COLD SPRING HARBOR LABORATORY OF QUANTITATIVE BIOLOGY

ANNUAL REPORT 1968

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DIRECTOR'S REPORT

To start with, let me express the strong debt which everyone connected with Cold Spring Harbor owes to John Cairns. When in June, 1963 he came here as director, he inherited a terrible mess. (We were badly in debt and most of our buildings in thorough disrepair.) Many outsiders thus thought his task insuperable, and that Cold Spring Harbor would soon cease to exist. But with much devotion, intelligence, and great indulgence of his free time, he again made the Laboratory a going concern. Without his achievement, I would have never taken over as director. Happily his decision to give up the directorship because of his desire to return to a full time research career, need not mean his loss from the lab and our community. We are now actively seeking means which will enable us to offer the long-term financial security which he and his family so warmly deserve. Hopefully we can soon announce that this has been achieved, and that he will decide to remain permanently as a senior staff investigator.

Now, about my plans for the future:

Since I have been coming regularly to Cold Spring Harbor for the past twenty years, my first eight months here as director do not represent an introduction to a new world or tradition. Over these two decades, fortunately, I see no major changes in its important qualities – the unhurried conversations still center on challenging scientific ideas and our uniquely beautiful and quiet site continues to provide a perfect setting for thoughts, perhaps fuzzy late in the evening, to coalesce over breakfast or lunch the following day. Also the fact that we are such a small community usually nullifies the differences between ages and social hierarchies, and virtually no discussion or evening party is limited to a privileged in-group. As soon as he arrives the most junior of students will be as well fed and housed as the most distinguished of our guests, and hopefully he soon will be doing experiments which he feels are important, and with perseverance he may hit jackpots, at least of a minor kind.

There also remains the fact that the Lab badly needs a real benefactor, but with much love it will probably survive without one. Of course, I dream an angel will appear soon and make me free of any serious worries for at least a month. Now most directors of other scientific institutions must also have this fantasy since Federal funds for the support of science are much more difficult to find now than at any time in the past decade, and this tight fiscal situation is likely to prevail for some years. Already a very large fraction of Federal research grants cover vital salaries, and so the recent cutbacks by the various government agencies are handled by restricting funds for supplies and equipment – compromises which all too easily lead to cautious experiments where some results, even if unexciting, can be obtained.

I believe, however, that despite these limitations the pace of our activity both year round and in the summer will increase markedly over the next several years. The reason why this can happen is that, as in the past, Cold Spring Harbor has never been representative of the average state of biology, but is and must be the place where trends of the future are anticipated. This was true of our role in the development of microbial and molecular genetics. Many of today's most distinguished investigators came here to take our summer courses before they made their famous discoveries, and it is our belief that while at Cold Spring Harbor they often learned what was important to do, and went back home to do so. To make this possible, we must choose both an exciting staff and bright students and, of course, the presence of either group helps breed the other.

We are therefore very concerned whether our current series of summer courses is still worthwhile, or whether we should move in a more avant-garde direction. Here I suspect that the Phage and Bacterial Genetics Courses remain highly valuable and should continue – we see no lessening in the quality of our students or in the problems with which they work. It is clear, however, that a great expansion of our efforts in teaching the genetics and biochemistry of higher cells is in order. A multitude of important problems (e.g. how the genetic material of a virus changes a normal cell into a malignant cell) may conceivably be solved in the next few years. So our Cell Culture and Virus Course, most ably taught these last several years by Phil Marcus and Gordon Sato, will become three courses next summer. One will center on animal cell culture, another on animal viruses, while the third will deal with cell transformations by tumor viruses. This expansion is made possible by the renovation this past year of the top floor of the James Laboratory as a teaching laboratory. The ground floor of James will also be focused toward animal cell work, as we hope to initiate there a year-round effort on the molecular biology of tumor viruses, an effort which should materially assist the related summer teaching programs. As in previous years, the Bacterial Genetics and Phage Courses will be taught in the Davenport Laboratory.

I also hope that the rebirth of an interest in fundamental physiological problems, as a result of Max Delbrück's sensory transducer workshop, will become a permanent feature of our summer program. Limitations in year-round research facilities now prevent us from considering year-round staff in this area. But in the summer, the healthy diversity which physiological thinking adds to our intellectual life has more than compensated for the accompanying restrictions in living quarters for students primarily interested in genetically oriented problems.

Soon, I hope we can overcome the severe limitations now imposed by the housing shortage. Except during the symposium, virtually everyone connected with the Lab lives on the site, a feature which all of us feel must be preserved. Over the years, as the number of our courses has increased, we have had less and less space for summer investigators or for visitors wishing to spend even short intervals working or talking with our staff and guests. For a period, the additional facilities provided by the construction of the Page Buildings provided a breather, but next summer we shall face a ghastly housing crisis as we increase our animal cell teaching program. Preliminary plans for a new summer residence to house 32 additional people have thus been prepared and now we actively seek funds to permit the start of construction by early spring – if this does occur some new units can be completed before next summer's guests arrive.

We are also attempting to thoroughly revitalize the library, now completely repainted. Many long needed books and journals are on order and several of the refurnished reading rooms will be air conditioned to combat those hot and muggy days which put a damper on serious discussions while on the Blackford porch or on the Sandspit. In addition, work should be completed early next year on the rebuilding of the Osterhout cottage as a residence for me and my wife. Initially we intended merely to renovate it, but when the job was started it became clear that little of the original dwelling was salvageable. The new building, however, will have many lines of the original and we think that it will fit equally well into its central location.

A most important consideration in our decision to go ahead with these plans has been a basic change in our relationship to the Participating Institutions. In the past these institutions guaranteed help of up to $25,000 in case of extreme financial need. This left the almost unanswerable question of what was a real crisis and so the only real assistance provided by these institutions came from the most helpful individual

efforts of the trustees drawn from these institutions. Last fall, however, the Trustees voted an essential change in this relationship.

In the future the participating institutions are to help us directly with annual contributions of at least $2500. This is a most welcome change, obviously expressing their good will toward us, but also, I believe, reflecting the real subsidies given the universities by the attendance of their staffs and students in our various summer programs. This change assures us of some $25,000 of additional income each year, and we hope that eventually a sum twice that large will be forthcoming. This will happen if several more universities decide to help us directly. Already MIT and the Harvard Medical School have decided to make annual contributions, and several other universities, whose staff and students always make major use of our facilities, are likely to join in also.

This raises the question as to how committed we are to these institutions which help us financially. Should we treat them preferentially, favoring them when we choose the people for our various summer activities? Here my feeling is that we should try very hard not to—but we should also use all means at our disposal to persuade these institutions which we have in effect subsidized for many years to realize their obligation to help us. But there naturally will exist many schools and institutions much to small or distant to be expected to help us directly, who nonetheless will want to send people to Cold Spring Harbor. Here it is most important for us to make every effort to bring them here.

All in all I think we can look forward to the next several years with much enthusiasm. Hopefully we shall find ways to provide secure salary support for our permanent staff—only with this assurance are we likely to ensure the presence of outstanding scientists as permanent members of our community; and only by their presence are we likely to continue to attract the exceptionally talented younger scientists who wish to immerse themselves totally in serious research for one to five years and then move on to university positions.

It is thus clear that I and all who value this Lab have much to do to help it prosper. Fortunately this can happen in the atmosphere of a science bursting with excitement and full of unexpected challenges for all.

J. D. Watson

THE REPLICATION OF DNA

Laboratory of
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P. De Lucia

Energy Requirements for DNA Synthesis

The work in collaboration with Dr. David Denhardt (Harvard University), reported briefly last year, has been pushed about as far as it can go. Our conclusion is that duplication of the bacterial chromosome is a more demanding process than replication of a single-stranded template (such as ΦX DNA) and requires additional energy beyond that needed to furnish the precursors of the conventional DNA polymerase. The kind of experiments we have carried out cannot show whether the additional energy is required for making some other as yet unknown precursor, or for bringing the precursors to the region of DNA synthesis, or for providing the template on which synthesis is to occur (e.g., by unwinding the DNA). But the conventional picture of DNA synthesis, as a process mediated by the known polymerase acting in a more or less complicated fashion, does not appear to be sufficient to explain our observations. For this reason, we have recently turned to a more direct approach to the problem.

Kinetics of DNA Synthesis, and the Precursor Pools

Although thymine and its derivatives are used by *E. coli* only for DNA synthesis, there has been no systematic study of the kinetics of labeling DNA with radioactive thymine in relation to the specific activities of the various thymine-containing pools. Since this seemed to be the most direct way to find out the nature of the precursors of DNA, we undertook such a study a few months ago. From our results so far, it seems unlikely that TTP is the sole immediate precursor of polymerized TMP. However, more experiments must be done before we can make a definite assertion about the precursors of DNA synthesis in vivo.

Synthesis of DNA in vitro

Uncertainty about the real precursors of DNA synthesis in vivo prompted us to look for enzymes, other than the Kornberg enzyme, that can polymerize compounds other than the deoxyribonucleoside triphosphates. This project is made much more difficult by the presence of active kinases in most cell extracts which allow almost any potential precursor to be rapidly converted into the triphosphate and therefore be incorporated by the Kornberg enzyme. The task is further complicated by the possible requirement of an intact membrane-bound template DNA for replication to occur. For this reason, the in vitro studies are now focused on the T4 system because of its smaller molecular weight and the availability of kinase-deficient mutants.

Structure of Replicating DNA in the T4 Pool

The study of the T4 replicating DNA pool (reported last year by R. Werner in the report of the Carnegie Institution of Washington) has been elaborated in several ways. Originally, these experiments were designed to demonstrate the distribution of sites of replication in relation to each other and to the total DNA in the pool; from this came the picture of a specific site at which synthesis was initiated, resulting in a steady succession of replicating forks, each of which follows the path taken by its predecessor. The work has now been extended to determine the number of such sites on each parental DNA molecule; from this it appears that each molecule must possess a single site, which gives rise to forks that move in only one of the two possible directions away from the site. These results are of great importance for any general model of the mechanism of DNA synthesis, because they imply certain singular restrictions to the synthesis of DNA in vivo.

Production of P1 Transducing Particles

Study of the rare production of transducing particles, in single bursts, has now been extended to three markers (prophages), located far apart on the genetic map. It is now clearly established that the production by a bacterium of transducing phage for one of these markers increases the likelihood that the bacterium will have also produced one of the others. Thus the act of packaging pieces of the bacterial chromosome into transducing phage is to some extent a generalized phenomenon. However, it is clear that the frequency of such multiple events is not as high as would be expected were the packaging an all-or-none phenomenon. Further, this departure from all-or-none behavior is probably too great to be accounted for by any simple model invoking errors in frame during packaging.

MESSENGER RNA AND PROTEIN SYNTHESIS

Laboratory of
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Our investigations of messenger RNA in vitro protein synthesis have concentrated on two systems: first, the RNA bacteriophage R-17 whose genetic material is itself a messenger and, second, the more complicated DNA bacteriophage T-4 whose genetic material must be transcribed into messenger RNA. R-17 provides an opportunity to study the control of translation of a natural polycistronic messenger, while the T-4 system permits investigation of both transcription and translation processes involved in production of an active enzyme, lysozyme.

RNA Bacteriophage

The RNA of R-17 has a molecular weight of 1.1 x 10^6 and codes for 3 known proteins: coat protein, RNA synthetase and "A" protein (a phage structural protein), whose total molecular weight accounts for 75-85% of the total information available in the RNA. In spite of this seeming simplicity, the synthesis of these phage proteins inside the infected cell is under both temporal and quantitative control. The coat protein itself has been implicated in control of translation of the RNA synthetase.

RNA Fragments

We have taken advantage of an enzyme from *E. coli*, RNase IV, that makes only a limited number of cuts in R-17 RNA, to try to dissect the polycistronic messenger. As we showed previously, the first cleavage by the enzyme is at a site about ⅓ of the way into the molecule from the 5′ end, allowing separation of two fragment molecules from the native RNA.

The fragments have now been prepared in a more pure state and have been characterized with respect to size, heterogeneity, stability and intactness. Polyacrylamide electrophoresis of the fragments shows that they can be prepared in a homogeneous and rather pure form with very little cross-contamination with the other species (intact or fragment). After treatment with formaldehyde or dimethyl sulfoxide, the ⅓ and ⅔ fragments still sediment homogeneously, showing that they contain few if any internal breaks (experiments carried out by M. Farber as an Undergraduate Research Participant). From sedimentation rates in formaldehyde and mobilities in polyacrylamide, we calculate that the molecular weights are 0.44 x 10^6 and 0.74 x 10^6 for the ⅓ and ⅔ fragments, respectively, compared to 1.1 x 10^6 for the native RNA. Whether this specific cleavage is due to base sequence or to secondary structure is still unclear.

Coat Protein Binding

It is known that 5-6 coat protein molecules bind to phage RNA in 0.1M tris buffer, and that when coat protein is added to the in vitro protein synthesizing system containing phage RNA, synthesis of the RNA synthetase is depressed. Thus it has been postulated that coat protein binds at a site in the RNA that prevents translation of the synthetase gene.

We find that under the ionic conditions needed for protein synthesis, R-17 coat protein binds to its RNA in stoichiometric amounts; that is, one coat protein molecule is specifically and tightly bound per RNA molecule. If the input ratio of coat protein to RNA is increased beyond 10, some additional weak binding is observed, then a point is reached where 100-150 molecules are bound, forming a phage-like complex as observed by Sugiyama and coworkers.

We can ask where the single specific binding site is located by using the fragments of RNA. The experiments clearly show that only the ⅔ piece containing the 3′ end of the RNA binds coat protein specifically, and it does so with one protein molecule per RNA chain. Thus, apparently the binding site is preserved in the ⅔ fragment.

Protein Synthesis With Fragments

Our previous results showed that the ⅔ fragment is able to direct synthesis in vitro of the RNA synthetase as seen on polyacrylamide gels. The ⅓ fragment, on the other hand, is rather inactive as messenger and does not make synthetase. These results are being checked by use of suppressible mutants in the synthetase gene.

Both fragments make some material that has the fast mobility of coat protein and that lacks histidine, which is also typical of the coat protein or fragments of it. The current more pure fragment preparations still direct synthesis of this material, albeit at a reduced level compared to the native RNA. We are currently doing peptide analyses to decide whether or not complete coat protein molecules are made by either fragment. Thus the location of the coat protein gene remains obscure. The A protein is difficult to detect among the products of in vitro protein synthesis. We are now using the fragments and A mutants to try to locate this gene.

The fact that the synthetase gene and the coat protein binding site are both located in the ⅔ fragment is consistent with the notion that control is exerted by coat protein. Preliminary results suggest that the complex of one coat protein plus one ⅔ fragment does not direct synthesis of synthetase, while the appearance of its other products and the products of the ⅓ fragment are not affected by the presence of coat protein. Therefore, the specific binding reaction appears to be of functional significance, and details of it will be studied.

Bacteriophage T-4 Lysozyme

As shown previously, we can detect in vitro synthesis of active T-4 lysozyme upon addition of messenger extracted from T-4 infected cells to an extract prepared from *E. coli*. In all respects the appearance of this activity seems to be due to *de novo* synthesis of the active enzyme. Using this system as an assay for biologically active lysozyme messenger, we have further investigated some of the properties of the messenger.

In the T-4 infected cells, lysozyme activity is first detectable at about 12 minutes and it increases thereafter more or less linearly until lysis. If parallel samples are taken for preparation of messenger, we can ask when the lysozyme messenger appears in a form that can be translated in the vitro system. As nearly as we can tell, the messenger appears no sooner than the active enzyme. That is, no lysozyme messenger is found before 12 minutes and from this time on its amount increases. Bautz and coworkers find that lysozyme messenger can be detected at early times (i.e. 5 minutes) by use of hybridization techniques. Apparently this RNA that is homologous with the lysozyme region of the T-4 DNA either cannot be extracted intact, or is not translatable. The explanation for this is unclear.

Since we know the molecular weight of lysozyme we can calculate that a messenger RNA molecule would have to be about 10 S in order to include the sequence information. How big is the active lysozyme messenger that we extract from the T-4 infected cells? By assaying sucrose density gradient fractions for their ability to stimulate lysozyme synthesis, it is found that the activity is predominantly at 17-18 S, and not at S values less than 16 S. Therefore the messenger seems to be three times larger than is necessary for just the sequence information. This does not seem to be due to aggregation of the RNA. Currently we are attempting to use RNase IV or limited treatment with other nucleases to see if part of the RNA can be removed without destroying the lysozyme activity. Attempts are also being made to purify further the lysozyme messenger.

Various attempts have been made to synthesize lysozyme messenger *in vitro* using T-4 DNA and RNA polymerase of *E. coli* (furnished by R. Burgess and J. P. Richardson), either by adding the RNA to the protein synthesizing system or by use of a coupled DNA-RNA-protein system. Thus far no active messenger has been detected. This is not surprising since *E. coli* polymerase does not synthesize late messenger off of T-4 DNA as shown by hybridization experiments. In collaboration with B. D. Hall, we are trying the RNA polymerase from T-4 infected cells that is able to synthesize late messenger RNA.

STRUCTURE AND FUNCTION OF PHAGE DNA's

Laboratory of
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Segmental Distribution of Nucleotides in λ DNA, A. Skalka, E. Burgi, and A. D. Hershey

For some years it has been clear that the DNA molecule in particles of phage λ contains segments of unlike composition. This is conveniently demonstrated by breaking the molecules, which are initially uniform in density, into fragments. The fragments exhibit a density that depends on average nucleotide composition and varies with place of origin in the molecules. As shown by Nandi, Wang, and Davidson, one can accentuate the density differences by combining the DNA with mercury. We described in previous reports our principal methods of analysis (*Carnegie Year Book 65*, pp. 559-562) and preliminary results (*Carnegie Year Book 66*, pp. 650-651; reprint, pp. 6-7). This year we pursued the analysis about as far as the methods permit, with rather greater success than we had anticipated. We identified six intramolecular segments. A full account of the work has been published in the *Journal of Molecular Biology*. There we discuss the significance, actually obscure, of the proposed structure.

The Molecular Site of the Repressor Gene, P. Bear and A. Skalka

In bacteria lysogenic for phage λ, the functioning of most phage genes is inhibited by a protein, called repressor, that is the product of a gene called *c*I situated in the right half of the genetic map of the phage. As far as is known, only *c*I and an adjacent gene called *rex* function under these conditions, and other genes are not transcribed.

Phyllis Bear took advantage of the circumstance described above to locate the genes *c*I and *rex* in our physical map of the λ DNA molecule. Though she encountered many difficulties, her experiment is simple in principle. She broke λ DNA molecules into small fragments, sorted them into fractions of unlike nucleotide composition by density analysis, and passed equal volumes of each fraction, heated to separate the DNA strands, through membrane filters. This gave her a series of filters containing various amounts of DNA but representing, in effect, equal numbers of copies of individual genes. She then isolated labeled RNA from lysogenic bacteria, fractionated it by hybridization with λ DNA to enrich for phage-specific messenger, and tested the messenger for hybridization with the DNA fractions affixed to filters. Her results show that the RNA binds specifically to DNA of 43% GC content. This DNA originates mainly from the segment of fractional molecular length 0.17 whose center lies at fractional distance 0.38 from the right molecular end. Therefore *c*I and *rex* are two of perhaps five genes that could be accommodated in this segment.

Dove (*Ann. Rev. Genet.*, 1968), citing data from several sources, puts *c*I at fractional

distance 0.25 from the right molecular end. His estimate is consistent with Bear's, even though neither may be very exact.

The results verify in a direct way that repression of gene function specifically suppresses the transcription of the repressed genes. Bear's experiments also demonstrate a useful means for determining the GC content of RNA.

Transducing Phages, H. Yamagishi and A. Skalka

Phage λ has contributed in many ways toward an understanding of genetic recombination. To appreciate this contribution, one must clearly distinguish three kinds of recombination seen in λ.

The first kind, observed in ordinary phage crosses involving homologous chromosomes, is the molecular equivalent of the process described in textbooks of genetics.

In λ, ordinary recombination is catalyzed by the function of a phage gene, *red,* and by the function of an equivalent bacterial gene, *rec* (Signer and Weil; Echols and Gingery).

The second kind of genetic recombination seen in λ is responsible for the reversible insertion of prophage into the bacterial chromosome, a process for which Campbell invented the proper model *(Carnegie Year Book 66,* pp. 651-657; reprint, pp. 7-13). Prophage insertion and excision differ from the exchanges described above in three respects: matching nucleotide sequences cannot be confidently invoked *(Carnegie Year Book 66,* pp. 657-660; reprint, pp. 13-16), the exchanges occur at a unique site in each DNA, and they depend on the function of a phage gene *int* (Zissler; Gingery and Echols; Gottesman and Yarmolinsky).

The third type of recombination seen in λ brings about excision of prophage in such a manner as to give rise to structures in which both phage and bacterial genes are represented. The recoverable structures, usually chromosomes of transducing phage particles, consist of a continuous segment of prophage DNA joined to a continuous, adjacent segment of bacterial DNA (Arber, Kellenberger, and Weigle, 1957; Campbell, 1957). Kayajanian and Campbell (1966) identified this type of recombination as illegitimate crossing over, in the technical sense that it does not depend on linear homology between phage and bacterial chromosomes.

The generation of transducing phage lines by illegitimate crossing over of the sort described is a rare event largely inaccessible to direct analysis. It is not even clear as yet whether or not the process depends on *rec, red,* or *int* functions, because when these are not provided, transducing phage particles of an atypical kind make their appearance (Gottesman and Yarmolinsky; Gingery and Echols).

The origin of typical transducing lines of λ can perhaps be accounted for by a process that resembles recombination of the first kind except for one known difference: the interacting DNA's are not homologous. According to this view, recombination of the third kind would depend on a nonlinear pattern of matching nucleotide sequences in the DNA's of λ and its host, *Escherichia coli.* Then the only resemblance to normal prophage excision (recombination of the second kind) would be topological.

Yamagishi and Skalka undertook to determine whether or not matching nucleotide sequences in phage and host DNA's are distributed in the same manner as the observed exchanges that generate transducing phage lines. If they are, bacterial DNA recovered from *gal*-transducing phages must resemble the left half of λ DNA, and bacterial DNA recovered from *bio*-transducing phages must resemble the right half of λ DNA. This prediction follows from the model of Kayajanian and Campbell for the origin of transducing phage lines *(Carnegie Year Book 66,* pp. 651-657; reprint, pp. 7-13).

Yamagishi and Skalka retrieved bacterial DNA from the λ*dg*(A-J) of Adler and Templeton, and from the λ*dbio* M55-3 of Kayajanian. Skalka's previous work with λ*dg* illustrates the principal method *(Carnegie Year Book 66,* pp. 657-659; reprint, pp. 13-15). She found that the segment of bacterial DNA lying between *gal* and the prophage has the uniform composition 51% GC, the same as that of the bacterial DNA as a whole. The segment found in λ*dbio,* on the contrary, proves to contain subsections ranging in GC content from 37% to 50%. Thus a certain resemblance is evident between the *gal* region of *E. coli* DNA and the left half of λ DNA (Fig. 1), and between the *bio* region of *E. coli* DNA and the right half of λ DNA.

The distribution of matching nucleotide sequences, expressed in terms of results of the appropriate DNA-DNA hybridization tests, shows the following features. (1) Bacterial DNA recovered from either transducing phage binds more efficiently to λ DNA than does unselected bacterial DNA. (2) Bacterial DNA from λ*dgal* binds equally to right and left halves of λ DNA. (3) Bacterial DNA from λ*dbio* binds preferentially to right halves of λ DNA. Thus the distribution of matching nucleotide sequences is such as to permit and even encourage the kinds of illegitimate crossing over that generate transducing phages.

The results suggest a resemblance between the left half of λ DNA and the *gal* region of *E. coli* DNA, and another between the right half of λ DNA and the *bio* region of *E. coli* DNA. The resemblances are weak but are perceptible both in local composition and in sequence of nucleotides, a correlation that is obligatory if matching sequences are numerous and clustered *(Carnegie Year Book 65,* pp. 562-565).

We suggest as a general inference that all recombination processes represent variations on a single theme. Those giving rise to transducing phages are inefficient by design. Because illegitimate crossing over is a rare event, it can afford to sacrifice precision for the sake of adventure.

Phage φ80, A. Skalka

The phage φ80 is one of several species related in many ways to λ. Comparison of structure of the two DNA's might be expected to clarify relatedness, on the one hand, and to provide clues to interdependence of structure and function on the other, notwithstanding the perennial difficulty that these two aspects of comparative structure tend to defeat each other.

Skalka has analyzed structure and transcription of the DNA of φ80 by methods previously applied to λ. She finds, in molecules φ80 DNA, a GC-rich half that, by analogy with λ DNA, (Fig. 1), can be called the left half. Then it turns out that the two DNA's terminate in identical cohesive sites at their left ends, as shown by the fact that left molecular halves of either DNA can join only to right molecular halves of either. Both DNA's contain long segments of high GC content (55% in φ80) in their left halves, and both terminate on the left in short segments of lower GC content. Hybridization tests show a moderate cross-reaction (20% for the unfractionated DNA's), which is strongest in the left half of each. In both DNA's the late-functioning genes reside in left molecular halves, as determined by hybridization tests with messenger RNA.

Right molecular halves of the two DNA's terminate in identical cohesive sites, contain early-functioning genes, and show similar discontinuities in nucleotide composition. They differ appreciably, however, in average composition (45% GC in λ, 51% in φ80), and show relatively weak cross-reactions in hybridization tests. The central segment of low GC content found in λ has no counterpart in φ80 detectable either by density analysis or by hybridization tests.

Messenger RNA synthesis in bacteria infected with the respective phages is entirely similar, characterized by slow transcription of right molecular halves at early times and rapid transcription of both halves, but predominantly the left, at late times.

The relatedness of the two phages is therefore clearly evident as linear homology in their DNA's. The differences are instructive, however. Our work with λ was prompted in part by our biological prejudice that structural singularities necessarily serve some useful purpose. Some of Skalka's results with λ were compatible, for instance, with the hypothesis that the switch from early to late phases of transcription depended directly on some device sensitive to local nucleotide composition *(Carnegie Year Book 64,* pp. 526-527). If that were true, one should expect early and late messengers to differ markedly in composition even in a phage like φ80, where the average compositions of large DNA sections do not differ greatly. Since Skalka's results with φ80 fail to verify the prediction, she concludes that patterns of transcription are not directly dependent on nucleotide composition.

Skalka plans to extend comparative analysis along the lines indicated to a few additional phage species.

Origin of λ DNA Synthesis, S. Makover

If a replicating DNA molecule contains a number of growing points that started from a single origin and are moving in the same direction, a nucleotide sequence lying ahead of the first growing point should be represented only once in the replicating structure, whereas a sequence lying between the n^{th} *and* $n+1^{th}$ growing points should be represented $n+1$ times. Sueoka and his colleagues exploited this principle to determine the sequence of replication of genetic markers in *Bacillus subtilis.* Makover is using it to determine the sequence of replication of the segments of λ DNA of dissimilar nucleotide composition.

Makover finds, first of all, that the appropriate experiments are feasible. He treats bacteria with mitomycin to destroy their capacity for synthesis of their own DNA, infects them with phage, and feeds tritiated thymidine to the culture for just three minutes to label replicating phage DNA. He then extracts DNA from the cells, mixes it with differently labeled DNA from phage particles, breaks the DNA in the mixture to small fragments, and fractionates these according to nucleotide composition. He finds that the replicating and nonreplicating DNA's in the mixture form identical distributions except for a quantitative bias that he attributes to sequential replication of the several molecular segments. His results show that the 48.5%-GC segment is one of the first to be replicated. He hopes that further experiments will define both origin and direction of replication. What is more important, he may be able to verify the general model of replication in a single direction from a unique starting point.

Makover's results are consistent with other facts. A line of λ*dg* from which the 57%-GC section of DNA has been deleted is able to synthesize DNA (Brooks; Echols, Skalka, and others; also analogous experiments by Dove and Franklin). A chromosomal locus *(ri),* situated near the right end of the genetic map, is a plausible candidate for the starting point of DNA synthesis (Dove, Hargrove, and Haugli).

R. L. Sinsheimer, in whose laboratory the mitomycin treatment was first applied in experiments with λ, furnished the requisite bacterial strain.

COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

June 5th to June 12th, 1968

XXXIII. THE REPLICATION OF DNA IN MICRO-ORGANISMS

The 33rd Cold Spring Harbor Symposium on "Replication of DNA in Microorganisms" was attended by 354 scientists, including 7 from France, 4 from Switzerland, 3 from Germany, 3 from Japan, 3 from Italy, 2 from Scotland, and one each from Denmark, Australia, Holland, and New Zealand. In all, 92 papers were read. The meeting was supported by grants (or contracts) from the National Science Foundation, the National Institutes of Health, the Air Force Office of Scientific Research, and the United States Atomic Energy Commission.

The object of the meeting was to review progress made in our understanding of the mechanisms of the DNA synthesis that accompanies DNA duplication, repair and recombination. Despite the great advances made in the general area of molecular biology, this particular field involves mechanisms that are poorly understood. Orthodox genetic procedures have identified many genes whose products are involved in the initiation or continuation of DNA duplication, in repair of various kinds of defect, and in recombination. But for the most part the exact property of the enzymes involved is not known. Further, it is not known what methods are used in vivo to overcome the great mechanical problems inherent in duplicating very long DNA molecules. Thus we have the paradox that, although it was the elucidation of the structure of DNA that really started the field of molecular biology, the methods of synthesis of DNA remain unclear. This is by no means a totally academic problem: presumably the cancer problem will ultimately be seen as a problem in the control of DNA duplication, and it has been suggested that ageing may in part be due to somatic mutation (i.e., defects in repair mechanisms).

The meeting dealt with the in vitro replication of DNA (including a report by Khorana on the progress in his attempt at total synthesis of a gene), the immediate products of synthesis in vivo (in particular the fragmented intermediates described recently), the various kinds of in vivo repair process, the physical structure of replicating DNA and its relation to other cell structures, the regulation of DNA synthesis, and the various kinds of defect in these processes that have been observed.

THE PROGRAM

REPLICATION OF NUCLEIC ACIDS

ENGLUND, P. T., M. P. DEUTSCHER, T. M. JOVIN, R. B. KELLY, N. R. COZZARELLI, and A. KORNBERG: Structural and functional properties of *Escherichia Coli* DNA Polymerase.

GOULIAN, M.: Initiation of the replication of single-stranded DNA by *Escherichia coli* DNA polymerase.

GELLERT, M., J. W. LITTLE, C. K. OSHINSKY, and S. B. ZIMMERMAN: Joining of DNA strands by DNA ligase of *E. coli*.

OLIVERA, B. M., Z. W. HALL, Y. ANRAKU, J. R. CHIEN, and I. R. LEHMAN: On the mechanism of the polynucleotide joining reaction.

KHORANA, H. G., H. BUICHI, M. H. CARUTHERS, S. H. CHANG, N. K. GUPTA, A. KUMAR, E. OHTSUKA, V. SGARAMELLA, and H. WEBER: Progress in the total synthesis of the gene for ala-tRNA.

GANESAN, A. T.: Studies in in vitro replication of *Bacillus subtilis* DNA.

BAUTZ, E. K. F., F. A. BAUTZ, and W. RUGER: A biological assay for in vitro repair of phage T4 DNA.

ROSENBERG, B. H., and L. F. CAVALIERI: Shear sensitivity of the *E. coli* genome: Multiple membrane attachment points of the *E. coli* DNA.

AUGUST, J. T., A. K. BANERJEE, L. EOYANG, M. T. F. de FERNANDEZ, and K. HORI: Synthesis of bacteriophage Qβ RNA.

WEISSMANN, C., G. FEIX, H. SLOR: In vitro synthesis of phage RNA: the nature of the intermediates.

SPIEGELMAN, S., N. R. PACE, D. R. MILLS, R. LEVISOHN, T. S. EIKHOM, M. M. TAYLOR, R. L. PETERSON, and D. H. L. BISHOP: The mechanism of RNA replication.

MARINO, P., M. I. BALDI, and G. P. TOCCHINI-VALENTINI: Effect of Rifampicin on DNA-dependent RNA polymerase and on RNA phage growth.

INTERMEDIATES IN DNA SYNTHESIS

OKAZAKI, R., T. OKAZAKI, K. SAKABE, K. SUGIMOTO, R. KAINUMA, A. SUGINO, and N. IWATSUKI: In vivo mechanism of DNA chain growth.

NEWMAN, J., and P. HANAWALT: Intermediates in T4 DNA replication in a T4 ligase deficient strain.

RICHARDSON, C. C., Y. MASAMUNE, T. R. LIVE, A. JACQUEMIN, B. WEISS, and G. FAREED: Studies on the joining of DNA by polynucleotide ligase of phage T4.

SADOWSKI, P., B. GINSBERG, A. YUDELEVICH, L. FEINER, and J. HURWITZ: Enzymatic mechanisms of the repair and breakage of DNA.

KIDSON, C.: Analysis of DNA fine secondary structure in relation to replication and transcription.

REPAIR OF DNA

HANAWALT, P. C., D. E. PETTIJOHN, E. C. PAULING, C. F. BRUNK, D. W. SMITH, L. C. KANNER, and J. L. COUCH. Repair replication of DNA in vivo.
HOWARD-FLANDERS, P., W. D. RUPP, B. M. WILKINS, and R. S. COLE: DNA replication and recombination after UV-irradiation.
SETLOW, J. K., M. L. RANDOLPH, M. E. BOLING, A. MATTINGLY, G. PRICE, and M. P. GORDON: Repair of DNA in *Haemophilus influenzae*. II. Excision, repair of single-strand breaks, defects in transformation and host-cell modification in UV-sensitive mutants.
TAKAGI, Y., M. SEKIGUCHI, S. OKUBO, H. NAKAYAMA, K. SHIMADA, S. YASUDA, T. MISHIMOTO, and H. YOSHIHARA: Nucleases specific for ultraviolet light-irradiated DNA and their possible role in dark repair.
GROSSMAN, L., J. KAPLAN, S. KUSHNER, and I. MAHLER: Enzymes involved in the early stages of repair of ultraviolet-irradiated DNA.
GANESAN, A. K., and K. C. SMITH: Recovery of recombination-deficient mutants of *Escherichia coli* K-12 from ultraviolet irradiation.
TOMIZAWA, J., and H. OGAWA: Breakage of DNA in *Rec*+ and *Rec*− bacteria by disintegration of radiophosphorus atoms in DNA and possible causes of pleiotropic effects of *Rec*A mutation.
STEINBERG, R. A.: Effects of ^{32}P-decay on chromosome transfer by a radiation-sensitive Hfr strain of *Escherichia coli*.
BUTTIN, G., and M. R. WRIGHT: Enzymatic DNA degradation in *E. coli:* Its relationship to synthetic processes at the chromosome level.
FRIEDBERG, E. C., and D. A. GOLDTHWAIT. Endonuclease II of *E. coli*.
STRAUSS, B., M. COYLE, and M. ROBBINS: Alkylation damage and its repair.
ALBERTS, B. M., F. J. AMODIO, M. JENKINS, E. D. GUTMAN, and F. L. FERRIS. Studies with DNA-cellulose chromatography. I. DNA-binding proteins from *E. coli*.

GENETICS OF REPLICATION

GROSS, J. D., D. KARAMATA, and P. G. HEMPSTEAD: Temperature-sensitive mutants of *B. subtilis* defective in DNA synthesis.
MENDELSON, N. H.: Can defective segregation prevent initiation?
KOHIYAMA, M.: DNA synthesis in temperature-sensitive mutants of *Escherichia coli*.
BERNSTEIN, H.: Repair and recombination in phage T4. I. Genes affecting recombination.
BALDY, M. W.: Repair and recombination in phage T4. II. Genes affecting UV sensitivity.
DRAKE, J. W., and E. F. ALLEN: Antimutagenic DNA polymerases of bacteriophage T4.
SPEYER, J. F., and D. ROSENBERG: The function of T4 DNA polymerase.
CHIU, C-S, and G. R. GREENBERG: Evidence for a possible direct role of dCMP hydroxymethylase in T4 phage DNA synthesis.
MOSIG, G., R. EHRING, and E. O. DUERR: Replication and recombination of DNA fragments in bacteriophage T4.
AMATI, P., and R. FAVRE: Phage DNA synthesis in bacteria infected with T4 light particles.
KOZINSKI, A. W.: Molecular recombination in the ligase-negative T4 *amber* mutant.
SESNOWITZ-HORN, S., and A. ADELBERG: Proflavin treatment of *Escherichia coli:* Generation of frameshift mutations.

STRUCTURE OF REPLICATING DNA

DAVISON, P. F.: DNA replication: Physical factors.
WANG, J. C., and N. DAVIDSON: Cyclization of phage DNAs.
THOMAS, C. A., JR., T. KELLY, JR., and M. RHOADES: The intracellular forms of T7 and P22 DNA molecules.
FREIFELDER, D.: Studies with *Escherichia coli* sex factors.
HUDSON, B., D. A. CLAYTON, and J. VINOGRAD: Complex mitochondrial DNA.
SINSHEIMER, R. L., R. KNIPPERS, and T. KOMANO: Stages in the replication of bacteriophage ØX174 DNA in vivo.
DENHARDT, D. T., and A. B. BURGESS: DNA replication in vitro. I. Synthesis of single-stranded ØX174 DNA in crude lysates of ØX-infected *E. coli*.
RUSH, M. G., and R. C. WARNER: Molecular recombination in a circular genome—ØX174 and S13.
HSU, Y.: Regulation of the propagation of HR virus plasmid.
GILBERT, W., and D. DRESSLER: DNA replication: the rolling circle model.
FRANKEL, F. R.: DNA replication after T4 infection.
FRANKEL, F. R., C. MAJUMDAR, S. WEINTRAUB, and D. M. FRANKEL: DNA polymerase and the cell membrane after T4 infection.
WERNER, R.: Initiation and propagation of growing points in DNA of phage T4.
HUBERMAN, J.: Visualization of replicating mammalian and T4 bacteriophage DNA.
WEISSBACH, A., P. BARTL, and A. SALZMAN: The structure of replicative lambda DNA—electron microscope studies.
TOMIZAWA, J., and T. OGAWA: Replication of phage lambda DNA.

ORIGINS OF REPLICATION

NAGATA, T., and M. MESELSON: Periodic replication of DNA in steadily growing *E. coli:* the localized origin of replication.
CARO, L. G., and C. M. BERG: Chromosome replication in some strains of *Escherichia coli* K12.
WOLF, B., M. L. PATO, O. B. WARD, and D. A. GLASER: On the origin and direction of replication of the *E. coli* chromosome.
VIELMETTER, W., and W. MESSER: Growth direction and segregation of the *E. coli* chromosome.
CERDA-OLMEDA, E., and P. HANAWALT: The replication of *Escherichia coli* chromosome studied by sequential nitrosoguanadine mutagenesis.
LePECQ, J. B., and R. L. BALDWIN: The starting point and direction of λDNA replication.
MAKOVER, S.: A preferred origin for the replication of lambda DNA.

BACTERIAL MATING

BONHOEFFER, F., and W. VIELMETTER: Conjugational DNA transfer in *E. coli*.
FISHER, K. W. and M. B. FISHER: Nalidixic acid inhibition of DNA transfer in *E. coli* K12
COHEN, A., W. D. FISHER, R. CURTISS, and H. I. ADLER: The properties of DNA transferred to minicells during conjugation.
GUILD, W. R., A. CATO, JR., and S. LACKS: Transformation and DNA size: two controlling parameters and the efficiency of the single strand intermediates.
RUPP, W. D., and G. IHLER: Strand selection during bacterial mating.
OHKI, M., and J. TOMIZAWA: Asymmetric transfer of DNA strands in bacterial conjugation.

REPLICATION AND THE CELL MEMBRANE

BOTSTEIN, D., and M. LEVINE: Intermediates in the synthesis of phage P22 DNA.
RYTER, A., Y. HIROTA, and F. JACOB: DNA-membrane complex and nuclear segregation in bacteria.
HIROTA, Y., A. RYTER, and F. JACOB: Thermosensitive mutants of *E. coli* affected in the processes of DNA synthesis and cellular division.
SUEOKA, N., and W. G. QUINN: Membrane attachment of the chromosome replication origin in *Bacillus subtilis*.
EARHART, F., G. Y. TREMBLAY, M. J. DANIELS, and M. SCHAECHTER: DNA replication studied by a new method for the isolation of cell membrane-DNA complexes.

REPLICATION OF TEMPERATE VIRUSES

SIGNER, E., H. ECHOLS, J. WEIL, C. RADDING, M. SHULMAN, L. MOORE, and K. MANLY: The general recombination system of bacteriophage λ.
SIGNER, E. and J. WEIL: Site-specific recombination of bacteriophage λ.
GINGERY, R., and H. ECHOLS: Integration, excision, and transducing particle genesis by bacteriophage λ.
KAISER, A. D., and R. WU: Structure and function of DNA cohesive ends.
GOTTESMAN, M., and M. YARMOLINSKY: The integration and excision of the bacteriophage lambda genome.
MOUSSET, S., and R. THOMAS: Dilysogenic excision: an accessory expression of the termination function?
EISEN, H., L. H. PERIERA da SILVA, and F. JACOB: The regulation and mechanism of DNA synthesis in bacteriophage lambda.
CALEF, E., and Z. NEUBAUER: Active and inactive states of the CI gene in some λ defective phages.
MATSUBARA, K. and A. D. KAISER: λ dv, an autonomously replicating DNA fragment.
DULBECCO, R.: The state of the DNA of polyoma virus and SV40 in transformed cells.
ROSNER, J. L., L. R. KASS, and M. B. YARMOLINSKY: Parallel behavior of F and P1 in causing indirect induction of lysogenic bacteria.
IKEDA, H., and J. TOMIZAWA: Prophage P1, an extrachromosomal replication unit.

REGULATION OF REPLICATION

BIRD, R., and K. G. LARK: Initiation and termination of DNA replication after amino acid starvation of *E. coli* 15T⁻.
HELMSTETTER, C. E., S. COOPER, O. PIERUCCI, and E. REVELAS: On the bacterial life sequence.
CLARK, D. J.: The regulation of DNA replication and cell division in *E. coli* B/r.
SGARAMELLA, V., S. SPADARI, and A. FALASCHI: Isolation of the hybrid between ribosomal RNA and DNA of *Bacillus subtilis*.
YOSHIKAWA, H., and M. HAAS: On the regulation of the initiation of DNA replication in bacteria.

SUMMARY

HOTCHKISS, R.: Metabolism and growth of gene substance: 1968.

POST GRADUATE TRAINING COURSES

Summer 1968

Many new fields have been developing in biology during the last ten years that do not fall into any particular subject but equally involve biochemistry, biophysics and genetics. As a result, most research workers have had to enlarge the extent of their professional competence: the biochemist has at last been forced to familiarize himself with genetics, and the geneticist has had to learn some biochemistry. This process of re-education, which could only be carried out with difficulty in most universities, tied as these are to a rigid curriculum, is being accomplished through a series of courses for qualified scientists held each summer at Cold Spring Harbor. The courses are given by a staff drawn from institutions all over the world and have already been attended by many hundreds of scientists drawn from disciplines as far apart as medicine and nuclear physics. In conjunction with these courses, the Laboratory invites about 50 prominent investigators as seminar speakers. This program of seminar speakers provides an extensive review of current research in these fields.

During the summer of 1968 four courses were given, designed to acquaint the student with some of the techniques used in sensory transducer processes, bacterial genetics research, bacterial virus research, and in microbiology of animal cells and viruses. The courses consisted of intensive laboratory and discussion periods and a series of seminars. In addition to the instructors in charge of the courses, several other investigators took part in teaching and in seminars.

1) SENSORY TRANSDUCER PROCESSES—June 14 to July 5, 1968

The course involved experiments with four organisms, namely, bacteria, Phycomyces, Bombyx, and Mytilus. It illustrated methods of quantitatively studying bacterial chemotaxis responses of Phycomyces to light; of the male silkworm moth to the sex lure emitted by the female (bombycol); and the mechanosensitivity of certain motoric cilia on the gills of the mussel, Mytilus.

The experiments gave rise to detailed discussions and propositions for improved and varied quantitative approaches to these problems. Many of these were tried out during the course. Many others were taken home for further study.

INSTRUCTORS

J. Adler, University of Wisconsin
M. Delbrück, California Institute of Technology
K.-E. Kaissling, Max Planck Institut, Seewiesen, Germany
U. Thurm, Max Planck Institut für Biologie, Tübingen, Germany

STUDENTS:

Allan, David A., M.S., Queens College, New York (Physics)
Bernstine, Edward G., A.B., Princeton University (Mol. Biol.)
Chen, Victor K., M.S., State University of New York, Buffalo (Biophysics)
Cohen, Robert J., M.S., Yale University (Mol. Biol.)
Doetsch, Raymond N., Ph.D., University of Maryland (Prof. Microbiology)
Feher, George, Ph.D., University of California, San Diego (Prof. Physics)
Gamow, Rustem, I., Ph. D., University of Colorado, Boulder (Mol. Biol.)
Hertel, Rainer A., Dr. rer. nat., Michigan State University (Plant Physiology)
Jesaitis, Algirdas J., B.S., California Institute of Technology (Mol. Biol.)
Kauffman, John W., Ph.D., Northwestern University (Prof. Materials Science)
Petzuch, Margarete, Dr. rer. nat., University of München, Germany (Physics)
Reiner, Albey M., M.Sc., Harvard University (Microbiology)
Schwartz, Joseph A., Ph.D., Richmond College, Staten Island, N.Y. (Physics)
Streisinger, George, Ph.D., University of Oregon, Eugene (Prof. Mol. Biol.)
Villet, Ruxton, D. Phild, University of Oxford, England (Chem. Eng., Biochemistry)

PHYCOMYCES WORKSHOP

After completion of the Sensory Transducer course, the laboratories were used for a *Phycomyces Workshop* under the direction of M. Delbrück. The Workshop was attended by:

K. Bergman, Graduate Student, California Institute of Technology
A. Jesaitis, Graduate Student, California Institute of Technology
Margarete Petzuch, Ph.D. (Physics) California Institute of Technology
Jean Matricon, Ph.D., Solid State Physics Institute, Faculté des Sciences, Orsay, France
Patricia Kenehan, University of Pennsylvania
Susan Godfrey, Graduate Student, University of Pennsylvania

Research during the Workshop concentrated on:

(1) The avoidance response,
(2) polarized light effects.
(3) search for temperature-sensitive mutants with abnormal receptor pigment,
(4) writing of a comprehensive Phycomyces Review, scheduled to appear March 1969 in Bacteriological Reviews.

During both the Sensory Transducer Processes Course and the Workshop Kostia Bergman was in charge of laboratory operations and Toby Bergman in charge of the kitchen and other technical assistance.

2) BACTERIAL GENETICS—June 17-July 8, 1968

INSTRUCTORS:

Dr. Sol Goodgal—University of Pennsylvania School of Medicine
Dr. Julian Gross—Microbial Genetics Research Unit, Hammersmith Hospital, London
Dr. Joseph Gots—University of Pennsylvania School of Medicine

Dilution and plating techniques; mode of origin of bacterial variants; induction of mutation; isolation and characterization of auxotrophs, mutagen specificity and reversions; sexual recombination and genetic mapping in *Escherichia coli;* transduction and determination of the linear order of mutational sites in *Salmonella typhimurium;* abortive transductions; characterization of suppressors and reversions by transduction; isolation and characterization of transforming DNA; transformation in *B. subtilis* and *H. influenzae.*

The course was taught for the nineteenth summer.

STUDENTS:

Becker, Michael A., M.D., National Institutes of Health
Bevill, Rardon D., Ph.D., Albert Einstein College of Medicine
Cassuto, Era N., Ph.D., Columbia College of Physicians & Surgeons
Chiu, Che-Shen, Ph.D., University of Michigan
Colowick, Maryda S., M.S., Vanderbilt University
Dayan, Jean, Ph.D., Columbia College of Physicians & Surgeons
Haenni, Anne-Lise, D.Sci., Rockefeller University
Hildebrandt, Sue Anne, B.A., Columbia University
Ishihama, Akira, Ph.D., Albert Einstein College of Medicine
Kovach, John S., M.D., National Institutes of Health
LeGal, Yves, Ph.D., University of Illinois
Livingston, David M., M.D., Laboratory of Biochemistry, National Institutes of Health
Maleh, Isaac, Ph.D., Brandeis University
Marver, Harvey S., M.D., University of California, San Francisco
Rogerson, Allen C., B.A., State University of New York, Stony Brook
Rubenstein, Kenneth E., Ph.D., University of Pennsylvania Medical School
Skoultchi, Arthur I., M.S., Yale University
Taichman, Lorne B., M.D., University of Wisconsin
Witonsky, Phil., Ph.D., Southwest Center for Advanced Studies
Zalkin, Howard, Ph.D., Purdue University

SEMINARS:

Paul Howard-Flanders, Yale University School of Medicine: "DNA Repair Mechanisms and Genetic Recombination"
Robert G. Martin, National Institutes of Health: "Regulation of the Histidine Operon"
Monica Riley, State University of New York at Stony Brook: "Recombination Mechanisms in *E. coli* Conjugation"
Stanley Falkow, Georgetown University: "Replication and Gene Expression of R Factors"
Paul Margolin, Public Health Research Institute of the City of New York, Inc.: "Initiation of Gene Expression"
David Zipser, Columbia University: "Stops and Starts"
P. H. Kourilsky, Institut de Biologie Physico-Chimique, Paris, France: "Studies on the mRNA of bacteriophage lambda"
Noboru Sueoka, Princeton University: "Chromosome Replication in *B. subtilis*"
Maurice S. Fox, Massachusetts Institute of Technology: "Transformation Heterozygotes in Pneumococcus"
Charles Helmstetter, Roswell Park Memorial Institute: "Regulation of Chromosome Replication and Cell Division in *E. coli*"
Walter Gilbert, Harvard University: "Repression and Genetic Control"
R. C. Clowes, Southwest Center for Advanced Studies: "Sex Factors in Bacteria"
Dan Morse, Stanford University: "Transcription and Translation of the Tryptophan Operon"
Jonathan Beckwith, Harvard Medical School: "Regulation of the LAC Operon"

Course Assistants: A. Bernheimer. Jr.. P. Cucinotta. S. Ehlinger, P. Feigin, M. Gladding, S. Resnick, M. Seaman.

3) BACTERIAL VIRUSES (PHAGE)—July 11-August 1, 1968

Genetic, physiological, and biochemical techniques were used in work with bacterial viruses. The life cycle of the temperate virus λ was the principal object of study.

The course was taught for the twenty-fourth time, under the following instructors:

INSTRUCTORS:

Dr. Harrison Echols--University of Wisconsin
Dr. Ethan Signer—Massachusetts Institute of Technology
Mr. Mark Willard—University of Wisconsin
Mrs. Lesley Moore Hallick—University of Wisconsin

STUDENTS:

Farron, Francoise, Ph.D., Cornell University
Ferretti, Joseph J., Ph.D., Dept. of Biology, Johns Hopkins University
Geer, Billy W., Ph.D., Dept. of Biology, Knox College
Gevers, Wieland, D.Phil., Rockefeller University
Goldberg, Allan R., Ph.D., Dept. Developmental Biology and Cancer, Albert Einstein College of Medicine
Grau, Oscar, Ph.D., Dept. of Biophysics, University of Chicago
Gunsalus, Carolyn, Ph.D., Biochemistry Division, University of Illinois
Hamkalo, Barbara A., B.S., Biology Division, Oak Ridge National Laboratory
Hou, Edward F., M.Sc., Miles Laboratories, Elkhart, Indiana
Huang, Wai Mun, Ph.D., Dept. of Biochemistry, Albert Einstein College of Medicine
Kleinkauf, Horst H. W., Dr., Rockefeller University
Leppla, Stephen H., B.S., University of Wisconsin, Molecular Biology Division
Lindahl, Alice, B.A., Dept. of Microbiology, New York University, Medical Center
McMilin, Kenneth D., B.A., University of Oregon, Eugene
Maleh, Isaac, Ph.D., Dept. of Biochemistry, Brandeis University
Meistrich, Marvin L., Ph.D., Bell Telephone Laboratories, Inc., Murray Hill, New Jersey
O'Neill, J. Patrick, A.B., State University of New York at Stony Brook
Richards, Oliver C., Ph.D., Dept. of Biochemistry, University of Utah
Robinson, John, Ph.D., Dept. of Bacteriology & Immunology, University of Western Ontario
Sadowski, Paul D., Ph.D., Albert Einstein College of Medicine

SEMINARS:

Franklin W. Stahl, Institute of Molecular Biology, University of Oregon: "The Life Cycle of T4 as Viewed by Genetic Experiments"
Rudolf Werner, Cold Spring Harbor Laboratory of Quantitative Biology: "Regulation of T4 Development: DNA Replication"
David Botstein, Dept. of Biology, Massachusetts Institute of Technology: "P22"
Walter Gilbert, Dept. of Biology, Harvard University: "ØX"
Dr. A. Marvin, Dept. of Molecular Biology & Biophysics, Yale University: "Filamentous Phages"
June Rothman Scott, Rockefeller University: "PI"
James D. Watson, Harvard University and Cold Spring Harbor Laboratory of Quantitative Biology: "RNA Phages"
Mark Ptashne, Harvard University: "The λ Repressor"
Ethan Signer, Massachusetts Institute of Technology: "Phage, Man, and the Universe"
Harrison Echols, Dept. of Biochemistry, University of Wisconsin: "Lytic Growth of λ-Early and Late Genes"
Harrison Echols, Dept. of Biochemistry, University of Wisconsin: "Repression of λ"
Ethan Signer: "Integration and General Recombination by λ"
Harrison Echols: "Induction, Transducing Phage Formation, and Miscellaneous Quirks of λ"
Ethan Signer: "λ DNA replication"
Harvey Eisen, Institut Pasteur: "The Paris View of λ"
Cyrus Levinthal, Dept. of Biology, Columbia University: "Regulation of T4 Development: Protein Synthesis"
E. Peter Geiduschek, Dept. of Biophysics, University of Chicago: "Regulation of T4 Development: Transcription"
Ray Gesteland, Cold Spring Harbor Laboratory of Quantitative Biology: "Regulation of T4 Development: *in vitro* Protein Synthesis"

Course Assistants: P. Feigin, M. Gladding, M. Seaman.

4) QUANTITATIVE MICROBIOLOGY OF ANIMAL CELLS AND VIRUSES August 4-25, 1968

INSTRUCTORS:

Dr. Philip I. Marcus, Albert Einstein Medical College
Dr. Gordon I. Sato, Brandeis University

Preparation of primary and secondary chick and mouse embryo cell cultures; chick embryo cultures of heart fibroblast, lung, kidney, and iris epithelium; HeLa, BHK, and L-cell

growth in mass culture and in clones; isolation of clonal sublines; tests and elimination of PPLO in cell cultures. Spinner cell culture. Karyotype analysis. DNA and RNA synthesis in the cell life cycle—an introduction to quantitative autoradiography. Synthesis of viral-RNA: coverslip technique and sucrose gradient analysis. Growth and purification of Newcastle disease virus (NDV). Assay of NDV by plaque formation, hemagglutination, hemadsorption, and cell-killing. The hemadsorption-negative plaque test and intrinsic interference: detection of noncytopathic viruses. Assay and properties of Sindbis virus, vesicular stomatitis virus and the pox viruses. Quantitative neutralization of viruses with antibody. One-step growth curve. Effect of antimetabolites on viral and cell growth. Preparation, assay, and the mechanism of action of interferon. Histo-chemistry and fluorescent antibody techniques in virus infection. Pherotypic mixing of myxovirus, genetic recombination of pox viruses, complementation with Sindbis virus. Morphologic transformation of cells by Rous sarcoma virus and polyoma. Hormone production in cultured cells and study of specialized cell function. Synchronous growth in cell culture. Hybridization of cells. Antibody plaque techniques. This course was given for the ninth time.

STUDENTS:

Brown, Juanita L., B.S., Massachusetts Institute of Technology
Craig, Nessly C., Ph.D., Institute for Cancer Research
Cunningham, Dennis D., Ph.D., Moffett Laboratories, Princeton University
Engelhardt, Dean L., Ph.D., The Salk Institute
Jacobsen, Edward H., Ph.D., Dept. of Biology, Massachusetts Institute of Technology
Lengyel, Peter, Ph.D., Dept. of Molecular Biophysics, Yale University
Lindahl, Tomas R., Ph.D., Rockefeller University
Lipton, Allan, M.D., Sloan-Kettering Institute
Maitra, Umadas, Ph.D., Dept. of Developmental Biology & Cancer, Albert Einstein College of Medicine
Mudd, John A., M.S., Dept. of Microbiology & Immunology, Albert Einstein College of Medicine
Velazquez, Antonio, M.D., Dept. of Human Genetics, University of Michigan
Walter, Gernot, F., Ph.D., Dept. of Cell Biology, Albert Einstein College of Medicine
Wang, Richard J., B.A., Dept. of Cell Biology, New York University Medical Center
Wu, Henry C., M.D., Ph.D., Dept. of Biology, Massachusetts Institute of Technology

SEMINARS:

H. Eagle, Dept. of Biology, Albert Einstein College of Medicine: "Biochemistry of Cultured Mammalian Cells"
G. Todaro, Cell Biology Section, Viral Carcinogenesis Branch, National Cancer Institute, National Institutes of Health: "Contact-Inhibition of Cell Division and Viral Transformation"
P. Choppin, The Rockefeller University: "The Structure and Replication of Para influenza Virus SV-5"
G. Sato, Graduate Dept. of Biochemistry, Brandeis University: "Functional Cells in Culture"
R. Perry, The Institute for Cancer Research: "Ribosome Biosynthesis and Polyribosome Formation in Mammalian Cells"
J. Darnell, Dept. of Biochemistry, Albert Einstein College of Medicine: "Animal Cell Ribonucleic Acids"
D. Summers, Dept. of Microbiology, Albert Einstein College of Medicine: "The Function and Products of the Polycistronic Poliovirus RNA"
R. Simpson, The Public Health Research Institute of the City of New York, Inc.: "The Genetics of Myxoviruses"
J. Strauss/B. Burge, Dept. of Biochemistry, Albert Einstein College of Medicine: "Sindbis Virus Membrane Protein and other Sindbis-Specified Proteins"
Philip I. Marcus, Dept. of Microbiology, Albert Einstein College of Medicine: "Viral Interference"
J. Sambrook, The Salk Institute for Biological Studies: "The Biochemistry and Genetics of Pox Viruses"
L. Crawford, Medical Research Council, Experimental Virus Research Unit, Institute of Virology, Glasgow, Scotland: "Chemistry of the Small DNA Viruses"
V. Defendi, The Wistar Institute: "Transformation *in vitro* by the DNA viruses"
H. Hanafusa, The Public Health Research Institute of the City of New York, N.Y.: "Replication of RSV in Transformed Cells"
H. Green, Dept. of Pathology, New York University School of Medicine: "The Human-Mouse Hybrid Cell"
S. Pfeiffer, Brandeis University
T. Benjamin, Institute for Biomedical Research, American Medical Association, Chicago, Illinois: "Transformed cell-dependent polyoma mutants"
D. Baltimore, Dept. of Biology, Massachusetts Institute of Technology

Course Assistants: S. Carlin, P. Feigin, M. Gladding, R. Knecht, M. Seaman.

The renovation of the upper floor of the James laboratory building to accommodate the Animal Viruses course was completed in the spring of this year and the course was conducted in this new facility for the first time during August. The use of this new laboratory has effected significant improvements in the conduct of this part of our research training program.

UNDERGRADUATE RESEARCH PARTICIPATION PROGRAM

Summer 1968

Once again, the Laboratory was able to take on ten undergraduates for the summer, sponsored by the National Science Foundation's Undergraduate Research Participation Program. The object of this program is to give undergraduates, who are planning a career in research, the opportunity to sample the research life at first hand. Each undergraduate is therefore made a member of one of the research teams working at Cold Spring Harbor during the summer, and is given a particular project. At the same time, he has the opportunity of attending the lectures given to the various courses in microbial genetics, and of joining in the singularly unstratified society that is so characteristic of the place. This program for undergraduates has proved to be very successful in the past, as witness the number of molecular biologists who began their careers as undergraduates at Cold Spring Harbor. No doubt it will continue to be rewarding.

The following students participated in this program during the summer of 1968:

	Title of Project
Michael Brandt, Williams College *Supervisor:* Roger Hendrix	Proteins in λ-infected bacteria
Maryann Brunstetter, Univ. of Calif. *Supervisor:* K. Manly	Isolation of phage λ C_{III} deletion mutants
Stephen Dennis, Massachusetts Inst. Tech. *Supervisor:* Rudolph Werner	DNA replication
Michael Farber, California Inst. Tech. *Supervisor:* P. Spahr	Molecular weight determination on R17 RNA fragments
Lynn Greenwald, Cornell University *Supervisor:* J. Marmur	*Bacillus subtilis* SP02 prophage
Palma Longo, St. Bonaventure Univ. *Supervisor:* R. Gesteland	In vitro protein synthesis
Michael Lovett, Yale University *Supervisor:* S. Goodgal	DNA-negative mutants of *Hemophilus influenzae*
William Meadow, Amherst College *Supervisor:* J. Marmur	*Bacillus subtilis* phage SP02
Donald Syracuse, Dartmouth College *Supervisor:* J. T. August	Isolation of phage Qβ amber mutants
Peter Wayne, Harvard College *Supervisor:* J. Cairns	DNA replication

The work of at least 5 students, S. Dennis, M. Farber, L. S. Greenwald, P. Longo, and P. Wayne, will be published with joint authorship of student and supervisor. The work of some other students may be extended later and the results published.

SUMMER GUEST INVESTIGATORS

Originally conceived as an informal, summer haven where scientists may meet their colleagues, the laboratories at Cold Spring Harbor continue to play host to a group of active workers who spend the summer here. They come to teach the courses, pursue independent projects, write, and collaborate with others in related fields.

In the informal summer atmosphere at Cold Spring Harbor, the scientific activities are enhanced intellectually by the presence of this group.

SUMMER GUEST INVESTIGATORS AND ASSOCIATES

T. August, R. Fox; Albert Einstein College of Medicine.
S. P. Colowick, M. S. Colowick, B. Nagarajan, F. Womack; Vanderbilt University.
L. Crawford; Medical Research Council, Genetics Research Unit, Hammersmith Hospital, London, England.
H. Eisen; Institut Pasteur, Paris, France.
S. Goodgal, J. Bendler, J. Caster, S. Godfrey, J. Gunther, P. Kenehan; University of Pennsylvania.
M. Gottesman; National Institutes of Health.
R. Huskey; University of Rochester.
J. Marmur, H. Leffert; Albert Einstein College of Medicine.
R. Novick, L. Wyman; Public Health Research Institute of the City of New York.
M. Schwartz, N. Axelrod, R. Hendrix, N. Hopkins, J. Kirschbaum, E. Minkley, J. Pero, J. Miller, H. Tranum; Harvard University. I. Herskowitz, K. Manly; Massachusetts Institute of Technology.
E. Signer; Massachusetts Institute of Technology.
A. Tissieres; L'Ecole de Medecine, Geneva, Switzerland.

SUMMER RESEARCH REPORTS, 1968

RNA BACTERIOPHAGES

T. August,
R. Fox,
D. Syracuse

Two basic areas of interest were pursued: (1) the isolation of mutants of *E. coli* which allow lysis by RNA-phage Q-beta at 32° but do not allow lysis at 40°, and (2) isolation of mutants of phage Q-beta which cause hyperproduction of the phage-specific RNA polymerase.

In the isolation of temperature sensitive *E. coli* mutants, the bacteria were first treated with nitrosoguanidine. Sensitivity of the mutated cells to phage was determined by spraying a fine mist of lysate over colonies which were ten hours old. Bacteria which remained sensitive to the sprayed lysate were noted because of the irregular shape of their colonies. It is hoped that characterization of such mutants will shed light on the phage-host interrelationships. Of particular interest are bacterial mutants that affect phage RNA replication.

Mutants of the phage Q-beta were obtained by treatment with nitrous acid. It is known that phage defective in coat protein synthesis may in some cases induce increased levels of the phage RNA polymerase. Methods to isolate such phage Q-beta mutants were studied since polymerase hyperproducers would be a helpful tool for the purification of quantitative amounts of enzyme and for the elucidation of the general mechanisms of control.

STUDIES ON 3′, 5′-CYCLIC AMP AS AN ADENYLATING AGENT

S. P. Colowick,
M. S. Colowick,
B. Nagarajan

This work was an extension of the finding, made earlier in the year at Vanderbilt University in collaboration with Professors Osamus Hayaishi and Paul Greengard, that the adenyl cyclase reaction of bacteria is readily reversible:

$$\text{ATP} \underset{\rightarrow}{\leftarrow} \text{pyrophosphate} + 3', 5'\text{-cyclic AMP}.$$

In the work this summer, the partially purified enzyme from *Brevibacterium liquefaciens* was used to study this reaction in both directions, using the firefly lucifirase system for ATP measurements, and a pyrophosphatase for pyrophosphate measurements. Although side reactions prevented accurate measurement of the equilibrium constant, the results were in rough agreement with our earlier estimate, that

$$K = \frac{(\text{pyrophosphate})\ (3', 5'\ \text{cyclic AMP})}{(\text{ATP})} = \text{ca. } .065$$

at a pH 7.3 and 25°C, when the reactants are present as the Mg salts. This result is surprising, in that the equilibrium is strongly in favor of ATP synthesis. It can be calculated from these results that the standard free energy of hydrolysis of the 3′ ester bond of cyclic AMP is close to 2 Kcal. larger than that of the inner pyrophosphate bond of ATP. The result is significant biologically in that it predicts that 3′, 5′ cyclic AMP may exert its known effects on enzymes by converting them to adenylyl derivatives.

BACTERIAL TRANSFORMATION, PROPHAGE INDUCTION, AND GENETICS OF PHYCOMYCES

S. Goodgal,
M. Lovett,
J. Gunther,
J. Bendler,
J. Caster,
P. Kenehan
S. Godfrey

Mike Lovett: A selection technique was sought which would permit efficient isolation of *Hemophilus influenzae* mutants unable to replicate DNA at high temperature. Three approaches were tried. In the first, mutagenized cells were treated with 5-bromodeoxyuridine at high temperature on the assumption that those cells able to replicate DNA would incorporate this analog and become sensitive to visible light. This procedure was not successful due to the apparent inability of *H. influenzae* to take up 5-bromodeoxyuridine.

The second approach involved heavily labeling mutagenized cells with tritiated thymidine at high temperature followed by storage for long periods at −90° F. Cells capable of replicating DNA at high temperature would be expected to incorporate large amounts of tritiated thymidine and would be preferentially killed by the tritium decay during storage. Loss of viability was less rapid than expected and the experiments are still in progress.

This third approach involved depriving a nitrosoguanidine-treated multiple auxotroph of both proline and thymidine at high temperature, on the assumption that cells unable to initiate DNA synthesis would preferentially survive this treatment. Several mutants were obtained which showed some inhibition of DNA synthesis at high temperature. Characterization of these mutants is continuing.

An attempt was made to determine if a period of thymine deprivation resulted in the premature re-initiation of chromosome replication in a thymidine auxotroph. Premature re-initiation should change the relative dosage of various genes in transforming DNA extracted from such cells. The number of copies of markers near the origin of replication should increase relative to those distant from the origin provided that the DNA is extracted before the new round of replication has been completed. DNA was extracted from samples of a culture taken at various times after re-addition of thymidine and the relative gene dosage of four genetic markers was determined by transformation. First results suggest that there is a preferential increase in the frequency of certain markers. This is consistent with the hypothesis that a period of thymidine deprivation does result in premature initiation of chromosome replication in *H. influenzae*.

Jay Gunther: Studies of the nucleases of *H. influenzae* were continued. Previous work at Cold Spring Harbor and Philadelphia has shown that this organism contains an enzyme which converts double stranded DNA into mononucleotides and single stranded DNA. Experiments were undertaken this summer to determine what effect this enzyme has on the transforming activity of *H. influenzae* DNA. In a typical experiment, it was found that after 8% hydrolysis, 80% of the original transforming activity remains. These results support the hypothesis that the enzyme is an exonuclease releasing the 5′ mononucleotides from the ends of the DNA molecule. The partially purified enzyme shows an absolute requirement for manganese or magnesium ions and the nature of the divalent cation does not affect the loss in biological activity. In an effort to see if this enzyme plays a role in the development of the competent state, its activity was determined in extracts of competent and noncompetent cells. The specific activity of the exonuclease as well as that of a nuclease specific for single stranded DNA was found to be the same in extracts of both cell preparations. Therefore, the development of the state of competence does not involve an enhancement of the activities of these enzymes. These studies are being continued.

John Bendler: Studies were continued on the quantitative relation between marker linkage and physical separation of marker loci during genetic transformation in *H. influenzae*. Previous work suggests that for markers specifying cell functions, the frequency of joint transformation by DNA fragments bearing two markers decreases exponentially with the physical separation of the marker loci on the DNA, marker linkage being halved for about every 10 million dalton increment in the DNA segment between the marker loci. The transformation behavior of markers within the prophage S2 region was examined by transforming cells carrying defective prophage with DNA from cells lysogenic for other mutant prophage. Results indicate that additive distances are obtained for markers within the prophage region when the same exponential mapping function is employed, and that the calculated physical length of the prophage region is in good agreement with the observed size of S2 phage DNA.

John Caster: H. influenzae mutants altered in their capacity to undergo genetic transformation were isolated, following nitrosoguanidine treatment, by a replica plating technique. Preliminary analysis indicates that these mutants differ from the parental strain in their ability either to take DNA up from solution or to integrate it into their chromosome. A detailed examination of these strains will yield useful information about these processes.

A model for prophage induction following inhibition of DNA replication was also studied. It appeared possible that if premature initiation of chromosome replication points were induced subsequent to ultraviolet irradiation or thymine deprivation, the resulting replication of the prophage locus might dilute the cytoplasmic repressor concentration below effective levels, provided that the repressor concentration in established lysogens is low. To account for superinfection immunity, however, it appeared necessary to assume that superinfection in some way stimulated production of the product of the prophage immunity gene. If this were the case, superinfection of a lysogen should protect it from induction by ultraviolet irradiation. Preliminary experiments carried out this summer indicate that superinfection of *Escherichia coli* K-12 (λ) by phage

containing a defective immunity gene (λ_{cI90}) does not confer significant protection against UV induction, although superinfection with normal λ does appear to confer some protection. The latter result can be accounted for by function of the normal immunity gene in the superinfecting phage. Therefore, these results contradict the assumption that superinfection stimulates prophage immunity gene activity, and by implication cast doubt on the idea that prophage induction is solely a result of premature chromosome initiation.

Patricia Kenehan: Several *Phycomyces* heterocaryons containing nuclei of different auxotrophic mutants were characterized by assaying for parental segregants and heterocaryons. The results indicate that recombinants may be formed. Further testing of these "candidates" will be continued at the University of Pennsylvania.

A variety of materials were tested for their ability to replica plate *Phycomyces* colonies. A commercial fastening material (trade name Velcro) was judged to be the most satisfactory. The "hooked" side of the Velro tape is a mechanically sound device for grabbing small pieces of mycelium and transfer efficiency is high.

Susan Godfrey: The reversion rates of *Phycomyces* auxotrophs were compared upon treatment with nitrosoguanidine or methyl-methane sulfonate. Preliminary results indicate that the two mutagens are similarly effective. However, attempts to isolate new auxotrophic mutants from nitrosoguanidine-treated spore stocks were unsuccessful.

A study of stage I sporangiophores was begun in order to develop methods for halting sporangiophore development in this stage, preliminary to measurement of the phototropic and avoidance response of stage I sporangiophores, and to an investigation of the control of differentiation.

BACILLUS SUBTILIS PHAGES

J. Marmur,
L. Greenwald,
B. Meadow,
H. Leffert

Attempts were made to locate two *B. subtilis* prophages on the bacterial chromosome using genetic techniques by DNA-mediated transformation and by transduction with PBS1.

Lynn Greenwald and *Bill Meadow* undertook to locate the map position of prophage SPO2 in lysogenized cells. Defective lysogens were first obtained which were incapable of liberating SPO2 spontaneously; they were constructed either by lysogenizing *B. subtilis* with temperature sensitive mutants incapable of replicating at the restrictive temperature or by mutagenizing lysogens with nitrosoguanidine. PBS1 lysates and high molecular weight DNA obtained from donor cells (erythromycin resistant, wild type nutritionally and lysogenized with wild type SPO2) were added to cells carrying the defective lysogen. Transduced or transformed cells, selected for markers located across the *B. subtilis* chromosome, were now assayed for their ability to liberate SPO2 spontaneously. It was found that the SPO2 prophage was linked to the erythromycin resistance marker and thus close to the origin of replication of the chromosome as well as the majority of the cistrons coding for ribosomal and transfer RNA. When the same transforming DNA used in the linkage study was added to non-lysogenized, transformable cells, then "zygotic" induction took place. Attempts to prepare specialized transducing lysates from mitomycin C induced lysogenic cultures have thus far been unsuccessful.

H. Leffert attempted to locate the PBSX prophage. This phage is defective, can only be obtained by induction and, since it cannot form plaques, is assayed like a bacteriocin by its killing properties on sensitive bacterial strains. Wild type strains of *B. subtilis* can be induced to produce PBSX; a mutant strain, 44AO, sent to us by K. Bott which he obtained by acridine orange treatment yields, upon induction, incomplete phage particles which contain heads but no tails and possess no killing activity. 44AO, carrying various nutritional and antibiotic sensitive markers, was used as a recipient in genetic experiments in attempts to restore the ability to make complete phage particles with killing activity by exposing them to PBS1 lysates or transforming DNA obtained from wild type cells. Transductants and transformants (using low DNA concentrations) of 44AO to five different markers did not produce complete PBSX particles as assayed by the restoration of killing activity. At saturating transforming DNA concentrations, killing activity could be restored to strain 44AO, due to the simultaneous entry into the recipient cell of unlinked DNA molecules coding for the selected marker and killing activity. Mr. Leffert, a second year medical student at Albert Einstein College of Medicine, is continuing his study of the linkage of the markers involved in the restoration of killing activity to various chromosomal markers as well as the possibility that the killing activity (i.e., the phage tail) is coded for by an episome.

STUDIES ON EARLY FUNCTIONS OF BACTERIOPHAGE

M. Schwartz,
H. Tranum,
J. Pero,
R. Hendrix,
M. Brandt,
N. Hopkins
K. Manly
M. Brunstetter
N. Axelrod
E. Minkley
I. Herskowitz
J. Kirschbaum
P. Kimball
D. Schwartz
R. Huskey,
E. Signer,
M. Gottesman,
H. Eisen

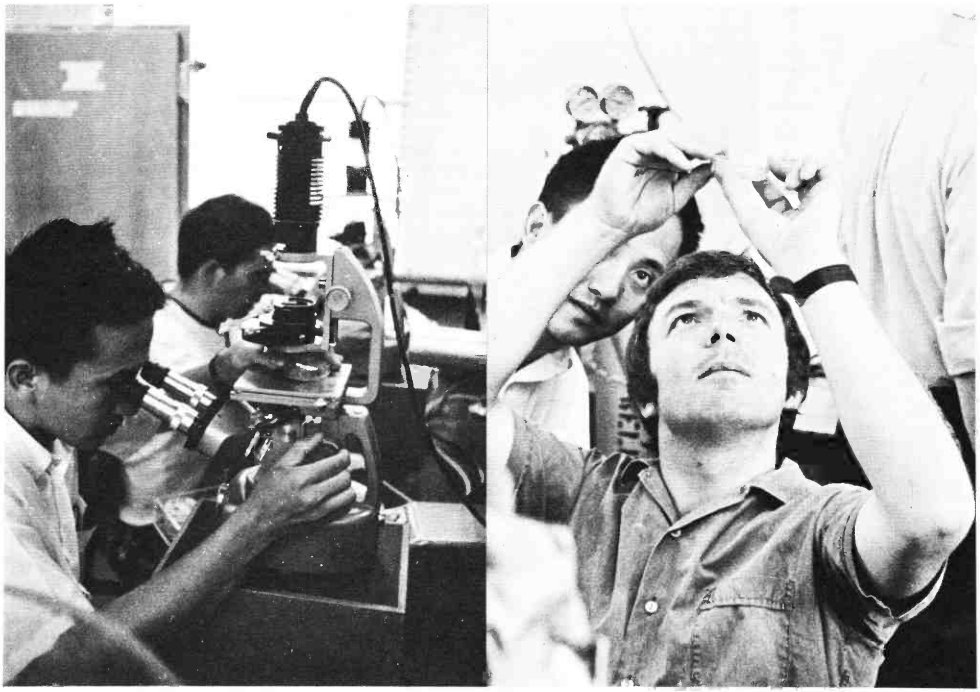
Bacteriophage λ, a virus of *Escherichia coli,* is the most extensively studied of the temperate bacteriophages. Its genetic map has been shown to contain 30 to 50 genes. Some of these, called early genes, are involved in the replication or recombination of the virus chromosomes, or in the regulation of the activity of other viral genes. Most of the work done by the group consisted of studying the functions and trying to isolate the protein products of some of these early genes.

Maxime Schwartz, assisted by *Henrietta Tranum,* made some studies on the function of cistron N, a gene whose activity is necessary for the expression of the majority of the other phage functions. He demonstrated that N function was expressed as early as one minute after induction of the phage and that the N product was functionally unstable (half life of about 5mn.). He also found evidence that the inducing activity of the N gene product on several other viral genes could not be expressed in the presence of the immunity repressor.

Janice Pero, by a totally different technique, supported this last conclusion. She showed that λ-exonuclease was synthesized by phage λ only if the cell contained an active N product and no active immunity repressor.

Roger Hendrix and *Michael Brandt* pursued a project on which Roger Hendrix had already been working for some time, consisting of identifying some of the viral gene products. Lambda phage proteins are made in 10-fold excess over host proteins if the host cells are appropriately treated with a heavy dose of ultraviolet light before infection. The proteins made under such conditions can be characterized by use of double label techniques and polyacrylamide gel electrophoresis. During the summer particular emphasis was put on proteins which seem to be products of genes involved in the recombination events leading to the integration of the phage chromosome into the host chromosome during the process of lysogenization. Also the use of a new deletion mutant isolated by K. Manly and M. A. Brunstetter (see below) gave preliminary evidence of the identification of the C_{III} gene product. C_{III} is a regulatory gene whose function is the subject of numerous discussions at the present moment.

Nancy Hopkins made some attempts to understand the role of this C_{III} gene as well as that of the C_{II} gene, another regulatory gene of unknown function. The effect of mutations in these genes on the pattern of DNA replication was studied. No conclusive results have yet been obtained.



Kenneth Manly and *Mary-Ann Brunstetter* isolated a new series of plaque-forming λ *bios*. Transducing phages of this type are interesting because they constitute a class of deletion mutants of λ and because their attachment region (substrate for the integration system) is modified.

A new class of λ *bio* was isolated, in which are deleted not only the viral genes involved in recombination and integration, but also the C_{III} gene. The mere existence of such phages shows that there are no genes essential for growth to the left of C_{III}. The isolation of such phages will undoubtedly allow several types of experiments involved in the study of the C_{III} gene function or the isolation of its RNA and protein products, in the physical mapping of λ, and in the study of the phage recombination system. In addition, λ *bios* have been isolated which may have deletions shorter than any of the known transducing phages of this type.

Nancy Axelrod did some studies to compare the phage and host recombination systems. A number of bacterial mutants deficient in recombination have been isolated by different workers. A class of these mutants, known as *rec* A, have at least two additional properties. First they are highly sensitive to ultraviolet light. Second, when they are lysogenic for a UV inducible phage, this phage cannot be induced by UV light in them. N. Axelrod's experiments were designed to test if the phage recombination system could complement the *rec* A mutations for these two last defects. The results obtained, although preliminary, indicated that this was not the case.

Edwin Minkley studied the action of the N gene on the expression of a late function, the synthesis of lysozyme. The experiments consisted in measuring lysozyme synthesis after shifting to the restrictive temperature cultures which had been infected for different lengths of time with a phage carrying a temperature sensitive mutation in gene N. The data showed that if the shift to the restrictive temperature was done when a fair amount of lysozyme had already been synthesized (20 to 50% of the maximum amount), the synthesis stopped immediately. This result seems to support the idea proposed by others that the N product is required during the whole life cycle of the phage. A detailed analysis of the kinetics, however, suggests a possible alternative hypothesis, which should be tested in the near future.

Ira Herskowitz went through the last steps of the preparation of a bacterial strain containing simultaneously the two known substrates of the phage integration systems, both the bacterial and the phage attachment sites. This strain will be used to study the specificity of the integration system. I. Herskowitz also demonstrated that a widely used strain of phage carrying two mutations in gene N had an abnormal density. He showed that this abnormal density was not a necessary consequence of the presence of the double mutation, since he succeeded in preparing a new strain with the same two mutations and a normal density. Revertants of this new strain will be looked for since they could be mutants not requiring N function for growth.

Joel Kirschbaum wanted to identify the products of genes involved in the replication of the viral DNA. He ran into the problem of getting high enough quantities of viral DNA to purify the proteins by their binding properties, as he was planning to do.

Paul Kimball went through the first steps of experiments devised to study the involvement of DNA synthesis in the two known phage directed recombination systems (*int* and *red*). He also did some experiments with the very interesting and very little studied phage μ.

Daniele Schwartz prepared bacterial strains containing temperature sensitive episomes which carry the attachment site for $\phi 80$, a temperate phage similar to λ. Derivative of these strains will be selected in which the episome, and thus the $\phi 80$ attachment site, will be integrated in different places of the bacterial chromosome. Phage $\phi 80$ will integrate in abnormal locations when infecting such strains. It will then give rise to transducing phages carrying a wide variety of bacterial genes. Such transducing phages will be of great interest both for the study of bacterial genetic systems and the study of $\phi 80$.

Robert Huskey, Ethan Signer, Max Gottesman, and *Harvey Eisen* stayed in the laboratory for only short periods of time. Not only did they pursue some of their current work on phage lambda during that time, but they also participated a great deal in several of the projects described above.

IN VITRO SYNTHESIS OF RNA AND PROTEIN

A. Tissieres,
P. Longo,
L. V. Crawford,
R. Gesteland

Study of the coupling of transcription and translation in an in vitro system.

It is now well established that messenger RNA, either phage RNA or RNA extracted from *E. coli* can be translated in vitro to make a polypeptide chain which very closely resembles a particular specific protein. Whether RNA synthesized in vitro by the DNA dependent RNA polymerase can be translated to make a specific protein is not yet clear.

By using preincubated ribosomes and supernatant proteins devoid of nucleic acids, a system was used which is entirely dependent upon the addition of T4 DNA and RNA polymerase, and which incorporates amino acids into peptide chains at a rate somewhat comparable to that given by a crude preincubated extract. The ribosomes were preincubated and then purified from the supernatant phase by centrifugation through a layer of sucrose. The supernatant was prepared by the two phase system of Albertsson in the presence of high NaCl concentrations, according to Capecchi. Some of the properties of this system were investigated as a preliminary study to finding out whether the synthesis of specific proteins, coded for by DNA could be demonstrated in vitro.

Synthesis of Polyoma RNA

The ability of RNA made from polyoma virus DNA using *E. coli* RNA polymerase, to act as messenger in the *E. coli* protein synthesizing system was tested. In both the coupled system (DNA-RNA-protein) and the uncoupled system, where RNA made previously was added to the protein synthesizing system, no activity for amino acid incorporation could be detected. Substantial quantities of RNA were made on the polyoma DNA template (tenfold synthesis in two hours at 37°C). Much of this RNA formed a fast sedimenting complex which is now being investigated to see if its formation is connected with the lack of messenger activity of the RNA.

Bacteriophage T-4 glucose transferase

Bacteriophage T-4 produces two enzymes at early times after infection that transfer glucose residues from UDPG to hydroxymethylcytosine in the DNA. In analogy with the in vitro synthesis of T4 lysozyme, RNA was extracted from T4 infected cells and tested for its ability to direct synthesis of glucose transferase activity in the same cell-free system. Using RNA extracted 10 minutes after infection as messenger, enzyme activity was produced during in vitro protein synthesis at a level that was 8-10-fold over background. This activity was clearly messenger dependent and was closely correlated with protein synthesis. Glucose transferase mutants are now being used to test these results critically.

R. Novick, L. Wyman

R. Novick: Most of the summer was spent completing a large manuscript. Preliminary studies were initiated on bacteriophage P1, with the object of determining whether there are two different replication control systems for this bacteriophage, one for lethal vegetative replication during lytic infection and the other for quiescent plasmid-like replication during prophage state. The rationale for the experiment was that if there are two such systems, it should be possible to isolate mutations affecting the replication control system for the prophage without affecting vegetative phage replication. A search was therefore begun for conditional mutations in which the prophage was cured during growth at high temperature, could maintain itself normally at low temperature, and vegetative replication was normal at both temperatures. Several promising mutants were found but have not yet been investigated further.

L. Wyman: Studies were continued on the staphylococcal plasmid system that we have been concerned with for some time. The summer's project for Miss Wyman was the isolation of recombination deficient staphylococci for the purpose of ascertaining whether the penicillinase plasmid had its own recombination system or not. A series of mitomycin-sensitive mutants were isolated and initial studies on their characterization were performed. Studies on their ability to support recombination were in progress at the end of the summer.

PHAGE CONFERENCES

August 28—September 5, 1968

Following the completion of the summer program, the auditorium and housing facilities were used for the annual phage conference, this year in the form of 2 three-day meetings, attended by most of the active phage workers in the country. They were attended by 209 people.

SECTION I – Single Stranded Phages – Aug. 28 - Sept 1, 1968
SECTION II – Double Stranded Phages – Sept. 1 - Sept. 5, 1968

SECTION I

RNA Phages–I–Organizer T. August

N. Federoff and H. P. M. Fromageot–Growth of the Bacteriophage f2 in *E. coli* treated with Rifampicin.
P. Silverman and S. Norton–The A Protein of the RNA Phage as a Trigger for RNA Release.
L. Wendt and H. Mobach–Evidence for Two States of F. Pili.
P. Model and R. E. Webster–A Novel Mutant of Phage f2.
Rolf Benzinger, Keith Yamamoto, Larry Lawhorne, G. Treiber and Bruce Alberts–Polyethylene Glycol Precipitation: A Mild and Effective Method for Concentrating and Purifying Bacteriophages.

RNA Phages–II

Anne M. Haywood–Cellular Location of MS2 RNA Synthesis.
L. Shapiro–Synthesis of Qβ RNA.
Rensing–The Qβ of RNA Polymerase–Qβ RNA complex.
Reuben, Levisohn, and S. Spiegelman–The *in vitro* Selection, Cloning and Properties of Mutants of Variant Qβ RNA.

RNA Phages–III

Tsutomu Sugiyama, Henry O. Stone, Jr. and Daisuke Nakada–Synthesis of Coat Protein and Coat-Protein Fragment Directed by an Amber Coat-Protein Mutant of the RNA Phage MS2.
Harvey F. Lodish–Bacteriophage f2 RNA: Gene Order and Control of Translation.
P. F. Spahr and R. F. Gesteland–Interaction Between R-17 Coat Protein and Intact or Fragmented R-17 RNA.
H. D. Robertson–The Mechanism and Control of RNA Synthesis by Bacteriophage f2.

Single Stranded DNA Phages—I—Organizer D. Denhardt

L. B. Lyons—Genetic Studies of Bacteriophage f1.
Hiromi Ishiwa and Irwin Tessman—Control of Host DNA Synthesis after Infection with Phages S13 and øX174.
E. F. Rossomando and N. D. Zinder—Structure and Mechanism of Stabilization of the Bacteriophage f1.
R. L. Wiseman, J. Lapointe and D. A. Marvin—Structure of the Filamentous Phage If1.
Jay Doniger and Irwin Tessman—An S13 Capsid Mutant that Fails to Inject its DNA.

Single Stranded DNA Phages—II

Timothy Henry—Phage M13 Proteins.
Ann B. Burgess and David T. Denhardt—øX174 Proteins made after Infection of *E. coli.*
David Pratt—The Gene for the Major Coat Protein of Phage M13.
A. Burton—øX and øR: A Problem for Phage Assemblers.
L. L. Larison—The Influence of the REP—Mutation on the Production of øX Specific Messenger RNA.

Single Stranded DNA Phages—III

B. Y. Tseng and D. A. Marvin—The Fate of Parental fd DNA During Replication.
R. Knippers and W. O. Salivar—The Fate of the "Parental Replicative Form" DNA of Bacteriophage øX174.
Dan S. Ray—Replication of the Single Stranded DNA of Bacteriophage MI3.
D. T. Denhardt—Synthesis of øX174 DNA in Extracts of øX-Infected Cells.

SECTION II

Lysogenic Phages—I—Organizer H. Echols

Sankar Adhya, Pat Cleary and Allan Campbell—A Deletion Analysis of Prophage Lambda and Adjacent Genetic Regions.
H. Yamagishi and A. Skalka—Distribution of Nucleotides and of λ-Matching Sequences in the Gal-Bio Region of *E. coli* DNA.
B. C. Westmoreland, W. Szybalski and H. Ris—Electron Microscopic Maps of the Deletions and Substitutions in Heteroduplex DNA Molecules of Phage λ.
H. A. Lozeron and W. Szybalski—DNA and Transcription Homologies Between Coliphages λ and φ80.
G. Veldhuisen and A. D. Kaiser—Intracellular DNA Produced Upon Induction of Head Mutants of λ.

Lysogenic Phages—II

L. Moore, H. Echols—Characterization of Recombination Deficient λ Mutants.
M. Shulman—Characterization of Recombination Deficient Mutants of Phage λ.
Kenneth Manly—Nondefective Biotin-Transducing λ Phage.
R. Weisberg and M. E. Gottesman—The Integration and Excision Defect of Lambda *dg*.
W. Spiegelman—Phage Production from a Terminal Deletion Prophage.
John S. Parkinson—Deletion Mutants of Lambda.

Lysogenic Phages—III

Roger Hendrix—Proteins without Genes and Genes without Proteins in Phage Lambda.
M. Schwartz—On the Function of the N Cistron in Phage Lambda.
D. Rabovsky and M. Konrad—The Timing of Two Early Phage Functions.
William S. Sly and Karen Rabideau—The Mechanism of λc_{17}CI Virulence.
Alan R. Kolber, Seymour Packman and William S. Sly—Additional Properties of λc_{17}, a Regulatory Mutant Related to Virulence.

Lysogenic Phages—IV

Robert M. Benbow and Paul Howard-Flanders—Transfer of Damaged Episomal DNA from Ultraviolet Irradiated F′ Donors to Lysogenic Recipients: Prophage Curing and Recombinant Formation.
I. C. Gunsalus, C. F. Gunsalus and A. M. Chakrabarty—Defective Transducing Phages Promoting Infectious Fertility in Pseudomonas.
L. Rutberg—Mapping of a Temperate Bacillus Phage.
R. L. Nutter and L. R. Bullas—Groups of Temperate Bacteriophages of Salmonella Potsdam.
S. Kumar, H. A. Lozeron and W. Szybalski—The Orientation of Transcription of the lac and trp Operons as Determined by Hybridization of mRNA with Separated DNA Strands of Coliphages φ80iλdlac and φ80pt.
R. Schleif—Inhibition of RNA Synthesis by Rifmycin.

Other Double Stranded Phages—I—Organizer J. Marmur

D. Brown and T. F. Anderson—Interaction of Bacteriophage T4 with host cell wall During the Latent Period.
S. Silver—Surface Alterations After Infection of *Escherichia coli* with Bacteriophages T2 and T4.
B. Rutberg—Hybrids Between T4B and T4D.
Dean Fraser and Karen Gabbard—Conformation of T2 DNA vs. Salt and Diamine Concentration.
Laura L. Winstead and Henry Drexler—On the Origin of Yielder Cells in Host Controlled Modification of T1.

Other Double Stranded Phages—II

E. J. Dubovi, I. Tessman and Y. C. Yeh—T4 Mutants Unable to Induce Ribonucleotide Reductase Activity.
M. Nishihara and H. V. Aposhian—Specific Labeling of Phage SP8 DNA with [^{14}C] Hydroxymethyldeoxyuridine.
Robert C. Miller, Jr.—DNA Synthesis and P^{32} Suicide Stabilization.
Michael M. Piechowski—The Condensation of T4 DNA in the Presence of Chloramphenicol.
R. B. Luftig and W. B. Wood—An Intermediate in T4 Phage Head Formation.
Andrzej W. Kozinski—Mode of DNA Replication in Ligase-Negative T_4AM. H39X.

Other Double Stranded Phages–III

D. Kennell–Continued Synthesis of Host RNA During Infection of *E. coli* by Bacteriophage T4.
G. Guthrie–Metabolic Regulation of the Lifetime of T4 Messenger RNA.
B. D. Hall and G. Hager–RNA Polymerase from T4-infected *E. coli.*
M. Adesnik and C. Levinthal–Messenger RNA Metabolism in T4 Infected *E. coli.* Electrophoretic Analysis on Polyacrylamide Gels.

Other Double Stranded Phages–IV

F. W. Studier and J. V. Maizel–Genetics and Physiology of T7.
Takashi Kasai and Ekkehard K. F. Bautz–Analysis of mRNA Transcription in Bacteriophage T4 Infected *Escherichia coli.*
R. Haselkorn, M. Vogel, D. S. McMahon and W. Mason–Protein Synthesis in T4-Infected *E. coli* B, I.
J. Wilhelm, J. W. Coolsma, D. S. McMahon and R. Haselkorn–Protein Synthesis in T4-Infected *E. coli* B, I.
David L. Wilson and E. Peter Geiduschek–*In vitro* Studies on a Template-Specific Inhibitor of RNA Synthesis.

NATURE STUDY COURSES

Children of Ages 6 to 16

During the summer of 1968, 19 courses in Nature Study were conducted in two monthly sessions. The enrollment this year was 302 students. The course offerings included:

General Nature Study (ages 6, 7)
General Ecology (ages 8, 9)
Plant-Insect relationships (ages 8, 9)
Bird Study (ages 10, 11)
Fresh-Water Life (ages 10, 11)
Seashore Life (ages 10, 11)
Insect Study (ages 10, 11)
Animals with Backbones (ages 10, 11)
Geology (ages 10, 11)
Advanced Ecology (ages 12-16)
Ichthyology-Herpetology (ages 12-16)

INSTRUCTORS:

Mr. Otto A. Heck, M.S., Assistant Professor of Biology at Trenton State College
Mr. Herbert Atkinson, M.Ed., Science Coordinator, Hicksville Jr. High School
Mrs. Barbara Church, M.Ed., Science Substitute Teacher at Central High School, Dist. #3
Miss Virginia Jones, M.S., Graduate Student in Nature and Conservation Education, Michigan State University
Mrs. Irene Parks, Science Teacher, South Woods Jr. High School, Syosset, N.Y.
Mr. Alex Pepe, M.A., Science Teacher, K-6, East Side School, Cold Spring Harbor, N.Y.
Mr. Larry Roel, Sophomore at Princeton University, and former student and assistant of the Children's Nature Study Program, Cold Spring Harbor Laboratory of Quantitative Biology

The Laboratory gratefully acknowledges the ninth-year contribution of the Huntington Federal Savings and Loan Association. This provided nature study scholarships for 12 students of the Huntington elementary schools.

NATURE STUDY WORKSHOP FOR TEACHERS

The 13th annual Workshop in Nature Study was offered from June 28th to July 26th, 1968. This program was designed to familiarize elementary and secondary school teachers with the natural environment of the Long Island area, including the animals and plants living there; and those aspects of the environment which affect these organisms. The course consisted of field trips to ponds, stream, seashore, woodlands, field and other natural habitats, for purposes of collecting and first-hand study, with indoor laboratory work-time divided between lectures and practical work. The experiences of the course are designed to help teachers in their classroom science activities. The Workshop consisted of 34 hours of lecture plus the additional hours spent as follows: The laboratory and field trip work accounted for 42 hours, while the completion of the course requirements utilized at least 10 additional hours and 2½ hours were spent in observation of a children's nature study course conducted by a naturalist-leader.

Twenty teachers attended the Workshop. Upon satisfactory completion of the requirements of the course, teachers were entitled to four points of in-service credit authorized by the New York State Education Department, Division of Higher Education. The instructor for the summer of 1968 was Mr. Otto A. Heck.

GRANTS AND CONTRACTS

May 1, 1967 to April 30, 1968

Grantor	Investigator	Total Award	Grant Number
	RESEARCH GRANTS		
National Science Foundation	Dr. Cairns	$50,900	GB-4055*
The Rockefeller Foundation	Dr. Cairns	17,000	RF-63042
National Science Foundation	Dr. Gesteland	98,900	GB-7209X*
National Science Foundation	Dr. Werner	66,900	GB-7222*
	TRAINING GRANTS		
National Institutes of Health	Summer Courses	50,527	GM-890-12
National Science Foundation	Phycomyces Workshop	20,000	GB-6114*
National Science Foundation	Undergraduate Research Program	14,000	GY-2575
	SYMPOSIUM GRANTS		
Atomic Energy Commission		7,000	AT(49-7)-3020
Air Force Office of Scientific Research		7,000	AFOSR-68-1542
National Institutes of Health		12,050	CA-02809-12
National Science Foundation		5,000	GB-7389

*These grants cover a two-year period

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Anna Marie Skalka
Carol Thomason
Hideo Yamagishi

FINANCIAL REPORT

For the period May 1, 1967—April 30, 1968

As of April 30, 1968 our assets were as follows:			
Cash	$ 52,681.21		
Accounts Receivable	80,278.38		
Inventory of book	15,342.98		
Prepaid expenses	2,183.87		
Investments in Government obligations, at cost which approximates market	29,585.70		
Restricted funds (cash and grants receivable)	237,835.34		
Plant funds:			
Unexpended	46,784.19		
Investment in plant	514,378.21		
Total		$979,069.88	
Our liabilities were as follows:			
Current funds:			
General:			
Accounts payable	$ 24,027.79		
Deferred income	7,740.00		
Fund balance	148,304.35		
Restricted:			
Fund balances	237,835.34		
Plant funds:			
Unexpended fund balance	46,784.19		
Investment in plant	514,378.21		
Total		$979,069.88	
For the year 1967-1968, our receipts were as follows:			
Grants and contracts		$196,183.17	
Indirect cost allowance on grants	$ 18,749.55		
Contributions:			
Sponsors and Friends	58,380.16		
Carnegie Institution of Washington	27,725.00		
Long Island Biological Association	5,000.00		
Wawepex Society	3,300.00	94,405.16	
Symposium registration fees	6,955.00		
Tuition fees	28,099.00		
Laboratory rentals	10,880.00		
Investment income	1,838.83		
Other sources	666.53		
Auxiliary activities:			
Symposium book sales	204,366.58		
Dining Hall	35,500.88		
Rooms and apartments	43,126.56	282,994.02	
Total			$640,771.26
Our expenditures were as follows:			
Research and education programs	$178,570.96		
Annual Symposium	27,101.15		
Library	4,320.28		
Operation and maintenance of physical plant	147,056.21		
General and administrative	72,733.09		
Scholarships	1,190.00		
Auxiliary activities:			
Symposium book	93,462.71		
Dining Hall	36,321.78		
Rooms and apartments	16,628.34	146.412.83	
Total			$577,384.52
Excess of Income over Expenditures 1967-1968			$ 63,386.74

This financial report indicates that the Laboratory ended its fiscal year 1968 showing a substantial excess of income ($63,386.74) over expenditures in contrast to the previous year in which there was an operating deficit of $10,089.61. This unexpected surplus, which brought about a much needed increase in our cash reserves, was created by two factors unanticipated when our 1968 budget was drawn up. By far the most significant is the tremendous increase in income from the sale of the Cold Spring Harbor Symposia volumes and the "Phage" book. In the previous fiscal year, sales of $107,373 were achieved. This year these sales increased by almost 100% to reach an unprecedented total of $204,367. As most of the sales of the "Phage" book occurred in fiscal '68, as did those of the enormously popular "Genetic Code" volume, we anticipate that our 1969 sales will be considerably lower.

Also contributing to our '68 surplus were delays in the construction of the James teaching lab, so that bills anticipated in '68 were not in fact received until early in fiscal '69. Given the various inflationary factors now operating, as well as an anticipated drop in book sales due to limitations in Federal money for science, we anticipate that, at best, our cash reserves will remain constant during the coming fiscal year.

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The Lilly Research Laboratories
Long Island Biological Association
The Merck Company Foundation
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for 1967-68, as of October 11, 1968

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