

ANNUAL REPORT 1964

COLD SPRING HARBOR LABORATORY OF QUANTITATIVE BIOLOGY



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**COLD SPRING HARBOR
LABORATORY OF
QUANTITATIVE BIOLOGY**

ESTABLISHED JULY 1, 1963

FIRST ANNUAL REPORT

CALENDAR YEAR 1964

COLD SPRING HARBOR, LONG ISLAND, NEW YORK

COLD SPRING HARBOR LABORATORY OF QUANTITATIVE BIOLOGY

Cold Spring Harbor, New York

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DIRECTOR'S REPORT

On July 1, 1963, the operation of the laboratories of the Long Island Biological Association and the Carnegie Institution of Washington formally became the task of the Cold Spring Harbor Laboratory of Quantitative Biology. The precise arrangements are a suitable subject for the first Annual Report of the Laboratory of Quantitative Biology, since much of the last year has been spent by the new management in sounding out these arrangements in order to determine how the Laboratory of Quantitative Biology should be supported and in what ways, if any, its role should differ from that of its parent organizations.

Since 1904, The Carnegie Institution of Washington had operated a year-round research program in laboratories built on land it leased from the Wawepex Society. These laboratories, plus various attendant buildings including a large library and a lecture hall, are leased (or subleased) for a nominal sum to the new organization until the year 2004, at which time the original lease on the Wawepex land runs out. In addition, the Laboratory of Quantitative Biology has a first option on two parcels of undeveloped land, owned by the Carnegie Institution within the general area usually considered as part of the Cold Spring Harbor complex. In its turn, the Laboratory of Quantitative Biology leases back to the Carnegie Institution the space occupied by Dr. Hershey and Dr. McClintock, and for this receives an annual grant from the Carnegie Institution to cover certain of the indirect costs of their research.

The second part of the reorganization concerns the Long Island Biological Association which, since 1924, has been responsible for the entire summer program and for some year-round research. All its own land and buildings are deeded to the Laboratory, and its lease of land from the Wawepex Society is transferred to the Laboratory.

The new Laboratory of Quantitative Biology, which is entrusted with the task of running these once-separate organizations, is supervised by a Board of Trustees, representing various "participating" institutions, much in the way the Brookhaven National Laboratory is supervised by Associated Universities, Inc. Thus, each participating institution guarantees some limited financial support which may be called for at any time, provided at least four of the other participating institutions have also agreed to produce their support.

The assets of the new organization are considerable. It has land and buildings worth perhaps two million dollars, and an international reputation for its year-round program of research; its annual symposium; and its advanced courses in microbial genetics. It is therefore spared what must be the greatest challenge of any institution created *de novo*, because it already has an "image."

Unfortunately, it had from the outset some conspicuous deficiencies—namely, a deficit budget, a dilapidated plant, and the absence of an endowment or equivalent source of income. What was not so clear was the exact size of this deficit or, more important, the exact extent of the physical dilapidation. For this reason, and because solicitation of support for any existing venture is seldom successful, the National Science Foundation provided a grant to pay for the cost of a Site Survey of all the buildings on the grounds by a firm of consulting architects and engineers.

The survey was completed in June, 1964. It showed that about \$750,000 would be required to return all the buildings to a sound physical condition. This is a rather large sum compared to the original capital investment at Cold Spring Harbor, which totalled about \$2.5 millions, though it is small compared to the cost of creating a new scientific institution (still less one of such international renown). For this reason, we would probably succeed in the end, were we to embark on a campaign to raise sufficient money to restore the physical plant.

However, the arrangement and types of buildings on the site were determined more often by historical accident than by intent; and there has been no opportunity, until the recent fusion of the two enterprises, to plan the site as a unified whole. Any act of general restoration should therefore be preceded by a general site development plan, so that what results finally will be a physical plant more economical to operate and more suited to its purpose than the somewhat haphazard collection of buildings we have at present. Such a site development plan will be prepared during the next year, hopefully with support from some federal agency. It should be completed by the time of next year's Annual Report.

Scientifically, the year has been notable for the sharp increase in the size of the summer program (described within this report). It seems that we are now at the stage where any further increase will demand additional housing.

During the year there have been a few changes in the scientific staff. In the Spring, Dr. Cedric Davern came here from Australia. At the end of the summer, Dr. Edwin Umbarger, who had been on leave in England, left to join the staff at Purdue University, and Dr. Joseph Speyer joined our staff, coming from the New York University School of Medicine.

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

John Cairns
Director

MICROBIAL BIOCHEMISTRY

Laboratory of
H. E. Umbarger,
R. O. Burns,
M. Freundlich,
P. Siu,
J. Calvo,
F. Størmer,
R. Bauerle

The program of the Microbial Biochemistry group was again devoted exclusively to mechanisms regulating the synthesis of the amino acids valine, isoleucine, leucine, and serine in microorganisms. As has been clearly shown in this laboratory and elsewhere, the formation of amino acids and other metabolites is controlled in two ways. In one, the enzymes concerned with the synthesis of a metabolite are not formed if the metabolite is supplied in the medium (enzyme repression). In the second, the function of the enzymes is prevented if the activity of the first enzyme in the sequence is inhibited by the metabolite itself (endproduct inhibition).

Three endproduct-sensitive enzymes were studied during the past year. One, 3-phosphoglycerate dehydrogenase, which catalyzes the first specific step in the biosynthesis of serine, was studied by Dr. Patrick Siu. Of particular concern with this enzyme was the fact that it exhibits an appreciable sensitivity to L-serine only when catalyzing the conversion of phosphohydroxypyruvate to phosphoglycerate, the reverse of the biosynthetic reaction. Nevertheless, the activity of the enzyme is thought to be regulated by serine in the intact cell. Attempts have been made to simulate more closely the conditions in the cell in a thus far unsuccessful effort to demonstrate an inhibition of the enzyme in the forward direction. This paradoxical behavior is shown by the enzyme in crude extracts and in partially purified preparations.

The second endproduct-sensitive enzyme studied was threonine deaminase, which catalyzes the first step in the biosynthesis of isoleucine. Much has already been learned about this enzyme but its study has been handicapped by the fact that its lability had made purification impracticable. During the past year, Dr. R. O. Burns has had considerable success in stabilizing the enzyme and has purified it in excess of 100-fold from extracts of *Salmonella typhimurium* containing "derepressed" levels of this enzyme. It is expected that this preparation will permit the study of the conformational changes accompanying binding of the inhibitor, isoleucine, and other small molecules to the enzyme.

The third endproduct-sensitive enzyme studied in this laboratory, acetohydroxyacid synthetase, catalyzes the formation of α -acetolactate and α -aceto- α -hydroxybutyrate, the earliest five- and six-carbon intermediates in valine and isoleucine biosynthesis respectively. Owing to the failure of workers in other laboratories to demonstrate the valine sensitivity of this enzyme in extracts prepared from *S. typhimurium*, a property routinely observed in this laboratory, a detailed study of the enzyme was undertaken by Dr. Ronald Bauerle. Like the corresponding *E. coli* enzyme which had been studied earlier, the optimal pH for both activity and endproduct sensitivity was pH8.0; Mg^{++} , diphosphothiamine (DPT) and a factor contained in boiled extracts were cofactors, and inhibition by valine was noncompetitive with respect to Mg^{++} and α -ketobutyrate. Unlike the results with the *E. coli* enzyme, however, the inhibition was also noncompetitive with respect to pyruvate and DPT. The enzyme could be rendered valine-insensitive by treatment with mercury, heat and urea. Very recently, Dr. Bauerle has been able to make substantial progress in the purification of acetohydroxyacid synthetase. He also developed an improved method for the demonstration of acetohydroxybutyrate.

Dr. Bauerle's work was greatly aided by the identification by Dr. Fredrik Størmer of the required factor in boiled extracts as flavine adenine dinucleotide (FAD). Dr. Størmer had purified and isolated the active component from a boiled extract of yeast. The work was initiated at Cold Spring Harbor and completed at Ullevål Hospital, Oslo, Norway. This finding was quite unexpected since FAD is usually associated with enzymes which catalyze reactions involving a transfer of hydrogen and there is no such transfer in acetohydroxyacid formation.

Considerable attention was also given during the past year to the question of repression of the enzymes in the pathways which are of interest to this group.

Dr. Joseph Calvo continued studies on the regulation of leucine biosynthesis in *Salmonella typhimurium*. Mutants resistant to the leucine analog, 5', 5', 5'-trifluoro-leucine, were isolated and most of these strains could no longer regulate the amount of leucine synthesized. From biochemical and genetic studies, the fluoroleucine-resistant strains could be divided into four classes:

Class	Linkage by transduction to		Excreted		Enzyme levels	
	<i>leu</i>	<i>ilva</i>	Leucine	Isoleucine plus valine	leucine pathway	isoleucine-valine pathways
1	—	—	+	+	high	high
2	+	—	+	—	high	normal
3	+	—	+	—	normal	normal
4	—	—	—	—	normal	normal

leu=genes for leucine biosynthesis

ilva=genes for isoleucine and valine biosynthesis.

Most of the fluoroleucine-resistant mutants isolated fall into class 1. In these strains, mutation to resistance resulted in the constitutive formation of the three leucine enzymes. Since the genetic lesion is unlinked to the leucine genes, Dr. Calvo has postulated that these organisms are unable to form the leucine-repressor even though leucine may be present in excess.

The class 2 mutants are most likely of the operator-constitutive type. That is, although they all presumably form the leucine-repressor molecule when exogenous leucine is present, the operator region is unable to respond to it. One of these mutants has been shown with some certainty to lie in the operator region of the *leu* operation.

Of the 130 fluoroleucine-resistant mutants tested only one was found to have characteristics described by the class 3 category. This mutant probably has a leucine condensing enzyme which is less sensitive to endproduct inhibition.

The class 4 mutants may very well have an altered uptake mechanism for fluoroleucine and perhaps for leucine as well.

Class 1 is of further interest since their organisms also exhibit constitutive synthesis of the enzymes on the pathway to isoleucine and valine. This observation indicates that a common factor is required for the regulation of the enzymes in the two systems. The existence of such a common factor is of particular interest since the regulation of the isoleucine-valine enzymes is multivalent.

Dr. Burns also investigated factors which affected the levels of the leucine enzymes in cells of *S. typhimurium*. It had been observed that with all procedures (genetic and physiological) employed for the derepression of the enzymes in the leucine pathway, the derepression was coordinate except when derepression was achieved by the limitation of valine. The latter observation correlated with the low level of leucine intermediates in *S. typhimurium* cultivated under these conditions. It was demonstrated directly in vitro and indirectly in vivo that α -ketoisovalerate and β -carboxy- β -hydroxyisocaproate were effective in preventing the thermal inactivation of the synthetase and isomerase, respectively.

An examination of 70 leucine auxotrophs of *S. typhimurium* that had been shown by the Bacterial Genetics group to have lesions in cistron I revealed that 24 of these strains formed less of the enzyme encoded by cistron II than did the others. A smaller number of strains blocked in cistrons II, III or IV were examined for possible effects on the function of cistron I but none were found. These observations are in accord with those of other workers who have demonstrated a polarity in the genetic information in other operons and that "modulations" in the reading of the corresponding message may result owing to certain base pair substitutions which have occurred as a result of mutation. Such substitutions are thought to impede the translation of the message or to increase the likelihood of ribosomes falling off the message. Thus the 24 strains referred to above are "modulated downwards." Interestingly, one strain was found which was "modulated upwards."

Dr. Martin Freundlich continued his study of the regulation of the enzymes leading to isoleucine and to valine for which repression was multivalent in the Enterobacteriaceae. A brief survey of several microorganisms, not members of the Enterobacteriaceae, failed to reveal the pattern of multivalency for these enzymes. During the year, Dr.

Freundlich collected and initiated studies on several mutants of *S. typhimurium* in which the pattern of regulation of these enzymes appears to be altered.

Dr. Patrick Siu's experiments with the enzymes in the pathway to serine in *S. typhimurium* indicated that these enzymes are not endproduct repressible and may very well be constitutive.

Another member of the group, H. E. Umbarger, was granted a leave of absence for the 1963-64 year. During this period he was a Guggenheim Fellow in the Department of Biochemistry, University of Leicester, England. While there, he initiated a genetic and biochemical study of mutants of *S. typhimurium* which had lost their ability to grow with acetate as the sole carbon source.

At the close of the 1964 summer season, the Microbial Biochemistry group was disbanded. It might be appropriate, therefore, on behalf of the nine investigators who, between 1960 and 1964, had been associated with the group as year-round staff members to express their appreciation to the individuals and groups who made it possible for them to have worked in the stimulating environment of Cold Spring Harbor. They are all especially appreciative of the unique opportunity which was theirs to collaborate with the members of the Bacterial Genetics group. All agree that much they took away with them was directly or indirectly contributed by that group.

The work in this laboratory has been concerned primarily with two aspects of bacterial genetics. These are: (1) the nature of the mechanism of 2-aminopurine mutagenesis, and (2) the organization and functional relationships among the elements which regulate the synthesis of leucine and tryptophan in *Salmonella typhimurium*.

MUTATION—A total of 127 independent leucine auxotrophic mutants have now been carefully investigated with respect to the manner of their response to back mutation induction by 2-aminopurine. These include at least 29 mutants of spontaneous origin, 49 induced by 2-aminopurine, 28 induced by X rays and 21 induced by nitrosoguanidine. Although all 2-aminopurine induced mutants could be induced to revert by 2-aminopurine, a total of 49 mutants from the other classes of auxotrophs revealed no detectable level of induced back mutation by 2-aminopurine treatment. The responding mutants fell into two rather distinct time classes with respect to the number of cell divisions following a pulse of 2-aminopurine, which were required for physiological expression of the back mutation. One class was apparently division-independent (D.I.) and produced physiological prototrophs immediately after incorporation of 2-aminopurine. The second class was division-dependent (D.D.) and required several cell divisions following 2-aminopurine incorporation before physiological prototrophy was expressed. When the data were analyzed in terms of frequency of induced reversions it was noted that almost all the D.I. mutants were reverted at quite high frequencies, whereas the D.D. mutants were mostly reverted at relatively low frequencies. This suggested that the two different time classes in fact represented the two different base pairs at the sites of mutation (A-T and G-C). Preliminary studies with additional mutagens such as hydroxylamine and 5-bromouracil indicated that the D.I. mutants contained G-C base pairs at the sites of mutation, whereas the D.D. mutants contained A-T base pairs. The mutants of spontaneous origin were equally divided between the two classes (15 D.I. and 14 D.D.). On the other hand, mutants resulting from 2-aminopurine treatment were primarily of the D.D. class (44 D.D. mutants and 4 D.I. mutants). This evidence implied that 2-aminopurine preferentially attacked the G-C sites causing transitions to A-T base pairs (D.D.) at the sites of mutation. This evidence for two simple time classes determined by the base pairs at each site has important implications regarding the mechanism of transcription into messenger RNA. The interpretation most consistent with this data suggests that the two bases, one from each strand of DNA, act together as a unit in determining which base is inserted at the homologous location in the messenger RNA. This appears to be true despite the fact that for any one gene the messenger RNA is presumably complementary to only one of the two strands of DNA. The evidence therefore leads to the hypothesis that messenger RNA is formed from DNA when it is in the double-stranded condition.

GENETIC REGULATING ELEMENTS—The discovery of the suppressors of the leucine operator mutant, leu 500, has led to the utilization of deletions for dissecting

**BACTERIAL
GENETICS**
Laboratory of
Paul Margolin,
Frank Mukai

the tryptophan gene cluster on the chromosome of *Salmonella typhimurium*. Three interesting points have emerged from this work: (1) The tryptophan cluster of genes is not a single operon, but consists of at least two operons. (2) There are interactions between mutations in one operon and the functioning of genes in the second operon. This implies that there exists a functional unit of regulation larger than an operon, which encompasses all the genes within a cluster of functionally related operons. (3) The polarity of the tryptophan operons is opposite to that of the leucine operon, indicating bipolarity of information transfer from the *Salmonella* chromosome.

Dr. Raphael Falk from the Hebrew University in Jerusalem spent three months in this laboratory investigating chromosomal aberrations in *Salmonella typhimurium*. Studying transduction mediated recombination frequencies, he found that the genes associated with leucine synthesis, arabinose fermentation, and azide resistance had the order: *ara-leu-azi*. This is in agreement with the order found in *E. coli*. Furthermore he found that the homozygous presence of a large multisite mutation in the leucine cluster increased the frequency of *ara-azi* co-transduction. This would be consistent with the interpretation of the multisite mutation as a deletion. Dr. Falk also carried out extensive tests for occurrence of functional inversions of the complete tryptophan gene cluster. Although very sensitive selective techniques were used, no functional inversions were detected. While negative results do not rule out the existence of such functional inversions, the inability to detect them is certainly compatible with the notion that they are not viable, perhaps because they conflict with a polarized transcription mechanism imposed by a portion of the chromosome which is larger than a gene cluster.

Although satisfying biochemical criteria for DNA synthesis, the DNA polymerases studied so far in vitro catalyze the synthesis of DNA macromolecules with an abnormal topology, in which the information of the input templates does not seem to be replicated. With the knowledge now available on the system of replication of chromosomes in vivo, it is easy to speculate on possible shortcomings of the in vitro system. It is obvious that the fragmented DNA molecules used as templates in this system are a poor facsimile of the chromosome, and it is also conceivable that the isolated DNA polymerase is not, in fact, the real DNA replicating enzyme.

The increasing number of examples of circularity observed for microbial chromosomes, together with the singularity of the replication site in such units of replication, suggest a degree of sophistication in the mechanism of replication that has certainly not been provided for in present in vitro studies of DNA replication. Thus it seems timely for a new look at the replication problem.

To this end we are endeavoring to isolate the *E. coli* chromosome intact, with its attached DNA replicating enzyme and any other proteins which may have indispensable replication functions.

In brief, the methods currently under development aim at lysing the cells in the situation in which the chromosomes are to be examined. *Cell Lysis*: Since the chromosome is to be released from the cell with its associated proteins in a functional condition, the usual method of detergent lysis cannot be used. One method of lysis which surpasses all so far tested involves the suspension of the cells in chloroform saturated 0.01M EDTA-Tris, pH 8.5, and then lysing the cells by the addition of 1/30 part of crude T6 phage lysate. The efficacy of this lysing system varies with both the physiological state and the strain of cell.

Characterization in the CsCl gradient:

Washed H³-thymidine labelled cells are lysed above a 5% sucrose pad floating on the CsCl solution in the centrifuge tube, thus minimizing the probability of chromosome breakage. The equilibrium distribution of the labelled DNA revealed two bands; one at 1.704 gm.cm⁻³ and the other at the meniscus. The presence of the latter band suggests incomplete lysis, or the retention of a proportion of the chromosomes in persistent protoplasts. These problems and others deriving from the extreme 'stickiness' of the isolated chromosome have not yet been solved. When suitable release of the chromosomes is achieved, their degree of intactness can be checked by various methods.

**ESCHERICHIA
COLI
CHROMOSOME**
Laboratory of
J. Cairns,
C. Davern

In Vitro Replication Studies:

In vitro replication of the DNA in crude lysates will be followed by isotope labelling, both by count assay and autoradiographic assay to perceive the morphology of the in vitro replication process. Ultimately, if such an approach to in vitro DNA replication is successful, these studies should be extended to organisms such as *Bacillus subtilis*, *Pneumococcus*, or *Haemophilus influenzae*, to check for the replication of transforming activity.

STRUCTURE OF PHAGE DNA'S

Laboratory of

A. D. Hershey,

E. Burgi,

N. Ledinko,

L. I. Ingraham,

E. Goldberg,

G. Mosig

Hershey and Burgi. The DNA of phage lambda consists of a double-stranded molecule about 15 microns long that apparently has short, single-stranded ends. The two ends are complementary in structure and can be reversibly joined (by thermal treatment) to form rings and multimolecular chains. Numerous types of evidence support the stated model, which is otherwise interesting for two reasons. First, it suggests a means for the transition from a linear structure (appropriate to the transfer of DNA from phage particle to bacterium at the start of infection) to a circular one (the form which λ DNA and perhaps all DNA's replicate in cells). Second, the model provides a means by which DNA fragments can join to each other in specific ways, which is a universal requirement for genetic recombination.

The DNA of phage lambda is also exceptional in consisting of two or more long intramolecular stretches differing in base composition. Owing to this fact, it is possible to separate specific parts of the molecule from each other. In this way it was shown that left ends of the molecules cannot join to each other but only to right ends, and right ends can join only to left ends. Other types of experiment showed that several parts of the molecule can interact, after denaturation, with the DNA of the bacterial host, *Escherichia coli*.

Ledinko. Lambda DNA contains about one methyl cytosine per 250 cytosine residues. It now seems likely that all DNA's possess at least two categories of structural features: the primary base sequence, determined in the act of replication; and local modifications under metabolic control. Little is known about the second category; but the examples recently discovered clearly suggest a molecular means of speciation, of gene interaction, and of metabolic control at the supra-gene level.

Ingraham. Phage T5 DNA contains mechanically weak spots at specified intramolecular locations. Their significance remains obscure but they are important to the general question of local singularities in DNA structure that modify DNA function.

Goldberg. When a bacterial spheroplast is infected simultaneously with a T4 phage particle and a T4 DNA molecule, the latter can contribute genetic markers to the eventual phage progeny. The genetic potency of the DNA survives fragmentation and denaturation of the molecules. The phenomenon will permit new approaches to several questions of genetic interest.

Mosig. The majority of particles in a population of phage T4 contain typical, genetically complete DNA molecules. A few particles are exceptional in one of several ways. One exceptional class consists of particles containing short DNA fragments and incomplete chromosomes. Two or more of these particles infecting a single bacterium can generate complete chromosomes again. The phenomenon illustrates the literal joining of DNA fragments by which genetic recombination occurs, and also provides new tools for observing local events at the site of rejoining.

COLD SPRING HARBOR SYMPOSIUM OF QUANTITATIVE BIOLOGY

June 5th to June 11th, 1964
XXIX. HUMAN GENETICS

The symposium was the 29th in the series initiated by the Long Island Biological Association in 1933. This year, however, it was organized by the newly-formed Laboratory of Quantitative Biology. As usual, many people had a hand in designing the program; in particular, we are indebted to Drs. Bearn, Cavalli-Sforza, Eagle, McKusick, Morton, Moulusky, Pontecorvo, Steinberg, and Stern. There were 44 speakers, of whom 12 came from outside the United States. More than 150 people attended the meetings.

There are many possible ways of organizing a symposium on Human Genetics, and many facets of the subject that could be included. It was felt, however, that comprehensiveness had to be sacrificed in order to keep the meeting to such a size that the participants might discuss their work as much outside the lecture hall as inside. This has been the tradition of the meetings at Cold Spring Harbor and has contributed greatly to their success and their formative influence on the progress of biology. Therefore some aspects of human genetics were deliberately omitted. There was, for example, no discussion devoted strictly to those chromosomal defects recently found associated with certain congenital anomalies, nor was there any section dealing specifically with chromosomal behavior.

The meeting opened with an address by Dr. Dobzhansky, in which he pointed out the pre-eminence of man as a subject for certain kinds of genetic research. He went on to discuss whether it is at present possible to formulate an optimum policy that man might pursue in order to maximize his own chance of survival. This subject entails both the philosophical difficulties of applying eugenic principles to man and the rather abstract notion of "genetic load," as well as a host of imponderables. So it was not surprising that few other speakers mentioned any possible application of their findings to the management of human affairs.

The body of the symposium was divided into three sections. The first of these sections dealt with the genetics of *Human Populations*. Several speakers described genetic studies of various small groups delineated by reason of religion, as in the Amish (McKusick), or geography (Gajdusek). Such groups usually show abnormally high frequencies of certain genetic characters, whether or not associated with overt anomalies, and this is known in some instances to have been the result of random genetic drift. Even in fairly large populations, mixing may be poor (i.e., inbreeding may be common)—the reason, once again, being religious (Dronamraju), social (Goldschmidt) or a mixture of geographic and social (Cavalli-Sforza)—and some rather complex migration effects may be observed (Morton). Eventually, in large populations, selective pressures become conspicuous, as in the now classical instance of the sickle-cell trait which partially compensates for the effect of endemic malaria (Allison). Generally, there is considerable difficulty in handling the potential information on the pedigrees of very large populations (Newcombe); this problem, of programing "nets," is shared to some extent by the linguist and the "dry" neurophysiologist. Aside from matters of pedigree analysis, for which human populations are uniquely suited, there is the practical consideration of distinguishing genetic load from adverse environmental effects; for example, it would be impossible to determine how much more infant mortality could be reduced in advanced countries until that part of the infant mortality due to the genetic load had been subtracted from the whole (Sutter).

The second section of the Symposium dealt with the *Genetics of Cells in Culture*. The difficulty of demonstrating chemical mutagenesis of human cells *in vitro* (Szybalski)

is conceivably due in part to the large number of isoalleles in such cells. Work on mutant cell lines has largely been directed at determining the biochemical or genetic abnormality that accompanies certain known mutations in man (Krooth, Koler). Apart from those markers known for obvious reasons to be on the sex chromosomes, a few other markers have been located on other chromosomes by study, for example, of heteroploid cells in vitro (De Carli). No doubt, with time, more extensive analysis will be made, particularly with antigenic mutants (Klein, Bodmer), now that mammalian cells can be hybridized in vitro (Littlefield). In one respect, mammalian cells are particularly suited for a study of events rigidly ordered with respect to the cell division cycle. It is possible to obtain much more precise synchronization of cell division in animal cells than in bacteria, because animal cells duplicate their DNA during only a very limited part of their division cycle; using such synchronized cells it is possible to determine, for example, which events in the life of a cell are most sensitive to radiation (Puck). Generally, however, our understanding of genetic mechanisms, at the molecular level, must continue to come from the study of unicellular organisms. The applicability of such studies to the genetics of higher organisms was reinforced by the demonstration that even in vivo the genetic code is apparently universal (Abel).

The last section of the Symposium dealt with studies on certain *Human Proteins*. Many species of human protein have been extensively investigated—in particular, hemoglobin, the serum globulins, and glucose-6-phosphate dehydrogenase. The methods for sequence analysis have in recent years become very sophisticated (Jones), and the ease of detecting abnormalities in certain human proteins by the very slight effects they may produce (effects that would presumably not be noticed by humans in a nonhuman victim) makes these proteins a particularly rich source of point mutations and genetic rearrangements (Smithies, Kirkman). In addition, hemoglobin synthesis has been studied in detail and displays the intricacies of the control mechanisms that may accompany such complex cell functions (Zuckerkanl, Baglioni).



POST GRADUATE TRAINING COURSES IN GENETICS

Summer 1964

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 brings 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 1964, three courses were given, designed to acquaint the student with some of the techniques used in bacterial virus research, in bacterial genetics research, and in microbiology of vertebrate cells and quantitative animal virology. The courses consisted of intensive laboratory and discussion periods, as well as formal seminars.

1) BACTERIAL GENETICS: June 20th to July 10th.

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*.

INSTRUCTORS:

S. H. Goodgal, University of Pennsylvania
P. Margolin, Cold Spring Harbor Laboratory
K. Sanderson, Brookhaven National Laboratory

SEMINARS:

Richard B. Setlow, Oak Ridge National Laboratory: "The Effects of U.V. on DNA and their Relation to Error Correcting Mechanisms"
M. Demerec, Brookhaven National Laboratory: "Evolutionary Differentiation of Genetic Material"
Louis Baron, Walter Reed Medical Center: "Genetic Transfer Between Bacterial Genera"
Bruce Ames, N.I.A.D. National Institutes of Health: "The Histidin Operon"
Luigi Gorini, Department of Microbiology, Harvard Medical School: "Streptomycin, Ribosomes, and the Reading of the Genetic Code"
Ellis Englesberg, Department of Biological Sciences, University of Pittsburgh: "The L-arabinose Gene-Enzyme Complex; Evidence for a New Type of Regulator Gene"
Austin L. Taylor, Laboratory for Molecular Biology, N.I.N.D.B., National Institutes of Health: "The Chromosome Map of *Escherichia coli* K12"
Donald Helinski, Department of Biology, Princeton University: "Some Aspects of the Colinear Relationship between Gene Structure and Protein Structure"
Noboru Sueoka, Department of Biology, Princeton University: "The Chromosome of *B. subtilis*"
Alexander Tomasz, Rockefeller Institute: "The Physiological Basis of Competence in *Pneumococcus*"
Francoise Levinthal, Department of Biology, Massachusetts Institute of Technology: "Studies on the Mode of Action of Colicines"
Philip Leder, The National Institutes of Health: "The Genetic Code, Older Questions and Recent Problems"

STUDENTS:

R. K. Brethauer, University of Wisconsin
L. W. Corwin, Walter Reed Army Institute of Research
R. L. Davidson, Western Reserve University
M. A. Eisenberg, Columbia University College of Physicians and Surgeons
G. Feher, University of California
M. Freundlich, Cold Spring Harbor Laboratory
S. A. Galton, Polytechnic Institute of Brooklyn
W. J. Gartland, Princeton University
D. Givol, National Institutes of Health
G. R. Greenberg, University of Michigan
L. Hirschbein, Princeton University
C. H. O'Neal, National Institutes of Health
W. D. Phillips, Du Pont de Nemours
R. J. Poljak, Johns Hopkins School of Medicine
M. R. Rao, University of Illinois
A. M. C. Rapin, Massachusetts General Hospital
L. C. Shapiro, Albert Einstein College of Medicine
D. F. Silbert, N.I.A.M.D., National Institutes of Health
T. A. Sundararajan, Harvard University
M. Watanabe, Albert Einstein College of Medicine

2) BACTERIAL VIRUSES: July 14th to August 3d.

Preparation and assay of virus; isolation of virus-resistant bacterial mutants; resistance patterns of bacterial mutants; serological classification of viruses; kinetics of neutralization of virus by antiserum; one-step growth; estimation of intracellular virus; bursts from single cells; isolation of virus mutants; chemical induction of virus mutants; genetic recombination in phage T4 and phage T1; fine structure mapping and complementation tests with rII mutants; ultraviolet inactivation and photoreactivation; multiplicity reactivation; inactivation of genetic markers and gene function; lysogenic bacteria and temperate viruses; lysogenization and transduction.

INSTRUCTORS:

R. S. Edgar, California Institute of Technology
C. Steinberg, Oak Ridge National Laboratory

SEMINARS:

A. D. Hershey, Cold Spring Harbor Laboratory: "Circular DNA molecules"
J. Marmur, Department of Biochemistry, Albert Einstein School of Medicine: "Bacillus Bacteriophages"
N. Zinder, Rockefeller Institute: "Little Phages"
R. Greenberg, University of Michigan: "Phage-induced enzymes"
C. A. Thomas, Jr., Johns Hopkins University: "Bacteriophage DNA molecules"
G. Mosig, Cold Spring Harbor Laboratory: "Natural Varieties of T4 Genomes"
K. Paigen, Roswell Park Memorial Institute: "Host-induced modifications"
S. Champe, Purdue University: "Extra cistronic suppression"
M. Meselson, Harvard University: "Phage Recombination"
Toshio Fukasawa, National Institutes of Health: "Modification of T-even Phages"
R. S. Edgar, California Institute of Technology: "Ontogenetic Phagogeny"
A. Campbell, University of Rochester: "Aspects of Lysogeny"

STUDENTS:-

D. F. Brown, Atomic Energy of Canada, Ltd.
L. R. Brown, Tulane University
J. M. Calvo, Cold Spring Harbor Laboratory
D. E. Duggan, Oak Ridge National Laboratory
E. R. Epp, Sloan-Kettering Institute
G. Feher, University of California
E. S. Kahan, Harvard University
A. Kaji, University of Pennsylvania
H. Kaji, University of Pennsylvania
M. A. Mufson, N.I.A.I.D., National Institutes of Health
D. Nathans, Johns Hopkins University
J. F. Niblack, University of Illinois
D. H. Parma, University of California
H. H. Peter, California Institute of Technology
R. J. Poljak, Johns Hopkins University
F. M. Rottman, N.H.I., National Institutes of Health
L. C. Shapiro, Albert Einstein College of Medicine
M. Watanabe, Albert Einstein College of Medicine
T. Yamane, Princeton University

3) MICROBIOLOGY OF VERTEBRATE CELLS AND QUANTITATIVE ANIMAL VIROLOGY: August 6th to August 25th:

Preparation of primary and secondary chick and mouse embryo cell cultures; preparation of chick embryo cultures of heart fibroblast, lung, kidney, and iris epithelium; HeLa cells and L cells—growth in mass culture and in clones; isolation of clonal sublines of cells; tests for PLO in cell cultures and elimination of P.P.L.O. Karyotype analysis of cloned populations of cells. DNA and RNA synthesis in the division cycle of mammalian cells—an introduction to quantitative autoradiography. Plaque assay of Newcastle Disease Virus (NDV) on chick embryo cells. Assay and properties of rabbit pox virus. Hemagglutination assay of NDV and influenza. Quantitative neutralization with specific antiserum (anti-NDV). Animal virus growth in monolayer cell culture, effect of antimetabolites on viral growth. Histochemistry of viral infection, phenotypic mixing of NDV, genetic recombination of poxviruses, morphological transformation of cells by Rous sarcoma virus.

INSTRUCTORS:

P. I. Marcus, Albert Einstein College of Medicine
G. Sato, Brandeis University

SEMINARS:

- H. Eagle, Department of Cell Biology, Albert Einstein College of Medicine: "Biochemistry of Cultured Mammalian Cells"
- G. Sato, Department of Graduate Biochemistry, Brandeis University: "In Vitro Studies on the Mechanism of ACTH Action"
- E. Robbins, P. I. Marcus, Albert Einstein College of Medicine: "Cell Ultrastructure and the Lysosome"
- C. Rappaport, Biology Laboratories, Department of Radiology, Yale University, School of Medicine: "A New Approach to the Growth of Differentiated Mammalian Cells in Culture"
- R. Perry, Institute for Cancer Research, Johnson Research Foundation, Philadelphia, Pennsylvania: "On the site of Cellular RNA Synthesis"
- S. Levine, Virology Division, Lederle Laboratories, Pearl River, New York: "The Rous Sarcoma Virus" Part I
- C. Morgan, Department of Microbiology, College of Physicians and Surgeons, Columbia University: "Electron Microscopy of Animal Virus Development"
- P. I. Marcus, Department of Microbiology and Immunology, Albert Einstein College of Medicine: "Surface Modification in Normal and Virus-Infected Cells"
- S. Penman, Department of Biochemistry, Albert Einstein College of Medicine: "Cellular Organization and Poliovirus Growth"
- H. Rubin, Virus Lab., University of California, Berkeley: "The Rous Sarcoma Virus" Part II
- W. Joklik, Department of Cell Biology, Albert Einstein College of Medicine: "Nucleic Acid Synthesis in HeLa Cells Infected with Vaccinia Virus"
- J. Darnell, Department of Biochemistry, Albert Einstein College of Medicine: "Following Newly Formed RNA in HeLa Cells"
- G. Hirst, The Public Health Research Institute of the City of New York: "The Genetics of Animal Viruses"
- L. Siminovitch, Division of Biological Research, The Ontario Cancer Institute, Ontario, Canada: "Proliferation and Differentiation of Hemopoietic Stem Cells"
- R. M. Franklin, Department of Pathology, University of Colorado Medical Center: "Replication of RNA Viruses"

STUDENTS:

H. M. Dintzis, Johns Hopkins School of Medicine
F. Falcoz-Kelly, Albert Einstein College of Medicine
E. J. Fleissner, Rockefeller Institute
R. A. Goldsby, DuPont de Nemours
S. W. Luborsky, National Institutes of Health
H. F. Lodish, Rockefeller Institute
K. Oda, Albert Einstein College of Medicine
A. L. Rubin, New York Hospital, Cornell University
G. H. Schwartz, N.I.A.M.D., National Institutes of Health
S. C. Silverstein, University of Colorado
E. Scarano, International Laboratory of Genetics & Biology, Naples, Italy
D. W. E. Smith, N.I.A.M.D., National Institutes of Health
G. deThe, N.C.I., National Institutes of Health
R. Wu, Public Health Research Institute of the City of New York

UNDERGRADUATE RESEARCH PARTICIPATION PROGRAM

Summer 1964

For the past six summers, ten undergraduate students have been selected each summer to spend ten weeks at Cold Spring Harbor, taking part in an undergraduate research program sponsored by the National Science Foundation. With the aim of encouraging careers in science for outstanding young students, the year-round and summer staff provide unique opportunities for these young people to learn at first-hand what a career in research is like. The supervisors channel students into projects which give them the satisfaction of interpreting their own data and revealing previously unknown information. In addition, the many informal discussions with graduate students, post-doctoral fellows and senior investigators on the grounds during these months provide excellent opportunities for intellectual growth and stimulation toward productive scientific careers.

The following students participated in this program during the summer of 1964:

Rosina O. Berry, Radcliffe College
Supervisor: Dr. Robert S. Edgar
Seth N. Braunstein, Princeton University
Supervisor: Dr. Samson Gross
Eric S. Brondfield, Harvard College
Supervisor: Dr. Robert S. Edgar
Barbara E. Bund, Radcliffe College
Supervisor: Dr. Sol Goodgal
Ann M. Gunsalus, Hiram College
Supervisor: Dr. Cedric Davern
Jack Michalka,
Philad. Coll. of Pharm. & Science
Supervisor: Dr. Sol Goodgal
Ethel M. Noland, Radcliffe College
Supervisor: Dr. I. C. Gunsalus
Henry M. Smilowitz, Reed College
Supervisor: Dr. Paul Margolin
Katherine Treible, Lycoming College
Supervisor: Dr. Gisela Mosig
Paul D. Wolfowitz, Cornell University
Supervisor: Dr. Edward B. Goldberg

Title of Project

Isolation of new T-Phage types and their characterization.
Isolation of leucine auxotrophs of *B. subtilis* for transformation experiment.
Characterization of "azure" mutants of T4.
Production by mutagens of temperature-sensitive mutants of *Hemophilus influenzae*.
Production by fluorouracil of temperature-sensitive mutants of an RNA phage.
Production of a defined medium for the growth of *Hemophilus influenzae* for transformation experiments.
Transduction of camphor-resistance by a *Pseudomonas* phage.
Studies on leucine-permease mutants in *S. typhimurium*.
Studies on complementation by amber mutants of T4 phage.
Studies on the kinetics of uptake of T4 DNA by spheroplasts.



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 INVESTIGATORS — 1964

Dr. Alan Bernheimer — New York University School of Medicine
Dr. Harriet P. Bernheimer — State University of New York, Downstate Medical Center
Dr. Sidney P. Colowick — Vanderbilt University
Dr. Robert S. Edgar — California Institute of Technology
Dr. Raphael Falk — Hebrew University, Israel
Dr. Bentley Glass — Johns Hopkins University
Dr. Edward Glassman — University of North Carolina
Dr. Sol H. Goodgal — University of Pennsylvania
Dr. Sampson R. Gross — Duke University
Dr. Irwin C. Gunsalus — University of Illinois
Dr. Rollin D. Hotchkiss — Rockefeller Institute
Dr. Richard Novick — Rockefeller Institute
Dr. Frances Womack — Vanderbilt University

REPORTS OF SUMMER GUEST INVESTIGATORS

Our summer research program was centered about two primary objectives: 1) to continue our studies on the mechanisms of bacterial transformation and 2) to retest the possibility of transformation in an enteric organism such as *Salmonella*.

With Jack Michalka we continued to perfect a synthetic medium developed by Dr. Herriott's laboratory and we feel we have succeeded, now, in obtaining excellent growth of cells in liquid medium and subsequent plating of these cells in agar medium with an efficiency close to that for broth medium. Several nutritional mutants have been isolated and we know that we can obtain a very large number of these.

Barbara Bund in the meantime has isolated approximately 70 temperature-sensitive mutants of *H. influenza*; a few of these have been successfully tested as recipients in transformation, and we hope to be able to map the *H. influenza* chromosome with respect to both dispensable and nondispensable functions.

N. Notani continued his experiments on the effect of shearing on the chromosomal material of *H. influenza*, and clearly demonstrated that large DNA particles (chromosomal particles) have lower transforming efficiency than particles which have been subjected to pipette shearing of the DNA. The phenomenon is not related to disaggregation of DNA as demonstrated by its lack of concentration dependence. The same shear effects are obtained at low and high concentration of particles.

Sol Goodgal attempted some transformation experiments with *Salmonella* and found that streptomycin-resistant cells could be found after treatment with DNA, but the numbers are quite low — 10 to 30/10⁸B, compared to a background of 0-2/10⁸B; and further work is required before one can assign the phenomenon of transformation to the difference.

With Dr. E. Goldberg, experiments were initiated to obtain spheroplasts of *Salmonella* in order to test for uptake of DNA and possibly the production of transformants. It appears that spheroplasts, or at least partial spheroplasts, can be obtained which can be made to revert to normal cells.

S. H. Goodgal,
N. Notani,
J. Michalka,
B. Bund

Finally, experiments were performed with reannealed *H. influenza* transforming DNA to determine the susceptibility of Herriott's reannealed hybrids to lamb's brain enzyme, which attacks denatured DNA preferentially by an endonucleolytic process. The hybrids are susceptible to attack, but single markers are also attacked at a much slower rate. We hope to compare the activity of the enzyme on native and renatured DNA and make a definitive decision concerning the nature of the hybrids.

S. P. Colowick
and M. S. Colowick

The purpose of our work was to obtain information about the function of the gene *rII*, which is essential for the multiplication of a bacterial virus (bacteriophage T4) in certain host cells (*E. coli*, strain K carrying the prophage lambda). Since thousands of different mutants have been obtained in which this gene is defective, and since the genetic fine structure is therefore known in great detail, it would be desirable to correlate this information with knowledge of the structure of the protein whose synthesis is presumably governed by this gene.

On the basis of certain information in the literature, we have proposed that the *rII* gene may give rise to a protein (enzyme) which increases the supply of chemical energy (ATP) for virus replication. We have therefore measured ATP in the host cells before and after infection with the virus. Upon infection with viruses containing a normal *rII* gene, we have found a marked rise in ATP content of the cells within 7 minutes, and a maintenance of this high level throughout the course of the viral multiplication. On infection with viruses in which the *rII* gene is nonfunctional, there is also an initial rise in ATP content, but this is regularly followed by a marked fall in ATP to about one-half of the original level. The results therefore support the view that the *rII* gene may be involved in energy supply.

I. C. Gunsalus,
M. R. R. Rao,
John Niblack,
Glen Gunsalus

The nearly limitless array of organic carbon structures left in nature by the growth of organisms and the laboratory activity of organic chemists presents a challenging problem to those organisms which return the carbon to essential metabolites. A different but similarly challenging series of questions arise for the biologist who wishes to understand these processes. The principal agents for return of carbon compounds to CO₂ and new cellular components are unicellular and occur in soil and water; among these are Pseudomonads, Corynebacterium, and Actinomycetes types. To ask about pathways, their control and the distribution and exchange of genetic information, microbial soil isolates were selected as biological agents and a series of terpenes were chosen for structural diversity and extended catabolic pathways.

Based on variability of phenotypic characters of Pseudomonad strains in general, and those degrading terpenes in particular, we initiated studies on the genetic control in these cells. Several phages were isolated from the cells, by ultraviolet induction. A number of spontaneous changes in phenotypic characters were selected as markers, and work was undertaken to prepare mutants to examine the genetic systems of these organisms both on the intra- and inter-cellular level.

Dr. Rao prepared a series of Pseudomonad mutants from a terpene-oxidizing strain by use of the chemical mutagens, principally N-methyl-nitrosoguanidine. Isolates blocked in the oxidative steps of the terpene pathway are now being studied to identify the sites of genetic lesion. Amino acid auxotrophs and streptomycin resistant strains were also prepared. It is proposed to use these as markers for genetic experiments on the carbon pathways, to determine if the biosynthesis of histidine and tryptophan proceed by pathways similar to those shown in the enteric bacteria, and if the genetic loci are similarly clustered.

John Niblack continued with physical characterization of a series of fluorescent pseudomonad phages isolated from a terpene oxidizing strain by ultraviolet induction by A. U. Bertland. The phage Pf2 contains double-stranded DNA of about 61% GC and molecular weight of ca. 25 x 10⁶. The cell DNA of strain CI contains about 63% GC as indicated by its melting temperature and preliminary base analysis. Thus the gross composition of the cell and phage DNA are not dissimilar. A knowledge of the

biological properties of these viruses and the recognition of a transducing system within the strains employed are objectives of these experiments.

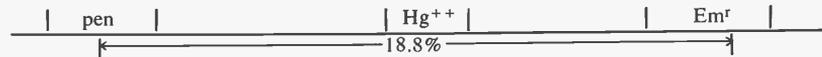
We wish to express our indebtedness to members of the permanent staff of the Cold Spring Harbor Laboratory, whose contributions to our education and experiments will be evident to those acquainted with these investigators and the spirit of the Laboratory.

(This work was supported in part by grant number AT(11-1)-903 of the Atomic Energy Commission to the University of Illinois.)

Work was continued on the penicillinase system in *Staphylococcus*, which consists of a series of different extrachromosomal elements each harbored by a different strain, but each carrying a penicillinase locus. The following new results were obtained:

1) In transductional crosses between particle α (penicillinase production, erythromycin sensitive - pen⁺, Em^s) and γ (pen⁺, Em^r) a recombination rate of 18.8% was observed between these two markers on scoring 6,000 transductants.

2) In a control cross, in which the recipient carried no penicillinase element and the donor carried γ , of 5,000 transductants 5 erythromycin resistant (Em^r) clones had deletions for the entire penicillinase locus. Of these, two had deletions also for a mercuric ion resistance locus (Hg⁺⁺) carried by the plasmid. On the basis of these data, a tentative genetic map was constructed, assuming linearity:



3) Work was continued on an "inhospitable" mutant, 258-94, of host strain 258. It was found that although the mutant was inhospitable toward the γ plasmid (i.e., the latter was maintained by this host in a highly unstable state, being lost with a high frequency), it manifested no inhospitality toward the β plasmid, which was maintained stably.

In connecting this latter finding with another new finding, namely that β and γ plasmids can form a stable diploid within the same (nonmutant) host, a unifying hypothesis was proposed. This is that there may be two separate binding sites within the host cell. One of these accepts specifically particles such as α and γ which are mutually exclusive in any given host and the other accepts particles such as β which can coexist in a diploid state in any given host, either with α or with γ . There would be, further, a compatibility locus on each particle which would bind specifically to the appropriate host site. Thus, it would be considered that in the mutant, 258-94, the binding site for the γ plasmid is altered while that for β is unaffected.

The genetic and physiological properties of a number of "azure" (*az*) mutants, isolated at Caltech, were studied. Unlike amber mutants, which grow on *E. coli* strain Cr but not B, the azure mutants grow on B but not Cr. Azure mutants fail to grow in most K12 strains and BB. The phage adsorb to and kill the restrictive host but no progeny are produced.

Recombination and complementation tests on eight independently isolated *az* mutants show that all are in the same cistron at four separable sites, the most distant showing about 5% recombination. About half of the mutants exhibit an *r* (rapid lysis) phenotype. Crosses to wild type showed that this phenotype was due to a second mutation distantly linked to the *az* mutation. The *az* gene is probably linked (~10%) to rII.

DNA synthesis in restrictive host cells infected with an *az* mutant was followed by incorporation of tritiated thymidine. It was found that normal levels of DNA are manufactured in the infected cells although aberrant kinetics of DNA synthesis are not ruled out. Infected cells lyse.

Thus *az* mutations, unlike amber mutations, are restricted to one gene whose function apparently is essential only in K12 strains but not in B strains. The *az* mutations are different both phenotypically and genotypically from the rII mutants which also show a comparable host-dependent conditionally-lethal gene function.

Richard Novick,
Prem Notani

E. S. Brondfield
and R. S. Edgar

Rosina O. Berry
and
R. S. Edgar

Preliminary to a comparative study of T-even-like bacteriophage, 61 phages active on *E. coli* strain B/5,1 were isolated from six different sewer treatment plants on Long Island. These phages were tested for homology with T-even phage by measurement of neutralization with anti-T4 and anti-T2 antiserum. Of the 61 phage, 34 were inactivated by one or both antisera, 27 were not. Of the 27 apparently non-T-even phage only three formed large plaques characteristic of T1, T3, or T7. These three were not detectably neutralized by anti-T7 antiserum.

In an attempt to demonstrate that all isolates were in some respect different from each other and thus of independent origin, mutant strains of B/5,1 resistant to 23 of the phage were isolated. All the phages were then tested for growth on these and seven other strains of bacteria. On the basis of these tests the 34 T-even-like phages were subdivisible into a minimum of 19 groups, the 27 non-T-even phages in 15 groups. In addition, the 23 resistant strains of bacteria could be separated into 14 nonidentical groups.

J. King
(Edgar group)

In the course of a search for amber mutants of T4D, a mutant was isolated which could no longer adsorb to *E. coli* strain B. Its host range is severely restricted, CR63 being the only host among various B and K strains tested to which it can adsorb. No revertants among 10^9 phage tested on *E. Coli* B were found, but on K strains revertants appeared. These were partial revertants, their host range being extended to all normal K strains, but still unable to plate on B strains. The partial revertants yielded full revertants when plated on B. When backcrossed with wild type these full revertants yield no detectable ($< 0.1\%$) mutant phenotypes, suggesting that they are true wild type revertants.

These facts suggest that there are two mutations responsible for the host range phenotype. Preliminary crosses place one of them in or closely linked to gene 37, the other in or closely linked to gene 34. Only one of the pair has been isolated as a single mutant, linked to gene 37. Genes 34 and 37 both control the synthesis and assembly of tail fiber components.

The adsorption of both the double mutant and the derived single mutant is temperature-sensitive. At temperatures below 25°C and above 35°C the rate of adsorption is a few per cent of wild type. The mutant phage particles themselves are heat-sensitive, both double mutant and single mutant being inactivated considerably faster than wild type at 45°C and 53°C .

Alan W.
Bernheimer,
Lois L. Schwartz

Protozoan cells, in contrast to mammalian cells, appear to be resistant to the action of several bacterial toxins of which staphylococcal alpha toxin is an example. The question arises whether insensitivity is to be explained (a) by a fundamental difference between protozoan and mammalian cells, that is, absence of toxin receptors in the former; or (b) by physical inaccessibility of receptors of toxin. The ciliates, Tetrahymena, were mechanically crushed between slides and cover-glasses, in the presence and absence of staphylococcal alpha toxin, and rate of deterioration of subcellular structures as macronucleus, micronucleus, mitochondria, and unidentified cytoplasmic granules, was observed by phase-contrast microscopy. No differences in rate of deterioration was found but more extensive observations are needed. A study has also been initiated on the possible effects of certain bacterial toxins on viruses.

Noncapsulated mutant strains of pneumococci derived from capsulated Type III cells give rise to two classes of capsular transformants when exposed to DNA from wild Type I pneumococci. These two classes are: 1) Type I cells which arise from an *exchange* of the capsular genome in the donor DNA for that in the recipient cell and 2) Type I-III cells which are partial diploids and arise from the *addition* of the capsular genome in the donor DNA to the recipient genome. Whether cells will be transformed to Type I or to Type I-III is not determined by the recipient cell but depends upon heterogeneity in the donor DNA fragments. Fractionation on methylated albumin columns of DNA from wild Type I cells was carried out in collaboration with Mr. Edmund Whang. In preliminary experiments, it was found that only those fragments of DNA giving rise to I-III pneumococci were recovered on elution from the column.

If the DNA from I-III cells is used as donor in various transformation reactions, cells which are phenotypically Type I may be recovered. These cells are also partial diploids but are not expressing a second capsule. The DNA-carrying Type I markers in such cells will only give the "addition" reaction in transformations with noncapsulated mutants derived from several different types of pneumococcus and will, therefore, transform noncapsulated mutants of Type III cells only to I-III.

In collaboration with Mr. Michael Erdos, an investigation was begun of the heat stability of the DNA from "atypical" Type I strains, mentioned above, and of the DNA from wild Type I strains. All donor strains carried a streptomycin resistance marker. In criti-heating experiments, it was found that the capsular markers from an "atypical" I strain were inactivated at a temperature two degrees lower than was the streptomycin resistance marker. In studying the effect of heating such DNA at sub-critical temperatures, it was found that only 0.3% of activity of the capsular markers was recovered after heating for 1 hr., and none was recovered after 2 and 3 hrs. heating. This is in sharp contrast to the streptomycin resistance marker in the same DNA preparation which still retained 75%, 51% and 27% activity after 1, 2, and 3 hrs. of heating.

PSEUDOMONAS WORKSHOP

Mechanisms and Control of Variability

A workshop devoted to the biology and chemistry of the Pseudomonads and their use as experimental material for solution of general biological problems was convened at Cold Spring Harbor, July 23rd to 25th, 1964.

Thirty-three investigators were in attendance representing those laboratories, in the United States and abroad, active in research with one or more of the Pseudomonad cell types or related organisms. The time and the place of this meeting were chosen to coincide with the Sixth International Congress of Biochemistry in New York City, July 26th to August 1st. Organized by Drs. I. C. Gunsalus and R. Y. Stanier, the meetings were attended by the following investigators:

BAUM, ROBERT H., Dept. of Chemistry, State University College of Forestry, Syracuse University, Syracuse, New York

BERTLAND, II, A. U., Biochemistry Division, University of Illinois, Urbana, Ill.

DAGLEY, S., Biochemistry Division, Dept. of Chemistry, University of Illinois, Urbana, Illinois

GRONLUND, AUDREY, 1222 Woodhead Road, Richmond, B. C., Canada

GUNSALUS, I. C., Dept. of Chemistry, University of Illinois, Urbana, Ill.

HARPRING, LINDA L., Biochemistry Division, Univ. of Illinois, Urbana, Ill.

HEDEGAARD, JENS, 36 Avenue Niel, College de France, Biochimie Generale et Comparee, Paris 17, France

HEGEMAN, GEORGE, 2321 Blake Street, Berkeley 4, California

HOSOKAWA, KEIICHI, Dept. of Bacteriology, University of California, Berkeley 4, California

HU, A. S. L., Dept. of Biochemistry, University of Kentucky, Lexington, Ky.

HUGH, RUDOLPH, The George Washington University, School of Medicine, Dept. of Microbiology, 1339 H. Street, N. W., Washington, D. C. 20005

JACOBSON, LEWIS A., Biochemistry Division, University of Illinois, Urbana, Ill.

JAKOBY, WILLIAM B., National Institutes of Health, Bethesda 14, Maryland

KALLIO, R. E., Dept. of Microbiology, University of Iowa, Iowa City, Iowa

KORNBERG, HANS LEO, Department of Biochemistry, University of Leicester, Leicester, England.

LEADBETTER, E. R., Biology Dept., Amherst College, Amherst, Mass.

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ORNSTON, L. N., Apt. 8, 2338 Blake Street, Berkeley 4, California

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PRAIRIE, BARBARA, Dept. of Biochemistry, University of Illinois, Urbana, Ill.

PRAIRIE RICHARD R., Dept. of Biochemistry, University of Illinois, Urbana, Ill.

QUAYLE, J. R., Dept. of Biochemistry, University of Sheffield, England

RAO, R. R., Biochemistry Division, University of Illinois, Urbana, Ill.

RITTENBERG, S. C., Dept of Bacteriology, University of California, Los Angeles 24, California

ROWDELL, VICTOR W., Dept. of Biochemistry, University of California, San Francisco Medical Center, San Francisco 22, California

SENEZ, JACQUES C., Laboratoire de Chimie Bacterienne C.N.R.S., 31 Chemin Joseph Aiguier, Marseille (9), France

SHUSTER, C. W., Department of Microbiology, Western Reserve University, Cleveland, Ohio

SNELL, ESMOND E., Dept. of Biochemistry, University of California, Berkeley, California

STADTMAN, E. R., National Heart Institute, National Institutes of Health, Bethesda, Maryland

STANIER, R. Y., Dept. of Bacteriology, University of California, Berkeley 4, California

TEAS, HOWARD, National Science Foundation, Washington 25, D. C.

TRUDGILL, P. W., Biochemistry Division, University of Illinois, Urbana, Ill.

PHAGE CONFERENCE

August 28-31, 1964

Following the completion of the summer program, the auditorium and housing facilities here were utilized for the annual phage conference, a four-day meeting attended by most of the active phage workers in the country. Fifty-eight papers were presented in the program listed below.

Friday — Chairman, Cyrus Levinthal

- Myron Levine and Hamilton O. Smith: Sequential repression of DNA synthesis in the establishment of lysogeny
Hamilton O. Smith and Myron Levine: The synthesis of phage and bacterial DNA in the establishment of lysogeny
Peggy Lieb: Studies of λ immunity substance using heat-inducible mutants
David Korn: Interference with bacteriophage DNA replication by inducing levels of Mitomycin C
Mark Ptashne: The detachment of a conserved λ prophage
Herbert Wiesmeyer: Prophage derepression following superinfection by heterologous phages
I. Takahashi: Lysogeny in *Bacillus subtilis*
Naomi Franklin: Temperate phage ϕ 80 and its *tryp* transducing ability
Dorothy Fraser: Growth of λ phage
June Rothman: Transduction mapping of the *gal-biotin* region and lambdaoid prophages

Friday — Chairman, Frank Lanni

- David Freifelder: Mechanism of inactivation of bacteriophage by X-rays
Nils A. Barricelli: A radiation genetic method to test whether one or both injected viral DNA strands duplicate
Frances C. Womack: Radiation genetic experiments with phage T4
Richard P. Novick: Genetics of penicillinase production in *Staphylococcus aureus*
N. L. Couse, M. M. Piechowski, and Millard Susman: Production of phages in the presence of acridine dyes
William V. Howes: The kinetics of attachment and injection of the lambda phages
Dwight Hall and Irwin Tessman: BU incorporation into thymine-requiring T4

Saturday — Chairman, Franklin W. Stahl

- E. Shahn and A. W. Kozinski: Further research on the fate of parental DNA of UV-inactivated phage under the conditions of rescue
Edward Goldberg: Assay of genes in T4 DNA
L. A. MacHattie: Circular T2 DNA molecules
Gisela Mosig: Variation among T4 DNA molecules
Hillard Berger: Deletion effects on recombination and heterozygosity in T4
George Streisinger: Nonrecombinant heterozygotes in phage T4
Jon Weil and Betty Terzaghi: Heterozygotes in phage T4
Edward Simon: A mechanism of recombination in T4
W. D. Fattig and Frank Lanni: The genetic map of T5
Eduardo Orias: Genetics of T7
Irwin Rubenstein: The physical properties of heat-sensitive T5 bacteriophage

Saturday — Chairman, Charles A. Thomas

- Kenneth Paigen: Cooperative infection with host-controlled lambda phage
Stanley Hattman: The functioning of T*-phage in restricting hosts
Richard Russell: Genetic studies on partial exclusion of T2 by T4
Irwin Tessman: Anti-suppression
Rebecca Hill: Segregation of genetic factors determining transmission of T4 amber mutants by *E. coli* K12 strains
M. G. Smith and K. Burton: Fractionation of DNA from phage-infected bacteria
Dean B. Cowie: Lysogeny and DNA homology

Sunday — Chairman, Seymour Cohen

- D. M. Green: The number and size of DNA molecules extracted from SP82 bacteriophage that are required for DNA infection of competent *Bacillus subtilis* determined by DNA infection alone and by marker rescue of DNA-infected cells
- Roy P. Mackal: Some aspects of the formation of λ virus by DNA in disrupted cell preparations
- K. Berns: Isolation of DNA of very high molecular weight
- F. William Studier: Sedimentation properties of phage DNA
- C. A. Thomas: Thermal chromatography of DNA
- David Freifelder and A. K. Kleinschmidt: Electron microscopy of single-stranded DNA
- Fred Frankel: A chromosomal nucleoprotein complex during the bacterial replication cycle
- Fred Frankel: T2 vegetative DNA as a nucleoprotein complex
- Z. Opara-Kubinska, H. Kubinski, and W. Szybalski: Interaction between denatured DNA, polyribonucleotides, and ribosomal RNA; attempts at preparative separation of the complementary DNA strands

Sunday — Chairman, George Streisinger

- R. M. Franklin, M. L. Fenwick, and R. L. Erickson: Double- and triple-stranded replicative forms of an RNA phage
- R. L. Erikson, M. L. Fenwick, and R. M. Franklin: Replication of R17 phage RNA: role of ribosomes and fate of incoming RNA strand
- Barbara Brownstein: The effects of streptomycin on the replication of RNA phage R-17, particularly the synthesis of viral RNA and some implications concerning the kinetics of phage protein synthesis
- Harvey F. Lodish and Norton D. Zinder: Early events in the replication of the RNA-containing bacteriophage f2
- Dean Fraser and Henry Mahler: Looking for small, two-stranded phage
- David Pratt and William Salivar: Growth and phage production by cells infected with the rod-shaped coliphage M13

Monday — Chairman, Sewell P. Champe

- Raymond Kaempfer: Accelerated breakdown of β -galactosidase-forming capacity following infection with T-even bacteriophage: a phage function suppressible by the F episome
- Tamiko K. Sueoka: A modification of leucyl-sRNA of *E. coli* B by T2 infection
- Cyrus Levinthal and Junko Hosoda: Studies on phage protein synthesis with polyacrylamide-gels
- R. S. Edgar: Genetic control of tail-fiber antigens in T4
- S. P. Colowick and M. S. Colowick: ATP levels after infection with *rII*
- Channa Shalitin: The ionic control of the T4 "clock"
- Gordon Edlin: A model for gene regulation by T4
- Ronald Rolfe: On the polarity for translation of the messenger for the head protein of bacteriophage T4D

NATURE STUDY COURSES

Children of Ages 6 to 14

During the Summer of 1964, fourteen sections of nine courses in Nature Study were conducted in two monthly sessions. The enrollment this year was 300 students. The course offerings included:

General Nature Study (ages 6, 7)	Entomology (10, 11)
General Ecology (8, 9)	Vertebrate Zoology (10, 11)
Earth Science (8, 9)	Seashore Life (10, 11)
Botany-Entomology (9, 10)	Advanced Ecology (12-14)
Fresh Water Biology (10, 11)	

INSTRUCTORS:

Mr. Marvin J. Rosenberg, Ass't. Prof. Biology and Education, State University of New York at Stony Brook, L. I.

Mr. Otto Heck, Biology Teacher, Island Trees High School, Levittown, N. Y.

Mrs. Barbara Sheehan Church, Science Teacher, Bellmore Schools, Bellmore, N. Y.

In addition to the instructors, each class had an assistant to help on the field trips and in laboratory work.

Several evening film showings were included in the courses, to which parents were invited.

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

The ninth annual Workshop in Nature Study was offered from June 29th to July 10th, 1964. 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. There were 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.

Upon satisfactory completion of the requirements of the course, teachers were entitled to two in-service credits awarded by the New York State Department of Education. Instructors were Marvin Rosenberg and Otto Heck. Special lectures and guided trips were conducted by Barbara S. Church.

**NATURE
STUDY
WORKSHOP
FOR TEACHERS**

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December, 1964

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Cedric I. Davern
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FINANCIAL REPORT

For the period May 1, 1963 — April 30, 1964

As of April 30, 1964 our assets were as follows:

Cash	\$105,102.69	
Accounts receivable	25,801.77	
Inventory of books	9,244.40	
Prepaid expenses	3,348.04	
Investments — U. S. Government Bond (market value \$98.88)	99.75	
Land, buildings and equipment	501,652.03	
Total		\$645,248.68

Our liabilities were as follows:

Accounts payable and taxes	13,797.54	
Grants and contracts, unexpended	72,664.42	
Deferred income	4,667.50	
Dr. William J. Matheson bequest	20,000.00	
Net worth	534,119.22	
Total		\$645,248.68

For the year 1963-64, our receipts were as follows:

Grants, contracts, research fees (exclusive of the Genetics Research Unit of the Carnegie Institution of Washington)	\$280,673.82	
Contributions:		
Carnegie Institution of Washington	30,812.50	
Long Island Biological Association	9,555.00	
Wavex Society	3,200.00	
Other	1,495.00	
Sale of Books (Symposium)	89,767.48	
Operating receipts (rentals, dining hall, etc.)	75,178.31	
Total		\$490,682.11

Our expenditures were as follows:

Research and educational programs	\$241,168.54	
Administration	46,262.67	
Plant operation and maintenance	64,667.87	
Publishing (book production & sales)	43,767.11	
Dining hall, rooms and apartments, etc.	61,803.95	
Total		\$457,670.14

Excess of Income over Expenditures 1963-1964 \$33,011.97

LABORATORY SUPPORT

As in previous years, the largest part of the support for the Laboratory has come from Federal grants. As a result of these grants, and a grant from the Rockefeller Foundation, the entire scientific staff now receive their salaries from outside sources. A list of all grants and contracts is given later in this report.

General support for the operation of the Laboratory has come, this year, from the Long Island Biological Association (\$9,555 in the fiscal year ending April 1964); from the Carnegie Institution of Washington (\$30,812.50); from the Hoyt Foundation (\$5,000); and from the Foundation for Microbiology through Dr. Selman A. Waksman (\$15,000). To all these groups we extend our gratitude for their support and encouragement. In addition, as mentioned briefly in the introduction, the National Science Foundation provided a grant of \$45,600 to pay for the cost of a survey of the buildings and grounds and the repair of the sewage disposal plant.

Totally, these various forms of support are insufficient to cover the proper operation of the Laboratory and the maintenance of its buildings and grounds. Last year, our Annual Report showed that, subtracting obligated funds from our cash balance, we had no unobligated cash reserve at all. During the last year we have achieved an excess of income over expense to the amount of about 7%; this has been accomplished by some neglect of maintenance, but has been essential in order to create a healthy cash position. To meet the real deficit, the Trustees of the Laboratory have, in the past few months, embarked on a campaign to raise money from industrial organizations and from the many scientists who have, at some time or other, benefited from the Laboratory's various programs. The campaign has not been going long enough to have produced a very large yield. Those scientists who have agreed to contribute \$50 per annum are listed separately as "Friends" of the Cold Spring Harbor Laboratory. Those organizations which have agreed to assist us are listed as "Sponsors" of the Laboratory.

**GRANTS
AND
CONTRACTS**
May 1, 1963
through
April 30, 1964

Grantor	Grant Number	Investigator	Award
	RESEARCH GRANTS		
National Science Foundation	GB-1690*	Dr. Cairns	\$116,400.00
National Institutes of Health	1-K3-GM-6887-01	Dr. Margolin	18,818.00
National Institutes of Health	GM-07178-05	Dr. Margolin	47,637.00
National Institutes of Health	GM-07675-04	Dr. Umbarger	19,662.00
National Institutes of Health	AI-03501-05	Dr. Umbarger	8,164.00
The Rockefeller Institute	RF-63042	Dr. Cairns	17,000.00
Ass'n. for The Aid of Crippled Children		Dr. Umbarger	6,500.00
Nat'l. Ass'n. for Retarded Children		Dr. Umbarger	6,500.00
	TRAINING GRANTS		
National Institutes of Health	GM-890-08	Summer Courses	33,144.00
National Science Foundation	GE-4192	Undergraduate Research Participation	14,000.00
	SYMPOSIUM GRANTS		
Atomic Energy Commission	AT(49-9)-2544		7,000.00
Air Force Office of Scientific Research	AF-AFOSR-426-64		5,000.00
National Institutes of Health	CA-02809-09		12,050.00
National Science Foundation	GB-2255		3,500.00
	SPECIAL GRANTS		
National Science Foundation	GB-2266	Site Survey and Repair of Sewage Disposal System	45,600.00
		Total	\$360,975.00

*This grant covers a two-year period.

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