COLD SPRING HARBOR LABORATORY OF QUANTITATIVE BIOLOGY

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COLD SPRING HARBOR, LONG ISLAND, NEW YORK

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The introduction to an annual report, as its name implies, is usually a general review of the preceding year. In it the writer recounts what has happened during the year and says how this fits in with the overall design set in previous years. If he is feeling optimistic, or wishes to appear so, he will go on to describe the steps he hopes to take next year.

The introduction to one's last annual report is more of an occasion. Although the accomplishments (and failures) of the preceding year should not be left out entirely, there is a challenge to produce a dispassionate account of the institution whose intimate workings, strengths and weaknesses, one has studied so long and so carefully.

It was a strange sensation to find myself, after a 12,000 mile journey to the richest country in the world, director of an institute that was at once world-famous and yet decrepit beyond belief, busily engaged in subsidizing its multifarious summer program and yet virtually bankrupt, possessed of devoted alumni scattered about the globe and yet newly under the guidance of a predominantly scientific board whose members were mostly not drawn from these alumni. It turned out, however, that this paradox was not new and any sense of despair that I felt had been felt at some time or another by my predecessors; indeed, one of them had even discussed suicide in an annual report. Further, it seemed that despite their renown all the independent "summer" institutes of the world, such as Woods Hole, Naples and Cold Spring Harbor, had experienced or were experiencing a degree of cliff-hanging that is almost unknown in the more sedate centers of learning.

The reason for the renown of these summer institutes is not hard to see. Science is constantly shifting. As new disciplines arise, others disappear - at least as usefully delineated entities. And so the universities have to keep abreast by adding new departments. The staff for these new departments cannot be drawn exclusively from the old, but must come in significant part from the independent research organizations which, in consequence, have an influence on the progress of science that is out of all proportion to their size or number. There is, however, an unavoidable interregnum before the universities have responded to the pressure, during which it is the job of the summer institutes to hold courses in the new disciplines and thereby populate them with practitioners. The unique role of the summer institutes is, therefore, to support heterodox disciplines in the making, and their year-round research activities should be thought of as subsidiary to their main purpose.

The reason for their troubles arises from the very qualities that make them successful. For they must champion newborn causes at the stage when these are not likely to get much support from conventional sources. And to have this versatility they must be small, even though to be small is to risk every vagary of staffing and budgeting without the protective inertia that comes with sheer size.

It was unfortunate that the fusion of the two units at Cold Spring Harbor (formerly under the Carnegie Institution of Washington and the Long Island Biological Association) should have occurred at a time when the post-war escalation of the sciences was drawing to a close and when the universities and colleges of the country were on the brink of an intense financial crisis. This greatly diminished the chance of acquiring the endowment that, until then, the presence of the Carnegie Institution had made almost unnecessary. No doubt with this problem in mind, a group of universities undertook to back the Laboratory in the event of exceptional need. However it seems to me that their subsequent reluctance to fulfill their obligations greatly reduced the chance that any foundation could be persuaded to step in with large scale support: after all, it is the first principle of fund-raising that you must get help from your nearest and dearest before you ask for help from strangers.

It is worth considering, at this point, the peculiar problem of a summer institute that has to balance its operating budget with earned income and whose scientific staff (even the director, as it turned out) have to be supported by outside grants each lasting between two and five years. Such an institute has to be content with a staff of transients who owe it little if any allegiance and whose research grants, in these days of 'cost-sharing', bring somewhat more expense than income. Only rarely can it expect to find someone who, though paid from his research grant, is willing to forgo the immunity which is the compensation for that precarious state and devote much of his time to the varied acts of institute housekeeping that are needed to keep going a program designed, among other things, to subsidize the summers of other scientists like himself.
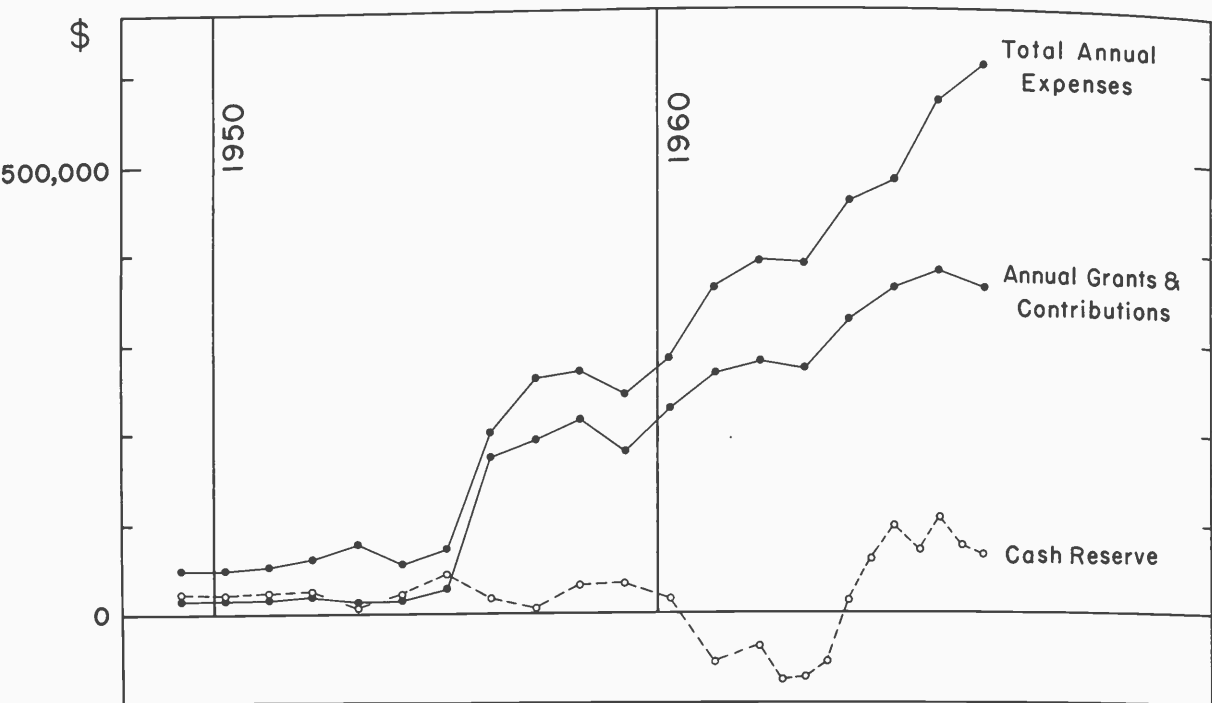
The trend in the finances of the Laboratory of Quantitative Biology and its predecessor, the Biological Laboratory of the Long Island Biological Association.

The Cash Reserve equals the total unobligated cash and investments and therefore excludes all money destined for expenditure on grants or held in certain trust funds and excludes, from 1963 onwards, funds held by the Long Island Biological Association.

The Annual Grants and Contributions represent the total support received each year from outside sources.

The level of the Total Annual Expenses is a rough index of the magnitude of the operation. In the years when the cash reserve does not change, the difference between Grants & Contributions and Total Expenses is made up by income from what might be called the Laboratory's hotel and publishing business.

Despite this basic problem of responsibility and allegiance, the Laboratory and its predecessor have managed to expand the size of the operation about tenfold in the past 13 years. This increase, which is shown in the accompanying figure, has been achieved partly by increased support from outside (in the form of grants and contributions from foundations, sponsors and friends) and partly by an increase in its publishing business. At the same time, the expenditure on salaries and materials for the maintenance of the buildings and grounds has been raised during this period from about $10,000 a year to slightly over $100,000 a year. As a result, the Laboratory is now in the position of having a balanced operating budget, a rather small albeit improved cash reserve, and a partly rejuvenated physical plant.

I shall now list what seem to me to be the successive steps needed to put the Laboratory in a secure position so that it can once again serve its proper pioneering role. First, the Laboratory must

acquire sufficient endowment to pay for as much scientific administration as is needed; this would take it back to where it was before the Carnegie Institution left the scene and would at least allow those who run the place to know that as long as they continue to run the place they will be paid for doing the job. Second, the participating institutions that dominate the Board of Trustees must participate more realistically; at present their lack of strong support seems to constitute a barrier to a successful appeal to the philanthropic foundations. Lastly, if the Laboratory is to conduct its operation and maintenance properly, it must either acquire a small amount of additional income or progressively turn its summer program over to less productive but more affluent scientists.

There is a school of thought which holds that the Laboratory should first staff itself on grants and only then try to legitimize itself with endowment; this was the dominant school in the middle of the 1950s, as the figure shows. However, the Laboratory at Cold Spring Harbor has been trying to get an endowment for 70 years and I cannot see that a fly-now-pay-later policy would do more than add to the financial burden, just as it did in the 1950s when pursuit of such a policy led to the maintenance problem we have been struggling with for the past few years. Nor does it seem likely that the coming of a large group of scientists, paid by grants, could be converted by some fiscal legerdemain into the equivalent of a steady income.

During the past year, both Dr. Davern and Dr. Speyer have left for tenured positions, at the University of California and the University of Connecticut respectively. Dr. Davern had been Assistant Director for two years, during which time he worked selflessly on behalf of the Laboratory and its numerous summer visitors. He is replaced by Dr. Raymond Gesteland, who arrived from Geneva at the end of August. Although Dr. Margolin joined the staff of the Public Health Research Institute of the City of New York last year, his laboratory in New York is not yet ready for occupancy and so he has stayed on at Cold Spring Harbor.

The following pages contain reports of the various activities of the Laboratory during the year. Since detailed reports of the Carnegie Institution's Department of Genetics appear in the Yearbook of the Carnegie Institution, only a brief report is given here.

John Cairns, Director.

**BACTERIAL
GENETICS**
Laboratory of
P. Margolin,
J. Kemper,
S. Kumar

Unstable Initiation of Gene Expression. The tryptophan operon of *Salmonella typhimurium* consists of five genes, *trpA-trpB-trpE-trpD-trpC,* with the operator and promoter at the *trpA* end and a second initiator between *trpB* and *trpE.* Deletion mutations which remove the operator, promoter, and part of the first structural gene, *trpA*, result in a loss of function of the physically intact second structural gene, *trpB.* We have been able to select for the spontaneous occurrence of clones in which the *trpB* gene has resumed functioning, as evidenced by the synthesis of the enzyme phosphoribosyl transferase and the ability to utilize anthranilic acid as a tryptophan precursor. These secondary mutant strains were given the designation RAX.

In the majority of RAX strains which arise spontaneously the renewed *trpB* function is unstable. Six strains with deletions which penetrate into *trpA* to varying extents were used for selecting 240 independent RAX strains and the degree of instability of each was determined. They ranged from more than 90% unstable to less than 1% unstable. The use of 3 to 5 subclones of each RAX strain showed that the percentage instability was a stable characteristic of each such strain. The size of the deletions in the original strains had no effect upon the range of instabilities of the RAX strains which they produced.

Preliminary tests of the nature of the elements which initiated unstable gene expression were made by carrying out genetic crosses, using RAX strains as donors in transductions. The use of appropriate linked markers allowed us to distinguish two types of RAX strains. For one of these, the evidence suggests that the renewed *trpB* gene expression is due to a duplication of the remaining tryptophan genes associated with a free episome, which supplies the initiating element for *trpB.* The instability may be due to frequent loss of the episome from the cells. The evidence from the transductions with the second type of RAX strain indicates that the unstable gene expression is a characteristic of chromosome associated genes. Our hypothesis is that in such cases initiation of *trpB* gene expression results from the temporary integration of episomes into the chromosome near the beginning of the *trpB* gene. The instability is due to the frequent excision of the episomes.

A Gene and Mutation Specific Suppressor. Mode of Action. The leucine operon of *Salmonella typhimurium* consists of four genes *leuA-leuB-leuC-leuD* with an operator at the *leuA* end. A leucine auxotrophic strain, *leuD700,* bearing a deletion of the distal portion of the *leuD* gene gave rise to a rare leu$^+$ revertant. The leucine prototrophy was found to be due to the presence of a suppressor gene, designated *supQ,* which was unlinked by transduction to the leucine-arabinose chromosomal region. The *subQ* suppressor does not suppress any mutations of the *leuA, leuB,* or *leuC* genes. Of 25 *leuD* mutations examined, 15 respond to the suppressor and 10 do not. The sites of the suppressible and nonsuppressible *leuD* mutations are interspersed among each other on the genetic map. Among the 15 suppressible mutations, two have been identified as deletions of the terminal portion of the *leuD* gene and nine as nonsense mutations. None of the nonsuppressible *leuD* mutations are nonsense mutations.

The second enzyme (isopropylmalate isomerase) of the leucine pathway consists of a complex of the *leuC* and *leuD* gene protein products. Our results may be explained by the following model. The *supQ* suppressor locus produces a protein which is an actual or approximate duplication of the *leuD*

gene product. When the *leuD* gene contains a suppressible mutation, its product is very drastically altered (deletion or frameshift mutations) or consists of only a peptide fragment (nonsense mutations). Such a product is unable to complex with the *leuC* gene product. The *leuC* gene product is thus free to complex with the *supQ* product and form an active isomerase. When the *leuD* gene contains a non-suppressible mutation (missense), it produces a slightly altered protein product (CRM) which can form an enzymically nonfunctional complex with the *leuC* gene product. Apparently the *supQ* gene suppressor product competes poorly with *leuD* CRM in formation of the isomerase complex. The above model predicts quite specific consequences with respect to the frequency of occurrence of secondary nonsense mutations and the effects of nonsense suppressor mutations. Preliminary tests have indicated the fulfillment of these predictions.

THE CHROMOSOME OF ESCHERICHIA COLI
Laboratory of
J. Cairns,
C. I. Davern,
P. Harriman,
P. De Lucia

Rotation, replication and transcription.

Two years ago we reported on an experiment designed to test the notion that the replication of the *Escherichia coli* chromosome was driven by some sort of active spinning device. This hypothetical spinner was pictured as being located at the origin of replication and driving the rotation of the DNA molecule about its axis, the torque being converted to unwinding of the double helix at the site of replication. In the simplest form of this kind of model, a break anywhere in the circular chromosome should arrest unwinding. The demonstration that a certain class of DNA P^{32} decay anywhere in the chromosome can instantly arrest the progress of DNA replication in certain radiation sensitive strains of *E. coli*, fulfilled this prediction. In contrast, RNA synthesis seemed unaffected by this lethal P^{32} decays. We were somewhat surprised at this observation, for it seemed reasonable to assume that RNA transcription would be affected to the extent that DNA was isolated from the hypothetical spinner by chromosome breakage. The possibility that overall RNA transcription was limited by some factor other than available DNA template could not be readily dismissed, so we decided to examine the sensitivity of specific messenger RNA transcription to DNA P^{32} decay.

While the facts of abortive transduction and the immediate expression of genes after conjugation transfer strongly suggest that chromosome fragments can be transcribed by RNA polymerase, they do not rule out the possibility that a molecule as large as that of the *E. coli* chromosome may not depend on rotation to drive transcription. Even so the postulate that rotation drives or facilitates transcription is a tenuous one (as is the reverse postulate of Maaloe's that transcription may drive rotation) because it seems that the direction of transcription is not constant throughout the genome (Beckwith, pers. comm.), and because the rate of transcription in the few instances where it has been measured [Leine 1965, Leine and Kollin 1967, Imamoto, et al. 1965] (50–100 nucleotides per molecule per second) is more than an order of magnitude slower than the rate expected from a rotation of 10,000 rpm necessary to account for the observed rate of DNA replication at 37°C.

If transcription is driven by spinning, the vulnerability of expression of a particular gene to random DNA P^{32} decays should vary with its distance from the spinner in the direction of propagation of torque. As yet it is not clear where the origin of replication is located on the chromosome, so the vulnerabilities of expression to P^{32} decay for two genes located about 180° apart on the circular map were measured, on the rationale that the transcription of one of the genes would have a DNA P^{32} sensitive target size of at least half the chromosome. The two genes chosen were those specifying the inducible enzymes B-galactosidase and D-serine deaminase respectively.

The radiation sensitive recombinationless (rec-) strain of *E. coli* (Meselson) was uniformly labeled with P^{32} at a specific activity of about 20 mC/mg for 3–4 generations. The cells were washed free of unincorporated label and stored away in frozen aliquots. Aliquots were withdrawn after various times of storage and the cells assayed for their viability (which provides a measure of chromosome lethal hits inactivating DNA replication in this strain) and for their capacity to be induced to synthesize the two enzymes. While profoundly affecting the viability of the cells, P^{32} decay hardly reduced the induced enzyme synthesis capacities of the two enzymes. Thus transcription is unaffected by P^{32} decay in rec-, indicating that if the immediate DNA synthesis arrest observed in such P^{32} damaged cells is due to the cessation of active unwinding, then active rotation is unnecessary for transcription.

Energy Requirements for DNA Synthesis

One prediction of our "spinner" model of DNA synthesis is that replication of the bacterial chromosome requires a source of energy in addition to the four deoxynucleoside triphosphates. In experiments carried out in the past year we have shown that bacteria growing on lactate stop synthesizing DNA, following the addition of cyanide or carbon monoxide, long before their pool of precursors is exhausted. Thus, not only can they be shown (by extraction and chromatography) collectively to con-

tain as the triphosphate the particular labeled base whose entry into DNA is being monitored, but they prove capable, in experiments done in collaboration with Dr. David Denhardt (Harvard University), of replicating single strands of ϕX174 introduced after DNA synthesis has stopped (i.e., the bacteria individually contain all the precursors of DNA). Further experiments of this kind will be carried out in the coming year.

Further studies of DNA breakdown after DNA P^{32} decay damage in rec-

Previous studies indicated that P^{32} decay damaged rec- chromosomes are liable to breakdown, the initiation of which is random in time and inhibitable by energy blocking agents. The liability to breakdown, like survival and DNA synthesis capacity, showed one-hit sensitivity to DNA P^{32} decay. We wondered whether undamaged chromosomes or episomes in the same cell as a damaged chromosome were also subjected to breakdown. An F'lac/ rec$^-$ lac$^-$ derivative of the Meselson rec$^-$ was constructed. This strain and the parental rec$^-$ lac$^+$ were uniformly labeled with P^{32}. The survival of the capacity to synthesize β-galactosidase was followed in samples which had received 2−3 chromosome lethal hits after incubation to allow P^{32} decay damaged chromosomes to break down. The sensitivity of β-galactosidase induction to post decay DNA breakdown was the same whether the enzyme was specified by the episome or by a chromosome lac gene, suggesting that the breakdown mechanism attacks damaged and intact replicons indiscriminately. However, severe catabolic repression would give rise to the same result, despite the fact that the enzyme inductions were performed in the absence of any exogenous carbon source to minimize catabolic repression.

This question was approached from another direction. P^{32} labeled F'lac/ rec- lac- was allowed to suicide, and the frequency of lac- segregants amongst the survivors scored. No increase over the spontaneous frequency (2%) of lac- segregants was observed after suicide down to 10^{-6} survivors. Since the F' episome comprises about 10% of the chromosome DNA, then the frequency of lac- segregants should have been enriched to about 50% if each cell contained 2−3 episomes. This lack of enrichment suggests that a DNA P^{32} decay in the episome is as lethal to the cell as one in the chromosome in this rec- strain, an interpretation which is compatible with the single hit inactivation kinetics of this strain.

Correlation between production of P1 transducing particles from different regions of the E. coli *chromosome.*

P1 bacteriophage can carry out generalized transduction. In a mass lysate of phage produced from a donor strain of bacteria there are a total of about 10^{-4} transducing particles (TPs) for a given marker (counting both abortive and stable TPs) for each plaque forming unit (PFU). With an average burst size of 100 PFU, this means that on the average one TP for a given marker is produced from every 100 P1 infected cells. One mechanism that would explain this finding involves the formation of one TP in every infected cell; since a TP contains on the order of 1% of the bacterial chromosome, the chance of that one TP containing a given bacterial marker would then be 1 in 100. At the opposite extreme would be a mechanism whereby the chromosomes of 1% of the infected cells are converted completely into TPs − this would result in 1% of the cells producing bursts of about 100 TPs each; in this case, one could ask whether PFU were produced in those cells that produce the TPs.

In order to determine which of the above possible mechanisms obtains, it is necessary to perform a type of single burst experiment. Lysogenic phage were used as bacterial markers because they can be transduced with the same frequency as bacterial markers and have the advantage that they can be easily scored; they do not require integration into the chromosome of the recipient strain to be counted, due to a mechanism similar to that of zygotic induction. Phages lambda and 21 have so far been used. By plating infected bacteria on a strain of bacteria on which both P1 and the lysogenic phage can plate and then analyzing the phage composition of the resulting plaques, it was found that cells which produce TPs also produce PFU. When cells lysogenic for two different lysogenic phages were infected with P1 and plated on a strain of bacteria lysogenic for P1, only TPs containing the lysogenic phage were able to produce plaques. These plaques were then tested for their phage content on appropriate indicator strains. Using this technique it was found that about 7% of the infective centers producing a TP for one phage also produced a TP for the other. This is higher than expected if the co-production of different TPs were a chance event, but is less than expected if two markers were always produced from the same bursts. Other phage markers are now being tested to see if the co-production of different markers is dependent on the distance between them.

**MUTAGENIC
POLYMERASES**
Laboratory of
J. Speyer,
J. Karam,
D. Rosenberg,
A. Lenny

The types of mutations induced by defective (ts gene 43) DNA polymerase alleles have been studied. Clear evidence for transition mutations has been obtained since this mutagenesis converts ochre to amber (UAA to UAG) and also promotes the interconversion between ochre (UAA) and opal (UGA) in both directions. Thus in DNA, AT and GC pairs are interconverted as a consequence of DNA synthesis by several defective alleles of polymerase. However, the relative extent to which these above three interconversions are promoted, as well as their total frequency of occurrence, varies with different mutagenic alleles of the T4 polymerase. Thus there is some mutagenic specificity. The frequency of the ochre - opal interconversion depends also on the location of the mutant site.

The above transitions are not the sole type of mutation produced: transversions may occur even more often than transitions. Evidence for transversions is less direct. Of 100 mutator-produced and revertible rII mutations, only about half were revertible with base analogues. Since the mutators apparently do not produce or revert frameshift mutations, the above rII's represent transitions and transversions. Many transversions are probably revertible to a pseudo-wild phenotype by the transition-inducing base analogues, since many sites in the rII gene have no stringent requirement for a particular amino acid. One may therefore conclude that

    a) Many silent mutations occur in this gene.

    b) An unknown fraction of the base analogue revertible rII mutants are transversions induced by the mutator; the rest are transitions.

    c) Hence most of the mutator-induced mutations are probably transversions.

Fifty of the above rII mutants have been mapped. No mutagenic "hot spots" were seen.

Other work is in progress that can demonstrate transversion. There are some T4 coat protein amber mutations revertible by mutators. The amino acid substitutions in these revertants are now being analyzed. From the knowledge of the genetic code the base change involved can then be deduced. Mr. Albert de Vries from Leyden University began this amino acid analysis here this past August, and it will be continued in Holland and in this laboratory.

A set of ochre rII mutations has been collected. These are being mapped to see if ochre sites are found that were not observed with base analogues. So far several such new sites have been noted where base analogue mutagenesis in other laboratories had not produced ochre mutants. However, an exhaustive screening for base analogue induced ochres has yet to be undertaken. Base analogue mutagenesis (limited to transitions) can induce ochres only from CAA, one of the two glutamine triplets. Thus there can be only a few base analogue inducible ochre sites in the rII gene. The new, mutator-produced, ochre sites therefore were probably formed by transversions.

Some 46 amber mutants in the polymerase gene have been mapped and found to be at 13 different sites. Many different isolates of gene 43 ambers thus involved the same mutation, and one occurred eleven times in the amber set. The ambers each display a characteristic temperature sensitivity pattern with various suppressor-carrying *E. coli* strains, which was correlated with the mapping data.

Some of the temperature sensitive mutants were also mapped. The mutagenic alleles were not clustered at any particular site.

Complementation was observed at 42°C. No complementation occurred between temperature sensitive and amber mutants of gene 43. The burst size of complementing pairs was very small (1.0 or less) but was sufficient to obtain complementation-dependent recombinants.

Several *E. coli* mutations that enable UGA mutations to be suppressed have been isolated by us. One UGA suppressor has the surprising property of also suppressing the amber mutation (UAG) but it does not suppress the similar ochre mutation (UAA). Another unexpected observation bearing on suppression mechanisms was made. We found that ochre suppressors, known to suppress both amber and ochre mutations, failed to suppress amber mutations at higher temperatures (38°C) even though ochre mutations located at the same codon site as the amber mutations were still suppressible at that temperature. The mechanism of both these unexpected phenomena is still unknown.

Dr. Jim Karam has studied the properties of several *ts* gene 43 mutants. He has found that in 5 cases studied the gene 43 product formed at 42°C is inactive but renaturable at lower temperature. However, once active, this DNA polymerase can be again denatured but not again renatured.

The coinfection of *E. coli* at high temperature with normal and temperature sensitive phage shows that when the temperature sensitive mutant is in excess it can prevent functioning of the normal gene product. There is a 90% reduction in burst size. On the other hand, amber gene 43 mutants do not have this dominant effect. In a mixed infection of a suppressor negative host with am gene 43 phage and wild type T4, the burst size is normal and the amber genome is replicated as is the wild type. We conclude that the *ts* gene product is dominant over wild type, and also that this gene product works trans. Trans mutagenesis has also been demonstrated. We hope to be able to decide soon whether the trans effect of gene 43 is limited to genomes involved in recombination with those genomes that coded for the active gene 43 product, or whether the gene 43 product can freely attach and replicate any phage chromosome in the host cell.

Dr. David Rosenberg has joined this group and has begun experiments designed to find out whether the gene 43 product, the phage DNA polymerase, is involved in recombination. His experiments are not completed. They ask whether mutagenesis by polymerase is localized in recombinant segments of the chromosome. Preliminary experiments with *ts* gene 43 mutants at temperatures that reduce the burst size by 90% failed to show any effect on either the recombination frequencies between rII mutants or on UV sensitivity.

STRUCTURE AND FUNCTION OF PHAGE DNA'S

Laboratory of
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P. Bear,
E. Burgi,
L. Ingraham,
S. Makover,
A. Skalka,
R. Werner

Nucleotide Distribution in λ DNA, Burgi, Skalka, and Hershey

Last year we described methods for analyzing the distribution of guanine and cytosine along the length of the λ DNA molecule. These methods depend chiefly on breaking the DNA into fragments of known average length and then sorting the fragments with respect to nucleotide composition. The resolution that can be achieved in this way depends on the length of the fragments. Pieces of fractional length 0.12, for instance, fall into three discrete classes. Pieces of fractional length 0.06 reveal four classes. These and other results show that the λ DNA molecule contains three large segments that differ in composition. From left to right these measure 0.44, 0.10, and 0.46 in fractional length, and 57, 37, and 46 mole per cent guanine plus cytosine (GC) in composition. The two central components come from the 46%-GC segment, which is therefore made up of two subclasses measuring 43% and 48.5% in GC content. The composition of right-terminal fragments of various lengths shows that the 43%-GC DNA is more abundant toward the molecular center than toward the molecular end. However, a short stretch poor in guanine and cytosine at the right end of the molecule (see below) complicates analysis of terminal fragments.

The molecular ends provide a special opportunity in that terminal fragments can be isolated individually, owing to the specific left-to-right joining of terminal cohesive sites. Burgi has found that left and right molecular ends of fractional length 0.14 do not differ appreciably in composition from the larger terminal segments from which they come. When reduced to fractional length 0.012, however, left ends contain only 48% GC, and right ends only 42%. These results support the anticipated conclusion that the shorter the fragment the less it is obliged to resemble its neighbors in composition. More interestingly, the two molecular ends show a common tendency toward diminishing GC content. Since the two terminal genes in the genetic map function late during the phage growth cycle, and late functions are generally associated with DNA of high GC content, it may be desirable to locate the terminal genes with respect to the changing base composition near the molecular ends.

Deletions in λ DNA, Skalka and Burgi

A mutant of phage λ known as λb_2 contains a DNA molecule 15 to 20% shorter than that of wild-type λ. It has suffered a deletion near the chromosomal center to the right of the host-range marker. The mutant grows normally except that it does not produce stable lysogens. Indirect evidence suggests that it has lost the crossover locus within which genetic recombination with the bacterium occurs during lysogenization. Burgi and Skalka have found that b_2 DNA lacks all or most of the 37%-GC segment present in the DNA of wild-type λ; therefore b_2^+ function resides in or adjacent to that segment, and the segment does not contain genes whose functions are essential during the lytic cycle of phage growth.

Skalka has also analyzed DNA from a defective strain of λ known as λ*dg* (A-J), which is a typical defective, *gal*-transducing phage whose deletion spans genes A through J in the left third of the genetic map. Her results reveal two features of the DNA of λ*dg* (A-J) that are best seen by analysis under somewhat different conditions. A large fraction of the DNA present in wild-type phage, including all the left-terminal 57%-GC section, has been replaced in λ*dg* (A-J) by DNA having the GC content characteristic of the DNA of *E. coli;* the 37%-GC section also has been deleted. The deleted DNA therefore

represents a continuous stretch measuring at least 54% of the wild-type molecular length taken from the left arm of the DNA molecule (but not including the extreme tip, because cohesive function remains). This physical structure, like the genetic results, is best interpreted as the consequence of a terminal prophage deletion. Given that interpretation, four conclusions follow: – 1. The 37%-GC segment must lie near the right end of the prophage map. 2. The locus of permutation points in the λ DNA molecule lies near or to the right of the right end of the 37%-GC section, at a position distant at least 54% of the molecular length from the left molecular end. 3. The 37%-GC segment cannot lie in a region of exact homology between λ and *E. coli;* if it did, loss of the λ homologue would be compensated by gain of the bacterial homologue in the origin of λ*dg*. 4. All λ*dg*'s should lack the 37%-GC section plus varying fractions of the 57%-GC section depending on the lengths of their deletions, that of λ*dg* (A-J) being one of the longest.

Independently of models, comparison of the DNA's of λb_2 and λ*dg* (A-J) shows that the genes A through J, present in b_2 but not in λ*dg*, must lie in the 57%-GC segment of the molecule. The physical data show that λb_2 has suffered a 10% deletion of DNA near the right prophage terminus and an additional 5–10% deletion of DNA of unknown location. We suggest as a plausible hypothesis that λb_2 arose by an illegitimate crossover deleting some of the DNA corresponding to both prophage ends.

Base-Sequence Similarities between λ *and coli DNA's, Ingraham and Hershey*

From previous work we concluded that base-sequence similarities between the DNA's of phage λ and *E. coli* are generally though not uniformly distributed throughout the length of λ DNA. As far as it goes, this conclusion is consistent with the hypothesis that the illegitimate crossovers giving rise to diverse lines of transducing phage depend on irregularly distributed base-sequence similarities.

The preferred crossover locus, responsible for normal prophage insertion and excision, must be something different. To account for the constancy in DNA content of phage λ through its lysogenization cycle, that locus must contain either a unique crossover point or a region of matching base sequences that is colinear in the DNA's of λ and coli. Skalka concluded that the λ*dg* she analyzed had arisen from a prophage inserted by crossing over near the right end of the 37%-GC section of the DNA molecule. To examine base-sequence similarities in this region, we broke λ DNA into fragments of fractional length 0.32, removed rejoined ends, and then selected mercury complexes of the remaining central sections that were rich in adenine and thymine. Such fragments should contain most of the 37%-GC section and an adjacent part of the 46%-GC section, and should be shorter than unselected fragments. When the selected fragments were reduced in size to about 0.1 of the original molecular length and were again analyzed by Hg-Cs$_2$SO$_4$ fractionation, two distinct components with densities corresponding to 37% and 46% GC, in the approximate ratio 1:1.5 were recovered. The effectiveness of the selection was also indicated by complete absence of a 57%-GC component. Both components recovered from the molecular centers proved to bind poorly to the DNA of *E. coli*. We also tested short right and left molecular ends (fractional length 0.14), donated by Elizabeth Burgi. They reacted with the DNA of *E. coli* just like the corresponding terminal thirds isolated in the experiment described above.

We conclude that the measurable base-sequence similarities between λ and coli DNA's are strongest (in λ DNA) near the right molecular end, weakest in the 37%-GC and 46%-GC sections near the molecular center, and intermediate near the left molecular end.

These results do not of course exclude the possibility of a critical region of exactly matching base sequences near *gal* in *E. coli* and near the molecular center in λ DNA, because genetic considerations suggest that the crossing over responsible for prophage insertion ought to be rather precisely defined, and a short matching sequence in a region of poor matching would be all to the good. The concentration of matching sequences in other parts of the molecule, however, argues for a recognition device that does not depend on homology alone. Signer and Beckwith (1967) and Zissler (1967) propose, in fact, that phage λ employs a special enzyme that somehow directs the normal insertion and excision of the prophage. In principle, such an enzyme could act by recognition of one matching base sequence among many.

Genetic Transcription in Bacteria Infected with Phage λ, *Skalka*

Skalka and Harrison Echols (University of Wisconsin) have studied the effects of mutational defects in λ on production of messenger RNA during phage growth. Their results, which are being published in detail elsewhere, identify two genes whose primary function may be control of transcription. Mutations in gene N block production of all messenger excepting a small amount similar to that formed when protein synthesis is inhibited by chloramphenicol; the simple inference, also suggested by the work of R. Thomas, is that a product of gene N directly initiates messenger synthesis characteristic of

the lytic cycle of phage growth. Mutations in gene Q, which do not block DNA synthesis or production of early-phase messenger, selectively depress transcription of genes responsible for late functions. The simple inference is that a product of gene Q specifically facilitates transcription of those genes. Needless to say, the facts are more reliable than the inferences at this time.

DNA Replication in Bacteria Infected with Phage T4, Werner

Last year Werner reported that a T4-infected cell contains a number of sites of DNA replication, about one for each molecular equivalent of phage DNA. His measurements were made at 45 minutes after infection of cultures growing at 25°C. Such cultures are entering a steady state of phage growth in which a constant rate of DNA synthesis is matched by an equal rate of phage particle formation to maintain an intrabacterial pool of replicating DNA of constant size. When bacteria are infected with phage T4, DNA synthesis starts about ten minutes later and attains a rapid rate very quickly, a rate that is clearly not proportional to the amount of phage-precursor DNA present in the cells. The approach to the steady state therefore calls for regulation of DNA synthesis. The infected cell could control its rate of DNA synthesis in either of two ways: by varying the number of growing points per unit length of DNA, or by varying the rate of synthesis at individual growing points. Werner has examined this question, and has found that the variable factor is the number of growing points. The rate of DNA synthesis at individual growing points remains constant.

Werner performed experiments of three types, described here in terms of specific examples.

Experiment 1. Infect thymine-requiring bacteria (*E. coli* B3) with thymine-requiring phage (T4 *td*8) and allow growth to proceed in medium supplemented with 2 μg/ml of thymidine. At minute 40, add 20 μg/ml of H^3 - labeled 5-bromouracil. At minute 42, add a large excess of unlabeled thymidine and permit growing points to move away from the labeled sections in the DNA. Extract DNA from the cells at minute 45, break samples into fragments of various sizes, and measure both the 5-bromouracil content of the DNA and the size to which the DNA must be broken to liberate pieces, identifiable by their density, that contain one heavy and one light strand. The critical size turns out to be 0.10 of the length of a T4 DNA molecule. The 5-bromouracil content of the DNA corresponds to 6.0 DNA molecules per cell. Therefore the individual cells contain an average of 6.0/0.1 or 60 growing points at 40 minutes after infection. In this experiment, the total amount of DNA per cell is not measured.

Experiment 2. Start the infection in medium supplemented with C^{14} - thymidine, then switch to H^3 - labeled 5-bromouracil by centrifugation and washing. The procedure is the same as in experiment 1 except for the additional measurement of the amount of DNA synthesized after infection. Because some cells lyse during centrifugation, the only reliable estimates are L, the average length of DNA segments of hybrid density, and F, the fraction of the recovered DNA containing 5-bromouracil in place of thymine. The ratio L/F gives the amount of DNA per growing point. This ratio measures 1.5 molecular equivalents at 45 minutes after infection.

Experiment 3. Start growth in thymidine-containing medium and add an excess of H^3 - labeled 5-bromouracil at time t. After an additional interval, Δt, extract DNA, reduce it to small fragments by sonication, and measure the ratio between H^3 counts in heavy DNA (both strands labeled) and in hybrid DNA (one strand labeled). This ratio increases in proportion to Δt, and results can be interpolated to find Δt corresponding to the ratio heavy/hybrid equal to 0.5. The interpolated Δt is roughly the interval during which two growing points move over an average segment of replicating DNA. Since the rate of movement of growing points is known, the length of DNA between growing points can be calculated. The distance between growing points, D, depends both on the time t at which 5-bromouracil is added and on the thymidine concentration of the medium. At 2 μg/ml thymidine, D = 0.17 of a T4 length when t = 20, and 0.29 of a T4 length when t = 40. At higher concentrations, D is greater at t = 40, about 0.73 of a T4 length.

Results of these three types of experiment permit the following conclusions.

1. Growing points first appear at 10 minutes after infection and increase in number at the rate of 3 per bacterium until they number 60 at 30 minutes, after which the number remains constant (experiments of type 1). The number of growing points found during a short pulse with 5-bromouracil does not depend on the amount of DNA in the cultures as influenced by the prevailing thymidine concentration.

2. Individual growing points move at the rate of 5% of a T4 length per minute in the presence of 5-bromouracil. This rate does not depend on the time after infection at which the measurement is made (experiments of types 1 and 2).

3. The distance between growing points in replicating structures varies from about 0.2 of a T4 length at early times to nearly 1 at late times (experiments of type 3). Replicating DNA can therefore take the form of a multiply branched structure.

4. During the steady state of phage growth, cultures maintained in the presence of excess thymidine produce 4.5 phage particles per bacterium per minute and synthesize DNA at the equivalent rate. If there are 60 growing points per cell, they are moving at the rate of 0.075 of a molecular length per minute. The local rate of DNA synthesis measured in the presence of 5-bromouracil is 0.05. Brief labeling with H^3 - thymidine or with C^{14} - 5-bromouracil shows the growing points move 1.5 to 2.0 times faster in the presence of thymidine than in the presence of 5-bromouracil. Thus each result checks fairly well with two independent measurements. The same is true at earlier times when the rate of DNA synthesis per cell is increasing.

Werner concludes that the rate of DNA synthesis in the presence of thymidine is controlled by the number of growing points, not by their rate of movement, and that there is no severe limitation to the number of growing points per length of DNA or per cell. A similar conclusion was suggested by Sueoka and his colleagues concerning replication of bacterial DNA in *Bacillus subtilis*.

According to Werner's results, growing points accumulate rapidly but move rather slowly and tend to remain clustered in the templates on which they originate. This conclusion suggests an unanticipated role for genetic recombination: to distribute growing points over the newly synthesized DNA. Eckhart found that genetic markers introduced into a T4-infected cell by a superinfecting phage replicate mainly after recombination with markers contributed by the primary infection. His finding can perhaps be explained, wholly or in part, by the clustering effect mentioned above.

Sedimentation Rates of Polynucleotides, Ingraham

Molecular weights of the polynucleotide chains released by denaturation of DNA can be measured from their rates of sedimentation in alkaline solutions according to an equation of the type $D_2/D_1 = (M_2/M_1)^{\alpha}$, where the D's and M's refer to distances sedimented and molecular weights of two DNA's spun in the same tube. The exponent α was estimated at 0.40 by Studier and at 0.38 by Abelson and Thomas, the difference possibly reflecting the use of different reference DNA's and different solvents. In any case, it remains uncertain whether or not the exponent α is really constant over a wide range of molecular weights. With the assistance of Dr. Gobind Khorana, Ingraham has made a partial check.

Khorana supplied two samples of C^{14}-labeled thymine deoxyoligonucleotides, one containing the heptanucleotide, the other mixed nucleotides of somewhat greater length. Since the mixed sample was larger, it was calibrated against the heptanucleotide and then used as a reference in other measurements.

In principle the check is simple. The molecular weight of single strands of λ DNA is 15.5 million, that of the heptanucleotide 2320. Measurement of the relative distances sedimented permits an estimate of α. Owing to the very great difference in sedimentation rates, the comparison has to be made in several steps. Ingraham used a sample of DNA broken by stirring and a sample of enzymically hydrolyzed DNA as intermediate references of unknown molecular weight. Other than the thymine oligonucleotides, the materials were prepared from P^{32}-labeled λ DNA.

Adjacent pairs in the molecular weight series were spun in concentration gradients containing 5 to 20% sucrose, 10^{-3} M ethylenediaminetetraacetate, 0.3 M NaOH, and 0.7 M NaCl. Time and speed of centrifugation were chosen to bring the faster-sedimenting member of the pair well down the tube.

Results for two or three trials of each kind are given in Table 2. They show that the molecular weight ratio 15.5 x 10^6/2320 corresponds to a distance ratio of 28.0 to 31.4. Substitution of these limits in the equation gives α = 0.378 to 0.392. Ingraham also verified that the exponent 0.38 serves in the range of molecular weights between 15.5 million and 320,000 under the conditions specified. The provisional molecular weights she cited last year are therefore about right.

COLD SPRING HARBOR SYMPOSIUM
ON QUANTITATIVE BIOLOGY

June 1st to June 7th, 1967

XXXII.
ANTIBODIES

This symposium was the 32nd in the series initiated by the Long Island Biological Association. As usual, many people had a hand in designing the program and adding to its usefulness in many ways. In particular, we are indebted to Drs. Edelman, Fazekas, Jerne and Nossal. There were 67 speakers and the meeting was attended by about 290 people.

PROGRAM

OPENING ADDRESS: SIR MACFARLANE BURNET: "The Impact of Ideas on Immunology."

STRUCTURE OF ANTIBODIES

PUTNAM, F. W., K. TITANI, M. WINKLER and T. SHINODA: "Structure and evolution of kappa and lambda side chains."

MILSTEIN, C. B., FRANGIONI, and J. R. L. PINK: "Studies on the variability of immunoglobulin sequence."

APPELLA, E. and R. M. PERHAM: "Structure of immunoglobulin light chains."

PRESS, E. M. and P. J. PIGGOTT: "The chemical structure of the heavy chain of human immunoglobulin G."

WAXDAL, M. J., W. H. KONIGBERG and G. M. EDELMAN: The structure of a human gamma G immunoglobulin."

CEBRA, J. J.: "Common peptides comprising the N-terminal half of heavy chain from rabbit IgG and specific antibodies."

EISEN, H. N., J. R. LITTLE, C. K. OSTERLAND and E. S. SIMMS: "A myeloma protein with antibody activity."

STONE, M. J. and H. METZGER: "The valence of a Waldenstrom macroglobulin antibody and further thoughts on the significance of paraprotein antibodies."

NIZONOFF, A., S. ZAPPACOSTA and R. JUREZIZ: "Properties of crystallized rabbit anti-P azobenzoate antibody."

POLJAK, R. J., D. J. GOLDSTEIN, R. L. HUMPHREY and H. M. DINTZIS: "Crystallographic studies of rabbit and human Fc fragments."

SINGER, S. J., L. I. SLOBIN, N. O. THORPE and J. W. FENTON, II: "On the structure of antibody active sites."

WOFSY, L. and D. C. PARKER: "Comparative studies of antibody active sites."

YOO, T. J., O. H. ROHOLT, and D. PRESSMAN: "Hapten binding activity in isolated light polypeptide chains from rabbit antibody."

KOSHLAND, M. E.: "Location of specificity and allotypic amino acid residues in antibody Fd fragments."

HAMERS, R. and C. HAMERS-CASTERMAN: "Evidence for the presence of the Fc allotypic marker As8 and the Fd allotypic marker As1 in the same molecules of rabbit IgG."

EVOLUTION AND GENETICS OF ANTIBODIES

HOOD, L., W. R. GRAY, B. G. SANDERS and W. J. DREYER: "Light chain evolution."

BAGLIONI, C., D. CIOLI, G. GORINI, A. RUFFILLI and L. ALESCIO-ZONTA: "Studies on fragments of light chains of human immunoglobulins: Genetic and biochemical implications."

SMITHIES, O.: "The genetic basis of antibody variation."

CRICK, F. H. C., L. HOOD, O. SMITHIES, W. J. DREYER, C. MILSTEIN, B. PAPERMASTER, N. A. MITCHISON, J. J. CEBRA, D. W. TALMAGE, C. BAGLIONI, G. EDELMAN, N. JERNE: "General discussion on theories of antibody variability."

NATVIG, J. B., H. G. KUNKEL and S. P. LITWIN: "Genetic markers of the heavy chain subgroups of human gamma G globulin."

HERZENBERG, L. A., J. D. MINNA and L. A. HERZENBERG: "The chromosome region for immunoglobulin heavy chains in the mouse: allelic electrophoretic mobility differences and allotype suppression."

POTTER, M. and R. LIEBERMAN: "Genetic studies of immunoglobulins in mice."

MAGE, R. G.: "Quantitative studies on the regulation of expression of genes for immunoglobulin allotypes in heterozygous rabbits."

SYNTHESIS OF ANTIBODIES

COHN, M.: "Natural history of the myeloma."

ASKONAS, B. A. and A. R. WILLIAMSON: "Biosynthesis and assembly of immunoglobulin G."

FLEISCHMAN, J. B.: "Synthesis of the rabbit gamma G heavy chain."

SCHARFF, M. D., A. L. SHAPIRO and B. GINSBERG: "The synthesis, assembly and secretion of gamma globulin polypeptide chains by cells of a mouse plasma cell tumor."

GREENBERG, L. J. and J. W. UHR: "DNA-RNA hybridization studies on gamma globulin synthesizing tumors in mice."

LENNOX, E. S., P. M. KNOPF, A. J. MUNRO and R. M. E. PARKHOUSE: "A search for biosynthetic subunits of light and heavy chains of immunoglobulins."

MELCHERS, F. and P. M. KNOPF: "Biosynthesis of the carbohydrate portion of immunoglobulin chains: Possible relation to secretions."

MOROZ, C. and J. W. UHR: "Synthesis of the carbohydrate moiety of gamma globulin."

KERN, M. and R. M. SWENSON: "Biochemical studies of the intra-cellular events involved in the secretion of gamma globulin in a cell-free system."

MACH, B., H. KOBLET and D. GROSS: "Biosynthesis of immunoglobulin in a cell-free system."

RALPH, P., M. BECKER and A. RICH: "Immunoglobulin synthesis in a cell-free system."

FAHEY, J. L. and I. FINEGOLD: "Synthesis of immunoglobulins in human lymphoid cell lines."

REISFELD, R. A.: "Heterogeneity of rabbit light-poly and peptide chains."

HABER, E., F. F. RICHARDS, J. SPRAGG, K. F. AUSTEN, M. VALLALLON and L. B. PAGE: "Modifications in the heterogeneity of the antibody response."

DUBISKI, S.: "Synthesis of allotypically defined immunoglobulins in rabbits."

CHOU, C.T., B. CINADER, and S. DUBISKI: Quantitative studies of antibody production by placque-forming cells."

PERNIS, B.: "Relationships between the heterogeneity of immunoglobulins and the differentiation of plasma cells."

FISHMAN, M. and F. L. ADLER: "The role of macrophage - RNA in the immune response."

COHEN, E. P. and K. RASKA, "Antigen - unique species of RNA in peritoneal cells of mice which do not adhere to glass."

DREYER, W.J., W.R. GRAY, and L. HOOD: "The genetic, molecular and cellular basis of antibody formation: some facts and a unifying hypothesis."

DIFFERENTIATION AND CELLULAR EVENTS

NOSSAL, G. J. V., K. D. SHORTMAN, J. F. A. P. MILLER, G. F. MITCHELL, and J. S. HASKILL: "The target cell in the induction of immunity and tolerance."

ADA, G. L., C. R. PARRISH, G. J. V. NOSSAL, and A. ABBOT: "The tissue localization, immuno-genic, and tolerance - inducing properties of antigens and antigen-fragments."

ELLIS, S. T., J. L. GOWANS and J. C. HOWARD: "Cellular events during the formation of antibody."

DUTTON, R. W. and R. I. MISHELL: "Cellular events in the immune response. The in vitro response of normal spleen cells to erythrocyte antigens."

FELDMAN, M. and R. GALLILY: "Cell interactions in the induction of antibody formation."

MAKELA, O.: "The specificities of antibody produced by single cells."

MITCHISON, N. A.: "Antigen recognition responsible for the induction in vitro of the secondary response."

GELL, P. G. H. "Restrictions on antibody production by single cells."

PAPERMASTER, B. W.: "The clonal differentiation of antibody - producing cells."

TILL, J. E., E. A. McCULLOCH, R. A. PHILLIPS and L. SIMINOVITCH: "Analysis of differentiating clones derived from marrow."

BUSSARD, A. E.: "Primary antibody response induced in vitro among cells from normal animals."

LITT, M.: "Studies of the latent period. I. Primary antibody in guinea pig lymph nodes 7½ minutes after introduction of chicken erythrocytes."

STERZL, J.: "Factors determining the differentiation pathways of immunocompetent cells."

WIGZELL, H.: "Studies on the regulation of antibody synthesis."

SIMONSEN, M.: "The clonal selection hypothesis evaluated by grafted cells reacting against their hosts."

FAZEKAS, S. de ST. GROTH: "Cross recognition and cross reactivity."

ANTIGENS: IMMUNE TOLERANCE

SELA, M., B. SCHECHTER, I. SCHECHTER, and F. BOREK: "Antibodies to sequential and confor-mational determinants."

RAJEWSKY, K. and E. ROTTLÄNDER: "Tolerance specificity and the immune response to lactic dehydrogenose isoenzymes."

WEIGLE, W. O. and E. S. GOLUB: "Kinetics of the establishment of immunological unrespon-siveness to serum protein antigens."

HAUROWITZ, F.: "Evolution of selective and instructive theories of antibody formation."

BENACERRAF, B., I. GREEN, and W. E. PAUL: "The immune response of guinea pigs to hapten-poly-L-lysine conjugates as an example of the genetic control of the recognition of anti-genicity."

SJÖQUIST, J., A. FORSGREN, G. I. GUSTAFSON, and G. STALENHEIM: "Biological importance of the Fc-region of gamma globulins."

METCALF, D. "Relation of the thymus to the formation of the immunologically reactive cells."

JERNE, N. K.: "Summary: "Waiting for the End."

PHYCOMYCES WORKSHOP
June 15th to August 15th

This summer the excellent facilities of the Phycomyces Course laboratories on the second floor of the Animal House were used for a two-month workshop centering on a variety of Phycomyces projects. For chemical work additional space in Jones Laboratory was used.

In June *W. Shropshire* came briefly to help set up the laboratory and to do some joint experiments with Bergman on the ATP content of adapted and of stimulated sporangiophores (spphs). The previously reported finding that stimulated spphs contain 30% more ATP could not be confirmed, neither here nor by Shropshire later at the Smithsonian in a much more extensive series of experiments.

In July, *R. K. Clayton* visited the Phycomyces group and gave a lecture on photosynthesis in purple bacteria.

G. von Ehrenstein and *J. Hogg* visited for a few days to discuss proposed work on the membrane structural proteins and on the peroxysomes of Phycomyces, respectively.

August 7-10, *W. Shropshire, D. Dennison,* and *K. Zrankel* visited the group for an informal review of the new findings.

Goodell and *Gamow* measured the oxygen consumption of single plucked spphs. It is equal to $10^{-5} cm^3$ O_2/min. During exponential increase in light intensity (sunrise experiment), the O_2 consumption differed by less than 5% from this value.

Foster measured the water uptake during growth of single plucked spphs. It drops gradually from about 0.4×10^{-6} cm^3/min (=10x volume increase rate) at early Ivb stage to a value only slightly larger than the volume increase rate, implying a surprisingly fast upstream of vacuolar sap during most of the growth. During and following a growth response, the water uptake appears to decrease drastically, possibly because the growth response is correlated with reduced transpiration, leading to increased turgor.

David and *Foster* measured the uptake and distribution of tritiated water into plucked spphs. These measurements nicely corroborated Foster's direct water uptake measurements. David made some preliminary studies of the uptake and distribution of P^{32} and of Fe^{32} into plucked spphs. It appears that these substances are taken up by stage I but not by stage IV spphs.

Berns and *Gamow* attempted to isolate soluble colored proteins from the cell contents of spphs of wild type and of albino mutants. Encouraging results were obtained which will be pursued on a larger scale by Berns in his laboratory.

Park, Eby, and *Goodgal* set up a large scale hunt for mutants of the ROB (*) strain, to be used in subsequent genetic crosses with the already available mutants of the DEL (-) strain.

Pat Kenahan studied avoidance responses, under three aspects: a) comparison of wild type and mutants; b) whether or not it occurs under conditions of 100% humidity (it does); c) whether it occurs when the air between the spph and the barrier causing the response is sucked or blown away (it does).

A great number of experiments were done by *Heisenberg, Bergman, MacLeod,* and *Delbruck* to characterize the responses of a number of interesting mutants isolated by Heisenberg and Eby during the past year.

These mutants may be classified as: a) *pigment mutants,* b) *night-blind mutants;* and the experiments are best summarized with reference to these two classes.

The *pigment mutants* differ from wild type with respect to carotenoid composition, as follows:

	β carotene	lycopene	colorless precursors
Wild type	100	0	0
R1	0	> 100	0
alb 5	2	0	0
alb 10	0.1	0	2

These mutants all react to light and they dark-adapt to low intensities with the same kinetics and down to the same threshold as wild type. The *phototropic action spectra* were very carefully

compared for *wild type* and *R1* at the wavelengths 485 mμ and 515 mμ. It was expected that 515 mμ would be relatively more effective on wild type than on R1 since the screening by β carotene at this wavelength would be almost nil, while the lycopene of R1 has a strong absorption maximum at 517 mμ. However, the difference found was in the opposite direction, and very small (10%). We do not understand this discrepancy but believe that it signifies an essential gap in our understanding of phototropic balance.

alb 10 tropes almost twice as fast as wild type. It also hunts with a large amplitude when illuminated from one direction only or from two directions making an angle of 120°. It also exhibits faster negative geotropism than wild type.

R1 is negatively phototropic in the far UV, like wild type, but the neutral wavelength is 310 mμ for R1, 300 mμ for wild type.

When immersed in fluid of high refractive index, wild type is *negatively* phototropic, due to a reversal of the lens effect. The refractive index for tropic neutrality n_n is around 1.31 for wild type when tested in light of 485 mμ. This value of n_n is somewhat below the n of the cell contents (1.35 for protoplasm, 1.335 for vacuole) and the difference is believed to be due to absorption by β-carotene acting as a screening pigment. If this is the correct explanation the albinos, lacking screening pigment, should have a higher n_n. Indeed it was found that for both *alb 5* and *alb 10* $n_n = 1.34$.

The *night-blind mutants* UV35 and CB51 have the remarkable characteristic that their reactions to light, including the kinetics of light and dark adaptation, are grossly similar to those of wild type in the intensity range above I = -7 on our scale (0.1 μ watt/cm^2), while they are almost totally nonreacting to lower intensities. The threshold of wild type and of the pigment mutants lies 10^5 times lower. The discovery of this class of mutants points to distinct photoreceptive mechanisms in the high and low range of intensities. In analogy with animal vision, one might therefore suspect that different visual pigments are involved. However, careful comparison of phototropic equilibrium of UV 35 and of wild type at 485 and 385 mμ showed no detectable differences.

PHYCOMYCES WORKERS

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I. R. Gamow, Dept. of Microbiology, University of Colorado Medical School, Denver, Colorado
William Goodell, Dept. of Biology, California Institute of Technology
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Walter Shropshire, Radiation Laboratory, Smithsonian Institution, Wash. D. C.
Ulrich Thurm, Max-Planck Institute fur Biologie, Tubingen, Germany
Kenneth Zankel, Sect. Genetics Dev. & Phys. – Plant Science Building, Ithaca, N. Y.

POST GRADUATE TRAINING COURSES
Summer 1967

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 1967 three courses were given, designed to acquaint the student with some of the techniques used in 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) BACTERIAL GENETICS: June 14 – July 5
INSTRUCTORS: S. Goodgal, J. Gots and J. Gross.

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.*

STUDENTS:

        James R. Carter, M.D., Massachusetts General Hospital, Boston, Mass.
        William W. Chan, Ph.D., Albert Einstein College of Medicine, Bronx, N. Y.
        Carolyn F. Gunsalus, Ph.D., Scripps Clinic, LaJolla, California and University of Illinois, Urbana, Illinois
        T. Hudnik-Plevnik, Ph. D., St. Louis University School of Medicine, St. Louis, Missouri
        Suzanne S. Hurd, Ph.D., University of Washington, Seattle, Washington
        Eliot Juni, Ph.D., University of Michigan, Ann Arbor, Michigan
        Jennifer Kahn, B.Sc., Columbia University, New York
        Roger H. Kennett, B.A., Princeton University, Princeton, N. J.
        Thomas A. Mahvi, Ph.D., Medical College of South Carolina, Charleston, S. C.
        Haim Manor, Ph.D., University of Chicago, Chicago, Illinois
        Janine Michel, "Prof. Agrege", Institut Pasteur, Paris, France
        Jerry L. Mosser, A.B., Rockefeller University, New York
        Harris S. Moyed, Ph.D., University of Southern California, Pasadena, Cal.
        Janice Pero, Harvard University, Cambridge, Massachusetts
        Vincenzo Pirrotta, A.M., Harvard University, Cambridge, Massachusetts
        Hans-Jurgen Rhaese, Ph.D., National Institutes of Health, Bethesda, Md.
        William L. Risser, B.A., Harvard University, Cambridge, Massachusetts
        Mark M. Rochkind, Ph.D., Bell Telephone Laboratories, Murray Hill, N. J.
        Onkar P. Shukla, Ph.D., University of Illinois, Urbana, Illinois
        Jiro Suzuki, Ph.D., The University of Chicago, Illinois
        Carl A. Westby, Ph.D., University of Pennsylvania, Philadelphia, Pa.

SEMINARS:

        Paul Howard-Flanders, Yale University School of Medicine: "Genetic Repair Mechanisms."
        Austin Newton, Princeton University: "Polarity Mutations and Translation of the *lac* Operon."
        Bruce Ames, National Institutes of Health: "The Histidine Operon."
        Werner Maas, New York University School of Medicine: "Studies on rec⁻ Merodiploids and the Function of the rec⁺ Gene."
        Harriett Ephrussi-Taylor, Western Reserve University: "Chemical Fate of Transforming DNA in Pneumococcus."
        Roy Curtiss III, Oak Ridge National Laboratory: "Mechanisms of Chromosome Mobilization Transfer during Bacterial Conjugation."
        Robert Herman, University of Minnesota: "Recombination of Chromosome and F-merogenote in *E. coli.*"
        Jonathan Beckwith, Harvard Medical School: "Gene Transposition in *E. coli.*"
        David Dubnau, Albert Einstein College of Medicine: "Genetic Mapping Studies with *Bacillus subtilis.*"
        Carol Mulder, Harvard University: "Naturally Occurring Cross-links in Transforming DNA."
        Arthur Pardee, Princeton University: "Permease Genetics and Cell-free Function."

STUDENT SEMINARS

    Jans-Jurgen Rhaese, "Inactivating DNA alterations"
    Jiro Suzuji, "TYMV RNA"
    Haim Manor, "Ribosome biogenesis in bacteria"
    Vincenzo Pirrotta, "Phage repressors"
    Janine Michel, "Early stages in sporulation"
    T. Hudnik-Plevnik, "Alternate pathway for thymine biosynthesis"
    Carl A. Westby, "First enzymes in purine biosynthesis"
    Suzanne S. Hurd, "Subunits of rabbit muscle phosphorylase"
    William Chan, "Subunits of rabbit muscle aldolase"
    Harris Moyed, "Plant auxins"
    Marc Rochkind, "Infra-red spectroscopy"
    James Carter, "Beta galactoside permease"
    Jerry Mosser, "Nature of competence in Pneumococcal transformation"
    Elio Juni, "Regulation of butanediol cycle"
    Onkar Shukla, "Biological degradation of menthols"

2) BACTERIAL VIRUSES: July 9 – August 3.
INSTRUCTORS: F. Stahl and C. Steinberg.

Preparation and assay of virus; serology; inactivation by ultraviolet light; the life cycle; mutagenesis; bursts from single cells; genetic recombination; physiological genetics; immunity; genetic structure of transducing phage; transduction.

STUDENTS:

    Cynthia Alff-Steinberger, Ph.D., New York University Medical Center, New York
    C. Thomas Caskey, M.D., National Institutes of Health, Bethesda, Maryland
    Fokke A. deVries, DRs., University of Leyden, Leyden, Netherlands
    Leonard X. Finegold, Ph.D., Dept. of Physics, University of Colorado, Boulder, Colorado
    Jay A. Glasel, Ph.D., Dept. of Biochemistry, Columbia University, New York
    Lawrence M. Gold, B.S., University of Connecticut, Storrs, Conn.
    David I. Hirsh, B.A., Rockefeller University, New York, N. Y.
    Pearl Horn, Ph.D., Dept. of Microbiology, New York University School of Medicine, New York
    Ann Jacobson, Ph.D., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee
    Seymour Joffe, M.D., Dept. of Neurology, University of Miami School of Medicine, Coral Gables, Florida
    Marvin A. Kastenbaum, Ph.D., Oak Ridge National Laboratory, Oak Ridge, Tennessee
    Sushil Kumar, Ph.D., Cold Spring Harbor Laboratory of Quantitative Biology, Cold Spring Harbor, New York
    Nancy Lundh, B.A., Dept. of Bacteriology, University of California, Los Angeles, California
    Corinne V. Michels, M.S., Dept. of Biological Sciences, Columbia University, New York, N. Y.
    Jane B. Mills, A.B., Dept. of Biochemistry, Columbia University, New York, N. Y.
    Vincenzo Pirrotta, A.M., Harvard University, Cambridge, Massachusetts
    Martin Posner, Ph.D., Physics Dept., Yale University, New Haven, Conn.
    William L. Risser, B.A., Harvard University, Cambridge, Massachusetts
    Gerald F. Vovis, B.A., Dept. of Biology, Western Reserve University, Cleveland, Ohio
    Hendrik J. Zweerink, B.S., Dept. of Microbiology, Tufts University School of Med., Boston, Mass.

SEMINARS

    Dr. Joseph Speyer, Cold Spring Harbor Laboratory: "Polymerase Mutagenesis."
    Dr. Rudolph Werner, Genetics Research Unit, Cold Spring Harbor: "T4 DNA Replication."
    Dr. Edward Goldberg, Bacteriology Dept., Tufts Univ., Boston: "Genetic vs Physical Measurements on the Chromosome of T4."
    Dr. Charles A. Thomas, Biophysics Dept., Johns Hopkins Univ., Baltimore, Maryland: "Virus Chromosome Structure."
    Dr. Frank Stahl, Univ. of Oregon, Eugene: "Genetic Recombination in T4 – a Review."
    Dr. Norton Zinder, Rockefeller University, New York: "Little Phages."
    Dr. Ann Skalka, Genetics Research Unit, Cold Spring Harbor: "Structure and Function of Lambda DNA."
    Dr. Ethan Signer, Biology Dept., Mass. Inst. Technology, Cambridge, Mass.: "Recombination and λ-Prophage Attachment."
    Dr. Max Delbruck, California Institute of Technology, Pasadena, California: "Phycomyces".
    Dr. Allan Campbell, Biology Dept., Univ. of Rochester, Rochester: "λ-Prophage Attachment and Recombination."
    Dr. T.F. Anderson, Institute for Cancer Research, Philadelphia, Pennsylvania: "Phage Adsorption, etc., as investigated by electron microscopy."

3) QUANTITATIVE MICROBIOLOGY OF ANIMAL CELLS AND VIRUSES

August 8 to August 27.
INSTRUCTORS: P.I. Marcus, G. Sato.

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-DNA: 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. Histochemistry and fluorescent antibody techniques in virus infection. Phenotypic 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 technique.

SEMINARS:
> *H. Eagle,* Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: "Biochemistry of Cultured Mammalian Cells."
> *E. Robbins,* Dept. of Cell Biology and Microbiology, Albert Einstein College of Medicine, Bronx, New York: "Cell Ultrastructure, Function, and Life Cycle."
> *E. Pfefferkorn,* Dept. of Microbiology, Dartmouth Medical School, Hanover, New Hampshire: "Conditional-lethal Mutants and the Genetics and Biochemistry of Arboviruses."
> *S. Shin,* Graduate Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: "Functional Endocrine Cells in Culture."
> *H. Hanafusa,* Public Health Research Institute of the City of New York, New York: "The Defectiveness of Rous Sarcoma Virus."
> *D. Summers,* Dept. of Microbiology, Albert Einstein College of Medicine, Bronx, New York: "The Function and Products of the Polycistronic Poliovirus in RNA."
> *H. Green,* Dept. of Pathology, New York University School of Medicine, New York: "Cell Division, Cell-Contact, and Oncogenic Viruses."
> *R. Lockhart,* Experimental Station, DuPont Laboratories, Wilmington, Delaware: "Interferon."
> *P. Choppin,* Rockefeller University, New York: "The Structure and Replication of the Parainfluenza Virus SV-5."
> *M. Weiss,* Dept. of Embryology, Carnegie Institute of Washington, Baltimore, Maryland: "Hybridization of Somatic Cells."
> *W. Joklik,* Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: "Nucleic Acid Synthesis in HeLa Cells Infected with Vaccinia Virus."
> *P. I. Marcus,* Dept. of Microbiology, Albert Einstein College of Medicine, Bronx, New York: "Intrinsic Interference: A New Type of Viral Interference."

STUDENTS
> Jorge E. Allende, Ph.D., National Heart Institute, Bethesda, Md.
> Friedlinde A. Bautz, Ph.D., Rutgers University, New Brunswick, N.J.
> Raymond W. Byrne, Ph.D., The Johns Hopkins University School of Medicine, Baltimore, Md.
> James J. Castles, M.D., National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.
> Richard L. Davidson, Ph.D., Western Reserve University, Cleveland, Ohio
> Leonard D. Garren, M.D., Yale University School of Medicine, New Haven, Conn.
> Thomas D. Gelehrter, M.D., National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.
> Fabio Gonano, M.D., Instituto di Patologia Generale, Modena, Italy
> Peter O. Kohler, M.D., National Cancer Institute, Bethesda, Md.
> Ming-liang Lee, M.D., University of Miami School of Medicine, Miami, Fla.
> Nicole S. Oeschger, M.S., The Johns Hopkins University, Baltimore, Md.
> Roger J. Radloff, Ph.D., California Institute of Technology, Pasadena, Cal.
> Jean-Paul Rebound, Ph.D., The Rockefeller University, New York
> James A. Robb, M.D., Yale University, New Haven, Conn.
> David H. Roscoe, Ph.D., Mass. Institute of Technology, Cambridge, Mass.

UNDERGRADUATE RESEARCH PARTICIPATION PROGRAM

Summer 1967

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 1967:

	Title of Project
Douglas Brown, Bellarmine College *Supervisor:* Dr. David Denhardt	Phage ΦX174
Judith Cohen, Columbia University *Supervisor:* Dr. Richard Novick	Staphylococcal RTF
Geoffrey Cooper, Mass. Inst. Tech. *Supervisor:* Dr. Maurice Fox	Transcription & Recombination
Palma Longo, St. Bonaventure Univ. *Supervisor:* Dr. Joseph Speyer	Genetic Suppression
Michael Lovett, Yale University *Supervisor:* Dr. Sol Goodgal	Bacterial Transformation
Michael McLeod, California Inst. of Tech. *Supervisor:* Dr. Max Delbruck	Albino Phycomyces
Gerald Rosen, Cornell University *Supervisor:* Dr. Maurice Fox	Bacteriophage Recombination
Robert Steinberg, Harvard College *Supervisor:* Dr. John Cairns	DNA Transfer
Jill Steinhardt, Goucher College *Supervisor:* Sidney Colowick	Bacteriophage rII function
Peter Wayne, Harvard College *Supervisor:* Dr. Cedric Davern	DNA Synthesis

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

T. August, Albert Einstein College of Medicine; R. Erickson, University of Colorado; R. Roblin, Harvard University

S. Colowick, M. Colowick, Vanderbilt University; J. Steinhardt, Goucher College

D.D. Denhardt, Harvard University

M. Fox, G. Cooper, J. Ebel, R. White, Massachusetts Institute of Technology; G. Rosen, Cornell University

H. V. Gelboin, National Institutes of Health

S. Goodgal, J. Gunther, H. Koslowski, J. Michalka, University of Pennsylvania: M. Lovett, Yale University

C. F. Gunsalus, I. C. Gunsalus, O. P. Shukla, G. J. Schmidt, University of Illinois

R. Novick, P. Guida, Public Health Research Institute of the City of New York; J. Cohen, Columbia University

F. Womack, K. Pate, Vanderbilt University

REPORTS OF SUMMER GUEST INVESTIGATORS:

RNA BACTERIOPHAGES

T. August,
R. Erickson,
R. Roblin,
C. Davern,
M. Murphy,
P. Wayne

1. *Studies of the nucleotide sequence at the 5′-terminus of RNA bacteriophage RNA -* Experiments concerning the 5′-terminus were carried out with Qβ phage RNA synthesized in vitro and with phage R17 RNA.

Previous studies have shown that by use of γ-P^{32}-labeled GTP, RNA synthesized in vitro in the Qβ RNA polymerase reaction can be labeled at the 5′-terminus, as p^{32}ppG – (Banerjee et al., 1967). The means for specific labeling of the 5′-terminus thus provides a new tool to aid in the isolation and characterization of 5′-terminal oligonucleotide segments of Qβ RNA. The research during the summer was devoted to the isolation and characterization of such labeled RNA. Working on this project were: Raymond Erickson, Richard Robin

Experiments were also carried out with R17 RNA made in vivo. This RNA was also shown to contain 5′-pppGp– (Roblin, J. Mol. Biol., in press). Digestion of this RNA and the Qβ RNA synthesized in vitro was carried out with pancreatic RNase, and the products separated on columns of DEAE-sephadex. The 5′-terminal sequence of both was found to be either pppGpPupPupPyp or pppGpPupPupPupPyp. These results are relevant to the question of whether messenger RNA need contain an initiator codon, AUG or GUG, at the 5′-terminus, and indicate that this is not a prerequisite. The question may thus be raised about other possible functions for this region of the RNA molecule. Participating in this study were: Richard Roblin, Raymond Erickson, Thomas August

2. A second area of interest was the isolation of conditional lethal mutants of *E. coli* defective in DNA synthesis, that would grow at 30° but not at 45°. Two selection techniques were utilized with bacteria treated with nitrosoguanidine. In one, bacteria grown in the presence of bromouracil at 45° were exposed to strong light in order to kill all those that continued to synthesize DNA at that temperature. The surviving colonies were thus enriched for mutant bacteria of the type desired, with a defect at high temperature that was reversible on shift to lower temperature. In addition, in order to isolate mutant bacteria that might exhibit an irreversible defect, the mutagenized bacteria were also replica plated to permit the simple isolation of temperature sensitive colonies.

With mutant bacteria derived in this manner, DNA synthesis was examined by the incorporation of radioactive thymine during growth at 45°. Investigation of other growth characteristics and of DNA polymerase activity in vitro was also initiated with certain of these isolates.

Associated with this project were: Cedric Davern, Mary Alice Murphy, Peter Wayne, Richard Roblin, Thomas August.

A new approach was made to the problem of the function of the r11 gene of bacteriophage T4. This approach is based on the assumption that the r11 gene controls a protein involved in the function of the membrane of the infected cell. The experimental procedure involves subjecting the infected cells [K12(λ)] to osmotic shock by the method of Heppel, a procedure which has been shown by him to remove specifically certain membrane-associated proteins from normal *E. coli* without killing the cells. In the present work, the ability of the shocked infected cells to produce phage has been found to be markedly reduced. Experiments are under way to determine whether addition of proteins present in the shock fluid will restore the ability of the shocked, infected cells to produce phage.

FUNCTION
OF THE
r11 GENE
S. Colowick,
M. Colowick,
J. Steinhardt

An attempt was made to find evidence that actively replicating ΦX DNA was bound to a component in the cell, for instance a membrane, that altered the behavior of the DNA during centrifugation in a gradient. Cells were infected with H3-labeled ΦX and lysed with lysozyme-versene. The resulting lysate was inserted into a CsCl-sucrose density gradient. A typical gradient was made by placing successive layers into a 5 ml centrifuge tube: for instance - 1 ml of 20% sucrose with 1.5 g per g CsCl (density about 1.8); 0.5 ml of 15% sucrose with 1.0 g/g CsCl (density about 1.6) (this layer contains the lysate); two 1 ml portions of density 1.4 and 1.2 g/ml; and a final 0.5 ml of 5% sucrose (density 1.0). The overall result of this is to produce a step gradient in both CsCl and sucrose. DNA should move toward the bottom of the tube during centrifugation while "membrane-bound" DNA would move up. Although some experiments indicated that some of the H^3 was moving rapidly to a lighter density than expected for DNA, it was not possible to demonstrate that this material had physiological significance. These experiments are being continued with different supporting material for the gradients.

STUDIES
ON THE
REPLICATION
OF ΦX174
D. T. Denhardt,
D. Brown,
J. Cairns

A research project conducted by an undergraduate (Douglas Brown) participating in the NSF-URP program involved the determination of recombination frequencies in several mutant strains of *E. Coli*. These mutants were derivatives of a ΦX sensitive strain and had lost the ability to replicate (but not absorb) ΦX. Earlier experiments had indicated that these strains were deficient in their ability to support bacterial recombination. The present work was designed to confirm and further quantitate the deficiency with different genetic markers. This work is being continued by Mr. Brown.

Experiments in collaboration with John Cairns were performed to see if bacteria which themselves were inhibited from synthesizing DNA could be stimulated to synthesize DNA by ΦX infection. The experiment entails adding H^3-thymidine to log phase cells; 20-30 sec later CN⁻ or CN⁻ plus iodoacetate are added and the rate of DNA synthesis (as measured by the incorporation of counts into a non-TCA-extractable form in the cell) very quickly falls to a low level; the culture is then split and part is infected with ΦX. The infected culture is observed to incorporate more counts than the uninfected culture. This work also is being continued.

Geoffrey Cooper, an undergraduate at M.I.T., investigated the relationship between enzyme induction and transformation efficiency in *B. subtilis*. A previous observation indicated that transformation of the histidase structural gene increased when histidase was induced in the recipient bacterium. Analogous experiments with threonine deaminase mutants gave qualitatively similar results, although the effect was smaller than in the case of histidase mutants. An attempt was made to repeat the observation on tryptophan synthetase mutants, assuming that analogues active as inducers in *E. coli* are also active in *B. subilis*. No stimulation of transformation efficiency was observed in these experiments. Attempts to isolate control mutants of histidase which also carry a structural gene mutation so as to determine the effect of control mutations on histidase transformation efficiency were made and are being continued.

TRANS-
FORMATION,
AND
INTERFERENCE
M. Fox,
J. Ebel,
G. Cooper,
G. Rosen,
R. White

An attempt was made by G. Rosen to clarify the relationship between genetic recombination and the ends of the T4 genome. He performed a cross between two parents mutant in the rII region and selected progency phage that were self-complementing, terminally-redundant herterozygotes. He had hoped to determine recombination frequencies in the rII region of this selected population, but the recovery of heterozygotes from the cross was unexpectedly low, preventing the determination of the appropriate recombination frequencies.

Ray White has been examining T4 DNA isolated from T4 infected *E. coli* using the T4 transformation system. Biologically active T4 DNA is found prior to the appearance of mature phage and appears to increase in parallel with gross phage DNA synthesis.

Judy Ebel, an M. I. T. graduate student, has been investigating the effects of ΦX174 infection on the liberation of λ from induced *E. coli* C(λ). The observation has been made that infection of *E. coli* C(λ) with an amber mutant of ΦX174 which lacks the ability to lyse such infected su5 cells results in a substantially reduced yield of λ phage following uv induction. Increasing fractions of the expected λ yield from uv-induced bacteria are observed when the ΦX174 infection is delayed.

INDUCTION OF HYDROXYLASE
H. V. Gelboin

Studies were performed on the induction of a microsomal hydroxylase in animal cell cultures. This enzyme plays an important role in vivo in the metabolism of drugs and carcinogens, thereby affecting the host response to the pharmacologic and toxic actions of these compounds. The enzyme is inducible up to 40-fold within 12-24 hours of exposure to the hydrocarbon inducer. During the summer, various cell types were tested for inducibility. Some of the conditions of the induction were determined, and chromatographic procedures for the isolation of hydroxylated hydrocarbon metabolites were established.

BACTERIAL TRANS-FORMATION
S. Goodgal,
J. Gunther,
H. Koslowski,
H. Lovett,
J. Michalka

Michel Lovett studied the uptake of DNA by *Salmonella typhimurium* and *E. coli* C with the objective of obtaining a transformation system for an enteric bacterium. Attempts to obtain transformations directly were unsuccessful in his hands as it has been with the many others who have preceded him. Using a variety of conditions of stress, pH, salt concentration, etc. with media normally used to grow salmonella or coli, no transformation and no appreciable uptake of DNA was obtained. With cells growing in a considerably enriched medium, brain heart infusion broth, Lovett observed a marked uptake of P^{32} labeled DNA. There was no appreciable breakdown of the DNA into acid soluble material. These experiments have been confirmed and merit continued investigation.

Jack Michalka: Mapping studies on the chromosome of *Hemophilus influenzae* using high molecular weight DNA were continued to confirm results which were obtained in earlier experiments. The DNA used in these experiments was sedimented in sucrose density gradients to determine the actual size of the fragments of DNA which were used in the transformations. The objective is to determine the physical distance separating markers, which are unlinked in transformations using DNA extracted by conventional methods but show some degree of linkage when extracted with low levels of shear.

Jay Gunther continued the work on the purification and characterization of the nucleases of *H. influenzae* which was begun at Cold Spring Harbor the previous summer. This summer he worked on the purification of a magnesium ion dependent nuclease which converts double stranded DNA into acid soluble material. This enzymatic activity was purified severalfold by precipitation with ammonium sulfate, chromatography on DEAE cellulose, and adsorption onto calcium phosphate gel. It has a pH optimum of about 7.6 and is inhibited by inorganic phophate. The acid soluble products were examined by chromatography on ion exchange resins and appear to be 5' nucleotides, although it is still possible some small oligonucleotides are produced. Final analysis of the mechanism by which this enzyme hydrolyzes DNA awaits a further characterization of the products and a study of its effect on the transforming activity of DNA.

Sol Goodgal (assisted by Helga Koslowski): Studies on the effects of enzymes on the linkage of markers of transforming DNA of *H. influenzae*.

A series of experiments were performed in which the linked markers Strep and C_{25} were inactivated by pancreatic DNAase and tested for the ability of polynucleotide ligase and DNA polymerase to restore biological activity. Most of our effort involved the development of methods for removing or preventing additional inactivation by contaminating nucleases or free radicals generated by sulfhydryl compounds (reducing agents). These efforts were successful and it has been possible to restore some biological activity to inactivated DNA. These experiments are continuing with a system in which we are attempting to elucidate the mechanism of recombination during transformation in *H. influenzae*.

GENETIC
CHEMISTRY OF
PSEUDOMONADS
Carolyn F. Gunsalus,
I. C. Gunsalus,
O. P. Shukla,
G. J. Schmidt

Building on knowledge acquired and mutants prepared in previous summers and with phages isolated in our laboratories in Urbana by A. M. Chakrabarty (Cold Spring Harbor, student and investigator, summer 1966), mapping by transductional analysis of the chromosomal organization of *Pseudomonas putida*, strain C1, for peripheral pathways of terpene oxidation was initiated. Amino acid auxotrophic and analogue resistance markers previously mapped were used as reference. Additional examples of convergent metabolism and relaxed enzyme specificity have been explored among the monocyclic terpenes with newly isolated organisms. Mutants which grow in D_2O media were prepared for use in genetic and chemical analysis from pseudomonads in both wild type (strain C1) and auxotrophic mutants.

The oxidation pathway for (+) -camphor, a bicyclic monoterpene, proceeds by a 10-step reaction sequence to isobutyryl coenzyme A plus 3 moles of acetate. The first 5 reactions require at least 11 polypeptides, several of which are specific for a single enantiomer; i.e., (-) -camphor oxidation requires several additional proteins induced by its presence. Phage pf16 and two host range mutants, h and h_2, which we have used in transductional analysis of tryptophan biosynthesis and for interstrain transfer of the mandelate group enzymes from *P. putida*, strain A312 (Stanier), to the terpene oxidizing strain C1, have been used to show linkage among camphor (cam) oxidation enzymes and of a fluorophenylalanine resistance locus (fpaA) to late cam enzymes. The locus fpaA1 shows 50% cotransduction with tryptophan gene cluster 1 (trp ABD) but is unlinked to trp clusters 2 (trp C) and 3 (trp EF). Similar scattering of tryptophan genes and linkage of trp to fpaA were observed in *P. aeruginosa* by Waltho and Holloway (J. Bact. *92*, 35, 1966), thus suggesting the possibility of inter-specific transfer should recognition and restriction barriers be overcome.

Organisms which grow at the expense of monocyclic monoterpenes of the menthol and carvol series have been isolated, the oxidation pathways and reactions partially elucidated, and mutants resistant to these highly bacteriostatic alcohols prepared. During the summer, methods of preparing carbon utilization mutants in these strains were explored and several mutants isolated. It appears possible that the added reactions and enzymes encountered will, with the genetic and chemical studies of bi- and acyclic terpenes, permit a general statement of mechanisms of selectivity in enzyme induction and action.

Structure analysis of several camphor oxygenase enzyme components, particularly the ferrosulfide subunit of a methylene hydroxylase, has been aided by isotopic labeling with iron and sulfide. A deuterated polypeptide would facilitate characterization of the reactive center. *P. putida,* strain C1 (w.t.) and a methionine auxotroph were therefore grown on increasing levels of D_2O with periodic mutagenesis and selection. Strains were obtained which grow on 99+% D_2O in an ammonium, glycerol, citrate, salts medium, supplemented with methionine in the case of the methionine auxotroph. The growth rates are about ¼ the rates in H_2O media; the growth yields are equal. Analyses of the whole cells and specific proteins for D/H ratios to determine enrichment levels are in progress as a preliminary to enzyme production and analysis.

EXTRA-
CHROMOSOMAL
INHERITANCE IN
STAPHYLO-
COCCUS AUREUS
R. Novick,
P. Guida,
J. Cohen

R. Novick: Studies into DNA bromouracil incorporation in *S. aureus* preliminary to attempts to isolate extrachromosomal DNA.

The organism incorporates, BUDR very poorly, if at all, in the absence of thymidine (Tdr).

Thymine starvation results in rapid DNA breakdown - with H^3Tdr-labeled cells, in the first 30 minutes about 20% of TCA-precipitable radioactivity is released into the medium. Thereafter, no further release occurs, but within two hours, in the absence of Tdr, at least half of the DNA is degraded into fragments of about 10^6 daltons. This process is enhanced by the presence of BUDR, in which case at least 80% of the DNA is fragmented.

In the presence of 10^{-6} M Tdr, BUDR inhibits the incorporation of H^3 Tdr into DNA and is itself incorporated. After two hours at 42° with BUDR, 20 $\mu g/ml$, about half the DNA is still unreplicated (i.e., light), the other half is hybrid but shows a broad band in CsCl, suggesting small molecular weight.

The conclusion is that it will be difficult to use BUDR as a clean density label for this organism. The mechanism of DNA breakdown is unclear.

P. Guida: High-frequency transduction (Hft). Conditions were worked out for a technique for examining large populations of colonies for Hft activity. Technique involves overlaying colonies with a layer of recipient organisms, incubating and looking for clusters of secondary transductant

colonies. The technique worked well and was used to isolate non-Hft mutants from an Hft donor strain.

J. Cohen: An attempt was made to demonstrate suspected chromosomal integration of a previously extrachromosomal gene for erythromycin resistance through the demonstration of linkage to chromosomal markers. The experiment consisted in mutagen-treating transducing phage grown on the erythromycin-resistant donor and examining subsequent erythromycin-resistant transductants for temperature sensitivity. In screening over 5,000 transductants, no linked temperature-sensitivity mutations were found. It is felt that further work is required before any negative conclusion can be drawn from these experiments.

HEXOKINASE MUTANTS IN YEAST
F. Womack, K. Pate

Current studies show the presence of two distinct forms of hexokinase in commercial baker's yeast, as well as in pure haploid yeast strains. Attempts to isolate hexokinase-negative mutants have been initiated, in order to determine whether these two forms are derived from different structural genes. The technique for isolation of the mutants, originally developed by Megnet, is based on the selection of cells which are resistant to 2-deoxyglucose. Wild-type cells containing hexo-kinase produce 2-deoxyglucose-6-phosphate, which is lethal to growing cells. Mutant cells lacking hexokinase should not be killed and should grow well if energy sources other than hexoses are provided. Several mutants resistant to 2-deoxyglucose have been isolated, but it has not yet been determined whether these mutants are lacking in hexokinase.

PHAGE
CONFERENCE

August 30—September 7, 1967

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 more than 200 people.

SECTION I - Temperature Phage
(Organizer: Harrison Echols)

Wednesday, August 30 — 7:30 P.M.
Properties of Phage DNA and DNA Replication

A. G. Mackinlay and A. D. Kaiser — "Two Steps in the Replication of Phage λ DNA."
L. A. Salzman and A. Weissbach — "Formation of Intermediates in the Replication of Phage Lambda DNA."
David Botstein — "Intermediates in the Synthesis and Maturation of Phage P22 DNA.
Don J. Brenner, Stanley Falkow and Dean B. Cowie — "Thermal Stability of Temperature Phage DNA Duplexes."

Thursday, August 31 — 9:15 A.M.
Developmental Control and Repression

M. Lieb — "Mapping and Complementation Studies of Mutations in the CI Region of λ."
W. Szybalski, K. Taylor, Z. Hradecna and A. Guha — "Transcription of Coliphage λ Genome in the Lysogenic and "Early" Induced States."
R. McMacken, B. Butler, A. Joyner and H. Echols — "Control of Late λ Genes by the c_{II} and c_{III} Products."
Finn B. Haugli and William F. Dove — "A New Clear Gene in Lambda."

Thursday, August 31 — 7:30 P.M.
Repression — Virulence

William F. Dove and Ermile Hargrove — "Partial Virulent Mutants of Lambda."
S. Packman and W. S. Sly — "Constitutive Killing by λ C17."
W. P. Diehl — "Prophage Induction by Superinfecting Virulent or Heteroimmune Phage."

Friday, September 1 — 9:15 A.M.
Integration and Vegetative Recombination

M. E. Gottesman, M. B. Yarmolinsky and E. Jordan — "Intergration-Negative (int) Mutants of λ Bacteriophage."
K. Manly, E. Singer and C. Radding — "Nondefective Biotin-Transducing λ Phages."
N. C. Franklin — "Deletions and Functions of the Center of the 080-λ Phage Genome. Evidence for a Phage Function Promoting Genetic Recombination."
R. Gingery and H. Echols — "Properties of λ Mutants Defective in Integration and Vegetative Recombination."
J. Weil and E. Signer — "Mutants of Phage λ Deficient in Genetic Recombinations."

Friday, September 1 — 7:30 P.M.
Integration and Repression Release

E. W. Six — "Prophage Site Specificities of P2 Phages."
Nagaraja Rao and H. O. Smith — "Secondary Attachment Sites for Phage P22."
M. E. Gottesman and V. H. Bridges — "Abnormal Integration and Excision of λ."
J. L. Rosner and Michael B. Yarmolinsky — "The Nature of the BR Factor."
Jennifer Patai Wing — "Induction of Phage P22 in a Recombination-Deficient Mutant of *Salmonella Typhimurium.*"

Saturday, September 2 — 9:15 A.M.
Phage-Host Interactions

W. S. Sly — "Host Killing by Thermal Induction of λ *Sus* Mutants."
M. Willard — "Effect of λ and λ dg Development on Phage and Host-Associated Bacterial Operons.
H. Boyer and D. Roulland-Dussoix — "Restriction and Modification of Phage Lambda by Diploids of of *E. coli* K12 and B."
R. Nagarajo Rao — "Mutants of Prophage P22 which do not Exclude Superinfecting Phage."

Saturday, September 2 — 2:30 P.M.
Transducing Phages

A. del Campillo-Campbell, G. Kayajanian, A. Campbell and S. Adhya - "Biotin Mutants of *E. coli.*"
Gary Kayajanian — "Transduction by Phage λ."
A. Guha, M. Tabaczynski and W. Szybalski — "The Orientation of Transcription of the *gal* Operon in Coliphage λdg."
I. C. Gunsalus and A. M. Chakrabarty — "Transduction Heterogenote from Inter-Strain Transfer of "Mandelate" Genes in *Pseudomonas putida.*"

SECTION II — Other Phages
(Organizers: Sewell Champe and Irwin Tessman)

Sunday, September 3 — 7:30 P.M.
Transcription from Phage Genomes

R. J. Crouch and B. D. Hall — "RNA Synthesis in Extracts from T4 Infected *E. coli.*
A. Guha and W. Szybalski — "A Late Switch in the Transcription from Only one to Both DNA Strands in Coliphage T4."
W. C. Summers and W. Szybalski — "Restriction of In Vivo Transcription of T7 Phage to the poly G-Binding DNA Strand."
G. Milanesi, E. Brody — "Temporal Sequence of Transcription of T4 DNA in Vitro by Purified *E. coli* RNA Polymerase.
L. P. Gage — "Program of m-RNA Transcription During Spol Development."
David L. Wilson — "Early m-RNA Synthesis In Vitro on Two Bacterio-phage DNA Templates with Homologous and Heterologous RNA Polymerase."
U. Bachrach and A. Friedmann - "Some Biological Properties of Internal Proteins from Coliphage T2."

Monday, September 4 — 9:30 A.M.
Phage Controlled Functions

Dwight H. Hall — "Mutants of Bacteriophage T4 Unable to Induce Dihydrofolate Reductase Activity."
C. P. Georgopoulos — "Nonglucosylated Mutants of Phage T4."
J. Hosoda — "Synthesis of DNA and Proteins by Ligase-Defective Mutants of T4."
J. Kan, T. Kano-Sueoka and N. Sueoka — "Characterization of Leucyl-sRNA in *E. coli* Infected with T2 Phage."
Elizabeth Kutter and John S. Wiberg — "Sucrose Gradient Characterization of Phage T4 Mutants Defective in Host DNA Breakdown."
F. Tomita and I. Takahashi — "Deoxycytidine Triphosphate Deaminase: A Novel Enzyme Found in PBS 1 Infected *Bacillus subilis.*"
R. Gesteland, W. Salser and A. Bolle - "In Vitro Synthesis of T-4 Lysozyme."
W. H. McClain and S. P. Champe — "Detection of the rII Protein of Phage T4."
Yvonne T. Lanni — "Genes in the T5 FST-DNA and Their Functions."
D. J. McCorquodale and Y. T. Lanni — "Patterns of Protein Synthesis in Cells Infected by Bacterio-phage T5 Having Amber Mutations in their FST Section."

Monday, September 4 — 7:30 P.M.
Single-stranded RNA and DNA Phages

Irwin Tessman, Ron Baker, Santosh Kumar, Ethel S. Tessman — "Direction of Translation Relative to the Circular Genetic Map of Phage S13."
Robert Shleser, Beverly Mannes, Hiromi Ishiwa and Ethel Tessman — "RF Made by Group IV Mutants of Phage S13."
David Denhardt — "Must ΦX DNA be Membrane-Attached in Order to be Replicated?"
D. Pratt — "Genetic Control of M13 DNA Replication."
H. D. Robertson, D. L. Engelhardt and N. D. Zinder — "In Vitro Translation of Phage f2 Replicative Intermediate."
W. J. Iglewski and R. M. Franklin — "Purification and Properties of the Complementary Strand of R17 RNA."
B. H. Iglewski and R. M. Franklin — "Association of Double-Stranded RNA with Polyribosomes in *Escherichia coli* Infected with Bacteriophage R17."

Tuesday, September 5 — 9:30 A.M.
Single-stranded RNA and DNA Phages. II.

Y. Hsu — "Regulation of Bacterial and Viral DNA Replication in HR Virus Carrier Cells."
B. Hohn, B. Y. Tseng, and D. A. Marvin — "The Filamentous Phage fd: DNA Replication."
Rolf Benziner — "Host-Controlled Restriction of Infectious Single- and Doublestranded DNA Derived from Bacteriophage fd."
Th. Hohn — "Self-Assembly of Defective Particles of the Bacteriophage fr."
R. Ziprin, B. Dorsett, J. Chu, L. Bernhardt, B. Berman and L. S. Jacobson — "Acid Inactivation of MS-2 Phage."
R. Wiener, R. Schwartz, H. Jacobson and L. S. Jacobson — "Estimate of the Inactivating Fraction of a Mutagenic Reaction."

Tuesday, September 5 — 7:30 P.M.
Structure and Replication of Phage DNA

Rudolf Werner — "Replication of Phage T4 DNA."
Fred R. Frankel — "Evidence for Long DNA Strands in the Replicating Pool After T4 Infection."
G. Mosig — "A Map of Distances Along the DNA Molecule Between Genetic Markers of Phage T4."
J. Renshaw and G. Mosig — "Sizes of DNA Fragments in "Light," Nonviable T4 Particles."
D. H. Parma — "Heterozygosis in T4D Partial Phage."
W. S. Reznikoff and C. A. Thomas, Jr. — "The Anatomy of the SP50 Bacteriophage DNA Molecule."
H. Yamagishi — "The Structure of PBS-1 DNA."
H. Yamagishi and I. Takahashi — "The Size of Transducing Fragments in Phage PSB 1."
Anthony A. Fuscaldo and Dean Fraser — "Characteristics of Coliphage C-1."

Wednesday, September 6 — 9:30 P.M.
Restriction, Suppression, Mutagenesis, etc.

J. Eigner and S. Block — "Relation of Four *E. coli* DNases to Restriction of T-Even Bacteriophages."
W. H. S. Smith and L. I. Pizer — "Restriction of T2 Infection by *E coli* Strain."
David Freifelder — "The Mechanism of X-ray inactivation of T4."
R. Hausmann, B. Gomez and B. Moody — "Abortive Infection of a Shigella Dysenteriae Strain by Coliphage T7."
H. T. Epstein, D. Housman, I. Mahler — "UV Repair and Phage Genetic Recombination in *B*. subtilis."
Stanley Person and Mary Osborn — "Conversion of Amber Suppressors to Ochre Suppressors."
Nils Aall Barricelli and Allan Lindh — "The Orientation of GC Base Pairs in T4 Mutants. Preliminary Results Obtained by Comparing Methylene Blue and Hydroxylamine Reversion Patterns."
H. Witmer and D. Fraser — "Ohotodynamic Inactivation of T3."
M. E. Bayer — "The Adsorption Sites of Bacteriophages on the Surface of *Escherichia coli.*"

NATURE STUDY
COURSES

Children of Ages 6 to 16

During the summer of 1967, twenty courses in Nature Study were conducted in two monthly sessions. The enrollment this year was 268 students. The course offerings included:

General Natures Study (ages 6, 7)
General Ecology (ages 8, 9)
Bird Ecology (8, 9)
Plant-Insect Relationships (8, 9)
Advanced Bird Study (10, 11)
Seashore Life (10, 11)

Geology (10, 11)
Animals with Backbones (10, 11)
Fresh-Water Life (10, 11)
Plant Ecology (12-16)
Insect Study (12-16)
Animal Ecology (12-16)
Icthyology-Herpetology (12-16)

INSTRUCTORS

Mr. Otto A. Heck, M.S., Assistant Professor of Biology at Trenton State College, Trenton, N.J., was in charge. The additional staff members were:

Mrs. Barbara Church, M.Ed., Science Teacher, former teacher at Jerusalem Ave. Junior High School, Bellmore, L.I., and Science Substitute at Central High School, District No. 3.

Mr. Alex Pepe, M.A., Science Teacher K-6, East Side School, Cold Spring Harbor, L.I.

Mr. Richard Ryder, a senior at Adelphi University, majoring in biology and a naturalist.

The Laboratory gratefully acknowledges the eighth year contribution of the Huntington Federal Savings and Loan Association. This provided nature study scholarships for 15 students of the Huntington elementary schools.

NATURE STUDY
WORKSHOP FOR TEACHERS

The twelfth annual Workshop in Nature Study was offered from June 30th to July 26th, 1967. 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, streams, seashore, woodlands, fields 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.

Twenty teachers attended the workshop. Upon satisfactory completion of the requirements of the course, teachers were entitled to four in-service credits awarded by the New York State Dept. of Education. The instructor for the summer of 1967 was Mr. Otto A. Heck.

GRANTS AND CONTRACTS

May 1, 1966 to April 30, 1967

Grantor	Investigator	Total Award	Grant Number
	RESEARCH GRANTS		
National Science Foundation	Dr. Cairns	$ 50,900.00	GB-4055*
National Science Foundation	Dr. Davern	76,200.00	GB-4001*
National Institutes of Health	Dr. Margolin	101,700.00	5-K3-GM-6887-03†
National Institutes of Health	Dr. Margolin	279,854.00	GM-07178-07†
National Institutes of Health	Dr. Speyer	245,728.00	GM-12371-03†
The Rockefeller Foundation	Dr. Cairns	85,000.00	RF-63042†
	TRAINING GRANTS		
National Institutes of Health	Summer Courses	47,865.00	GM-890-11
National Science Foundation	Phycomyces Workshop	20,000.00	GB-6114*
	SYMPOSIUM GRANTS		
Atomic Energy Commission		7,000.00	AT(49-12)-2963
Air Force Office of Scientific Research		7,000.00	AF 49(638)-1727
National Institutes of Health		12,050.00	CA-02809-11
National Science Foundation		8,500.00	GB-4500
	SPECIAL GRANTS		
National Science Foundation	Interim Support and Development	73,900.00	GB-3727*
New York State Science and Technology Foundation	Support of Graduate Teaching & Research Programs	31,130.00	SSF(5)-14

*These grants cover a two-year period
†These grants cover a five-year period

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FINANCIAL REPORT

For the period May 1, 1966—April 30, 1967

As of April 30, 1967, our assets were as follows:

Cash	$123,049.45	
Accounts receivable	46,857.51	
Inventory of books	37,958.84	
Prepaid expenses	2,641.63	
Investments—U. S. Government obligations		
(market value $29,754.30)	29,399.18	
Land, buildings and equipment	510,426.00	
Total		$750,332.61

Our liabilities were as follows:

Accounts payable	$ 27,722.95	
Unexpended grants and contracts	72,184.77	
Scholarship fund	781.28	
Deferred income	4,300.00	
Net worth	645,343.61	
Total		$750,332.61

For the year 1966-1967, our receipts were as follows:

Grants and contracts		$279,933.24
Contributions:		
Sponsors and Friends	$ 50,038.09	
Carnegie Institution of Washington	27,445.83	
Long Island Biological Association	409.17	
Wawepex Society	3,200.00	$ 81,093.09
Book Sales (Cold Spring Harbor Symposia		
on Quantitative Biology)	107,373.44	
Dining hall and dormitories	77,829.45	
Tuition fees	30,060.00	
Summer laboratory fees	6,200.00	
Symposium registration fees	8,610.00	
Interest on time deposit and		
U. S. Government obligations	2,711.78	
Miscellaneous	960.22	
Total		$594,771.22

Our expenditures were as follows:

Research and educational programs	$256,639.12	
Administration and general	94,104.66	
Plant operations and maintenance	131,325.33	
Publications	52,065.65	
Dining hall and dormitories	70,726.07	
(inc. provision for depreciation)		
Total		$604,860.83

Excess of Expenditures over Income 1966-1967   ($10,089.61)

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of the Cold Spring Harbor Laboratory of Quantitative Biology

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