity of the infecting agent to various treatments (DNase, heat, Versene) appears to be at marked variance with our findings.

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[1] R. D. Hotchkiss, *J. Cellular Comp. Physiol.*, **45**, (Suppl. 2), 1, 1955.

[2] A. D. Hershey and M. Chase, *J. Gen. Physiol.*, **36**, 39, 1952.

[3] A. D. Hershey, *Advances in Virus Research*, **4** (New York: Academic Press, Inc., 1957), 25.

[4] M. H. Adams, *Methods in Medical Research*, **2** (Chicago: Year Book Publishers, 1950), 1.

[5] D. Fraser and H. R. Mahler, *Arch. Biochem. and Biophys.*, **69**, 166, 1957.

[6] R. C. Williams and D. Fraser, *Virology*, **2**, 289, 1956.

[7] H. R. Mahler and D. Fraser, *Biochim. et Biophys. Acta*, **22**, 197, 1956.

[8] S. E. Luria, *General Virology* (New York: John Wiley & Sons, Inc., 1953), p. 164.

[9] M. R. J. Salton, *Bacteriol. Revs.*, **21**, 82, 1957.

[10] H. Fraenkel-Conrat and R. C. Williams, these PROCEEDINGS, **41**, 690, 1955.

[11] C. Levinthal and C. A. Thomas, Jr., *Biochim. et Biophys. Acta*, **23**, 453, 1957.

[12] Unpublished observations.

[13] M. R. Salton and K. McQuillen, *Biochim. et Biophys. Acta*, **17**, 465, 1955.

[14] N. D. Zinder and W. F. Arndt, these PROCEEDINGS, **42**, 586, 1956.

[15] M. S. Fox and R. D. Hotchkiss, *Nature*, **179**, 1322, 1957.
[16] N. K. Jerne and O. Maaløe, *Acta Pathol. et Microbiol. Scand.*, **40**, 362, 1957.
[17] Joan M. Sams, thesis, Indiana University, 1957.
[18] Janet Rowe, thesis, Indiana University, 1957.

*GENETIC INTERACTION AMONG STREPTOMYCETES: HETEROKARYOSIS AND SYNKARYOSIS**

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The streptomycetes comprise a group of coenocytic filamentous microörganisms having many biological properties in common with the true bacteria, although superficially resembling the asexual molds. The industrial importance of certain species has focused considerable attention on the group as a whole. A wealth of information is available relating to their taxonomy, morphology, biochemistry, and other traits,[1] but, at the time the present work was begun, no experimental evidence for the existence of a sexual cycle within this group was available. The purpose of these studies was to survey the genus *Streptomyces* from the aspect of genetic interactions, to throw some light on the genetic mechanisms operating within these microörganisms, and to develop methods for controlled breeding experiments. Standard techniques for recombination analysis[2] in bacteria and for the study of heterokaryosis and sexual or parasexual reproduction in filamentous fungi were modified and applied to the streptomycetes. There were two main approaches: genetic analysis employing biochemically marked mutant strains, and cytological examination. The property of vegetative reproduction by the formation of uninucleate conidia proved to be useful.

Cytological Studies.—A colony of streptomyces is composed of three major elements: vegetative mycelium, aerial mycelium, and conidia formed in chains at the tips of the aerial hyphae. The nuclear structure of these elements was studied by light microscopy, utilizing the thionin stain with modifications described by Hunter-Szybalska *et al.*[3] Conidia of all the streptomyces species employed in this study appeared to be uninuclear, a very convenient feature for genetic studies. These nuclei divided after the conidia had been incubated approximately 3 hours in nutrient broth at 28° C. Germination with the production of one to two germination tubes occurred as early as 3 hours (*S. griseus*) to as late as 6 hours (*S. fradiae*), with still greater variation for several deficient mutants. The resulting vegetative mycelium was of very small diameter, varying from 0.6 to 0.8 μ, and was filled with an average of 8 chromatinic bodies for every 10 μ of length. The aerial mycelium was of approximately double thickness and exhibited a more distinct nuclear structure. No figures indicative of hyphal fusion and exchange of nuclei were observed with the species employed, in accordance with the genetic data, which imply a low frequency of heterokaryon formation. Visual observation of anastomosis was recently described by Gregory,[4] and the nuclear cytology of *Streptomyces* sp. T-12 has been discussed by McGregor.[5]