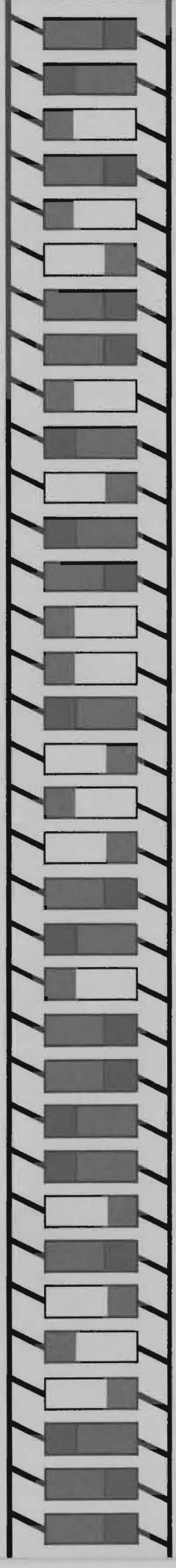
Annual Report of the Biology Division

1966

Southwest Center for Advanced Studies
(Formerly Graduate Research Center of the Southwest)



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Printed in the United States of America

History of the Southwest Center for Advanced Studies

The pioneer society of the Southwest played an important role in the developmental history of the United States. Emergence of the region into the highly technological culture of the twentieth century, however, has not been uniform, and in some respects the Southwest still lags behind other regions. It is clear that rapid cultural, economic, and industrial development is essential to the future health of the region, which in turn is bound up with the future of the entire nation. In the last analysis, such development springs from the educational foundation that the region provides. Undergraduate education in the Southwest is relatively good, but there is a need for more modern and extensive education at the graduate and postdoctoral levels. Regional universities have recognized this problem, but have encountered serious difficulties in attempting to solve it.

A new approach was provided by a group of Dallas citizens, headed by J. Erik Jonsson (the present mayor of Dallas), Cecil H. Green, and Eugene McDermott. They established the Southwest Center for Advanced Studies (originally chartered under the name Graduate Research Center of the Southwest) on 14 February 1961. By providing for the recruitment of many workers in related areas within a short period of time, and by permitting them to devote a major part of their time to research, it was hoped that a large number of scientists, active in modern research, could be assembled in one institution, which would then provide a nucleus for the activation of superior and extensive regional programs in higher education.

This experiment appears to have succeeded in its initial phase. The Center's objective in the first five years was to assemble a faculty of quality and to provide it with the opportunity to launch a variety of

President Gifford K. Johnson chats with Dr. Harris

President's Message

It is a pleasure for me to present the first annual report of the Biology Division. This newest addition to our academic family has grown rapidly since its inception about two years ago, and is now a vigorous research group.

The Division was purposely organized with primary strength in the area of the molecular biology of microorganisms. This should provide a firm foundation for future diversification into systems of greater biological complexity.

Emphasis to date has been on establishment and organization of the research functions of the Biology Division. We expect that the near future will see more extensive educational activity.

Gifford K. Johnson
President

modern research activities. During this founding phase, interaction with regional institutions was not a principal focus.

Dr. Lloyd V. Berkner, first President of the Center, and former President of Associated Universities, Inc., oversaw the establishment of the first research divisions in Atmospheric and Space Sciences, Geosciences, Mathematics and Mathematical Physics, and most recently Biology. In March, 1965, Gifford K. Johnson became President of the Center and Dr. Berkner is presently Chairman of the Board of Trustees. As of 1 June 1966, the Center employed 344 persons, including 77 members of the faculty and scientific staff, and 158 engineers, technicians, and students.

The Founders Building was the first building of the Center campus. This $3.5 million structure, of some 65,000 net square feet, was built with the private resources of the founders, according to a design developed with funds provided by the Ford Foundation. It is a versatile, functional, economical, and attractive science laboratory. Two other smaller structures have now been added to the campus.

Private support of the Center has been provided by local citizens and industries under the guidance of Stanley Marcus (President of Neiman-Marcus), Chairman of the Committee on Resources. This support since founding of the Center approximates $15 million. In addition, in the present fiscal year, the Center is carrying out approximately $4.5 million of research sponsored partly by private, but mostly by federal, research organizations, including NASA, NSF, DOD, NIH, and AEC.

* * * * *

The Center has adopted the following main planning objectives for the next five-year period:

a. To cooperate with academic institutions of the Southwest in advancing education and research towards a level comparable to that of leading academic regions of the nation.

b. To continue development of the Center as a basic research institution.

c. To continue development of the Center as a regional postdoctoral education institution.

d. To promote, adjacent to the Center campus, the development of scientific research and education facilities, serving either specific universities and colleges or specific Government agencies.

A significant step in the second five-year program has already been taken. On 2 August 1965, The Association for Graduate Education and Research (TAGER) was chartered by three private educational and research institutions, namely Southern Methodist University, Texas Christian University, and the Southwest Center for Advanced Studies. Four other academic institutions in the area, Bishop College, Austin College, the University of Dallas, and Texas Wesleyan College have also joined TAGER. The Association's purpose is to help affiliated institutions meet regional and national needs for more and better scientists, engineers, and scholars by pooling their existing resources, as well as by developing new resources and new approaches.

Development of the Biology Division

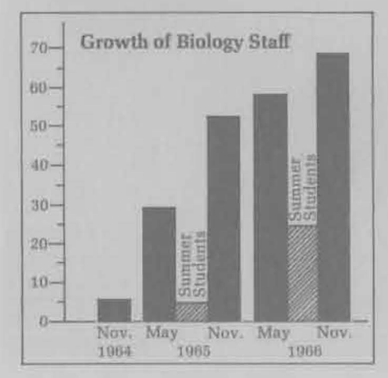
The Board of Governors of the Southwest Center for Advanced Studies in 1963 authorized initiation of a faculty in biology. An advisory committee, consisting of Drs. F. Seitz, J. Lederberg, J. Tukey, and S. Warren, was established, and Dr. Lauriston C. Marshall, Professor of Physics, made the first contacts to candidates in that year. The challenge of a major experiment in education involving the rapid development of a large faculty in molecular biology, a modern and exciting scientific field hitherto barely represented in the Southwest, resulted in some early and important acceptances.

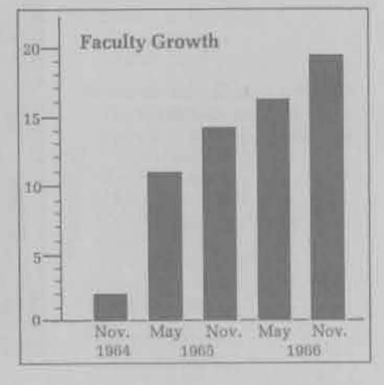
Carsten Bresch, Head of Biology, shows a model of a gene in a seminar for Center guests

In late 1964, Dr. Carsten Bresch, Head of the Division, began to assemble the present faculty. The Founders Building was completed in November 1964, in time to accept the first biology faculty members, and by spring 1965 the first laboratories were functioning. Since then the biology group has shown a steady growth. The diagrams below show the initial phase of rapid growth followed by a period of slower increase.

Dr. Bresch planned to concentrate the research efforts of the new division on problems concerning the structure of genetic material (nucleic acid, chromosomes) and the functional regulation of these genetic entities. It was expected that this program would later broaden to include studies on differentiation. However, it was also necessary, especially in view of the future educational role of the biology faculty, to cover as wide a range of modern biological subjects as possible. Accordingly, faculty was recruited whose interests and activities involved studies with animal and bacterial viruses, bacteria, fungi, protozoa, and human tissue cells.

The backgrounds of the faculty are almost equally divided among the disciplines of biology, chemistry, and physics. The faculty come from active scientific centers, including the Universities of California (Berkeley), Wisconsin, Chicago, Johns Hopkins, Berlin, Cologne, Frankfurt, Tübingen, Tokyo, and Brazil, as well as the laboratories of Cold Spring Harbor and Oak Ridge and of the British Medical Research Council. Some of the foreign scientists will later return to their homelands,





Reading room of library (Dr. Jagger)

but others will stay. Two of our German colleagues have recently received five-year Career Development Awards.

The Biology Division now occupies about 18,000 net square feet in the Founders Building. Its facilities include a media kitchen, a workshop, a dark room, a chromatography room, an isotope counting room, a cold room, an analytical-centrifuge room, constant-temperature rooms, and small animal quarters. Equipment includes an electron-microscopy facility, a high intensity X-ray machine, an ultraviolet monochromator, 9 spectrophotometers, an electrophoretic separator, a Coulter particle counter, 5 Spinco centrifuges (including one analytical), 4 radioactive isotope counters (planchet, strip, and scintillation), and a fermentor.

Our Natural Sciences Library is small, but modern and growing rapidly, having presently 3700 books and subscribing to 375 periodicals, of which about 140 are in the biological area. It is effectively supplemented by an inter-library service program which permits rapid exchange of the holdings of 10 academic institutions in the Dallas-Fort Worth area.

Our educational activities are now getting underway. At present 12 postdoctorals are members of our staff, 6 predoctoral students are working on their theses, and the number of summer students (undergraduate research participants) grew from 4 in 1965 to 23 in 1966. In the spring of 1965, the first biology course (Introduction to Molecular Biology) was given to students of Texas Christian and Southern Methodist Universities on our campus by our faculty. Three of the faculty gave a course in General Biophysics in the fall semester at Texas Christian University. One member of our academic staff holds a joint appointment as Assistant Professor at Austin College, where he teaches genetics. Our research seminars provide additional stimulus to interested faculty and students of neighboring institutions. (See page 24.)

In summary, although we have barely started work on our ambitious educational and research objectives, we have nevertheless made a vigorous beginning, having accomplished the assembly of an enthusiastic faculty, equipped with modern facilities and working in a pleasant environment.

Dr. Lang explains operation of the electron microscope to students

Individual Research Reports

R. H. BAUERLE, D. D. SMITH

Mechanism of Gene Expression and its Regulation (from June, 1966)

Current work involves a study of the nature and properties of the enzyme complex which catalyzes the two initial reactions of tryptophan biosynthesis in Salmonella typhimurium and continues studies already reported (Bauerle and Margolin, 1966 a,b; Margolin and Bauerle, 1966). It has been found that the complex apparently consists of two dissimilar subunits, components I and II, which are coded by the first two, operator-proximal genes of the trp operon, trpA and trpB respectively. The first reaction mediated by anthranilate synthetase (ASase), involves the conversion of chorismic acid to anthranilic acid requiring glutamine and Mg^{++} at ph 7.0. This reaction is catalyzed by the component I-component II complex and is strongly sensitive to allosteric (feedback) inhibition by the end product of the pathway, L-tryptophan. The second reaction, phosphoribosyl transferase (PRTase), involves the coupling of a phosphoribosyl moiety to anthranilate, and may be catalyzed by either the component I -component II complex or by uncomplexed component II. Significantly, the PRTase reaction is also inhibited by L-tryptophan, but only when carried out by the complex. This suggests that the ASase reaction and allosteric control by tryptophan is accomplished by sites on component I. However, union of component I and component II is required for activation of component I in the ASase reaction and simultaneously renders component II sensitive to tryptophan inhibition.

This conclusion has been supported experimentally since it has been found that under the proper conditions in vitro the ASase reaction can be catalyzed by uncomplexed component I. These conditions involve the substitution of ammonium ions for glutamine and a shift of the pH to 9.0. This reaction is also quite sensitive to tryptophan inhibition.

Preliminary steps in the purification of components I and II and of the complex have proved promising. Separate purification of uncomplexed components I and II, using as source material the proper trp mutants, by G200 Sephadex fractionation, has been accomplished.

Bauerle, R.H. and P. Margolin, Proc. Natl. Acad. Sci. 56, 111 (1966a).
Bauerle, R.H. and P. Margolin, Cold Spr. Harb. Symp. Quant. Biol. 31 (in press, 1966b).
Margolin, P. and R.H. Bauerle, Cold Spr. Harb. Symp. Quant. Biol. 31 (in press, 1966).

Biology faculty session

Academic Staff of Biology Division

1966

Professors
Carsten Bresch
Royston C. Clowes
Walter Harm
Daniel L. Harris
John Jagger
Claud S. Rupert

Associate Professors
Hans Bremer
Rudolf L. Hausmann
Wolfram Heumann
Dimitrij Lang
Harold Werbin

Assistant Professors
Ronald H. Bauerle
Myer L. Coval
Herbert Gutz
Klaus Haefner
Klaus Heckmann
Edwin B. Horowitz
Winfrid Krone
Michael H. Patrick

Research Associates
Horst Brunschede
Hermann Bujard
I. C. Felkner
Beatriz Gomez
Hideo Hirokawa
Adelaide C. Macfarren
Karl Mueller
Christine E. Smith
Derald D. Smith
Hiraku Takebe

Visiting Professor
A. D. McLaren

Cold room

H. BREMER, K. MUELLER

Mechanism of RNA Synthesis by RNA Polymerase (from March, 1966)

Current work on the mechanism of RNA synthesis in E. coli by the enzyme, DNA-dependent RNA polymerase, concentrates on 1) detection of nascent RNA synthesized in vivo in the complex of bacterial DNA and RNA polymerase which is present in crude bacterial extracts; 2) rationalization and improvement of the current methods for purification of RNA polymerase.

1. Nascent RNA synthesized in vivo.

During the synthesis of RNA on a DNA template, a complex must be formed consisting of the DNA, RNA polymerase, and nascent RNA Such complexes have been found in the in vitro RNA synthesis reaction (Bremer and Konrad 1964) but not so far in vivo. Its discovery may allow the determination of the number of polymerase molecules active in the cell under different physiological conditions and may also be regarded as an initial step in the isolation of the hypothetical larger complex of DNA, RNA polymerase, nascent RNA, ribosomes and nascent protein. The experimental procedure firstly requires preparation of crude extracts from E. coli grown under widely different physiological conditions. This is followed by a short incubation of the extracts with radioactive substrates for RNA synthesis and finally an analysis of the labelled products by sedimentation studies and by electrophoresis after hydrolysis. In vitro continuation of RNA chains initiated in vivo is recognized by both the more rapid sedimentation of the RNA and the absence of radioactivity in its initial nucleotides. Preliminary results obtained suggest that most polymerase molecules continue the in vitro synthesis of nascent RNA chains initiated in vivo.

2. Improvement of RNA polymerase purification.

Previous experiments indicated a heterogeneity of purified polymerase molecules (Bremer, et al., 1966 a, b), suggesting that the cell may employ different kinds of polymerases for the synthesis of different species of RNA. During purification, considerable losses of polymerase activity occur, some of them not easily detected since, when the activity is first measured in the crude extracts, the conditions and templates are different from those in the later assays; it is thus possible that special polymerases are selectively purified. In order to obtain a preparation which may be more representative of the population of polymerase molecules in the cell, attempts are being made to reduce the losses during purification. For this purpose the yield of polymerase activity (DNA-dependent and DNA-independent) is being studied after various treatments and fractionation procedures of crude bacterial extracts. The results so far indicate the existence of two classes of polymerase activity, one liberated from the bacterial DNA by various methods and then being easily inactivated (by RNA?); another part remaining attached to DNA (perhaps containing nascent RNA chains?) and being relatively stable. A method to liberate these DNA-bound polymerase molecules is now being sought.

Bremer, H. and M.W. Konrad, Proc. Natl. Acad. Sci. 51, 801 (1964).
Bremer, H., C. Yegian, and M. W. Konrad, J. Mol. Biol. 16, 94 (1966a).
Bremer, H., M. W. Konrad, and R. Bruner, J. Mol. Biol. 16, 104 (1966b).

C. BRESCH, K. MUELLER, G. WUESTHOFF

Studies on Inactivation and Reactivation Phenomena of Cowpox Virus

Heat-inactivated pox virus can be reactivated if cells are simultaneously infected with virus particles having undamaged protein coats (see review, Joklik, 1965). Attempts to perform this reactivation in vitro (Abel, 1963) have led to controversial conclusions. In a typical experiment, the heat-inactivated virus was incubated with an extract of infected cells (ICE), inferred to contain decoating activity (DCE). Increases in plaque count obtained were suggested to be due to a modification of a heated virus particle to produce an infectious subviral entity. However, two other interactions between heated and unheated particles, previously not taken into consideration, have recently been demonstrated:

I. One possible source of error in the quantitative determination of active titers in the reaction mixtures could have been due to the masking of active virus by an excess of inactive particles. This suspicion has been verified by adding a known amount of active particles to virus samples heated for different periods of time. After inactivation to a survival of 10^{-5}, the heated virus preparation becomes able to suppress the plaque formation of active virus. This capacity is lost on further heating, since if active particles are now added, normal plaque counts are obtained. The standard procedure to prepare heated virus, as used in the controversial experiments, results in a preparation exerting maximal interfering power. However, there are differences in suppressing activity found in individual preparations.

   Other experiments concerning the nature of the interference do not disagree with the notion that the mechanism involved is the production of an interferon, well known in many systems, which is stimulated by a factor with transient existence produced by the heating of a virus preparation. An inhibiting substance (interferon?) has been shown to occur in the growth medium of chick-embryo fibroblast cells infected with heated pox virus. This inhibitor is produced from about 8 hours onwards after infection. It is stable at pH2 and is not dialysable.

   Details of the inactivation kinetics of the virus and the production and disappearance of the interferon-inducing factors are still under investigation.

II. If interference is avoided in reactivation experiments by either diluting the heated virus or by the choice of a heated preparation with little suppressing effect, another type of interaction between heated and active particles can be inferred from the following observations:

   In agreement with Abel, we find an increase in the number of plaque-forming units in heated virus preparations after treatment with infected-cell extracts (ICE), above the background number contained in the ICE preparation. However, in our experiments the "reactivated" viral entities are neither sensitive to DNAse nor to ultrasonic treatment. On the contrary, sonication leads to an additional increase in titer. Furthermore, no "reactivation" is found, when all viral background activity in the ICE is either removed by centrifugation or destroyed by UV before mixing with heated virus. In contrast, UV treatment of the heated

Harvesting animal viruses

virus prior to mixing with ICE does not influence the titer increase. This may suggest that the additional plaque formers arising in "reactivation mixtures" originate from virus activity in the ICE rather than from the heated material. The gain in titer might result from a dispersion of active particles from viral clumps which may attach to aggregates of heat-inactivated particles, a process probably enhanced by sonication. With this assumption, no enzymatic action would be required for the observed increase in titer. The finding that a mixture of heated virus with a diluted, purified virus suspension shows a comparable titer increase might be considered as additional support to this notion.

Abel, P., Z. Vererbungsl. 94, 249 (1963).
Joklik, W.K., Progr. med. Virol. 7, 44 (1965).

Dr. Clowes discussing an experiment with a co-worker, Eric Moody (left)

R. C. CLOWES

Extrachromosal Factors in Enterobacteria

This work investigates the characteristics, interactions and possible origins of extrachromosomal elements, particularly those which act as sex factors. These include the fertility factor (F), certain factors which give rise to a lethal antibiotic or colicin (C factors) and other factors (R factors) which determine resistance to a series of antibiotic drugs and which are infectiously inherited between strains of Enterobacteria.

One aspect of this work investigates the relationship of F-pili (Brinton et al. 1964) in the conjugation process. In collaboration with D. Lang, we have established that similar structures, to which male-specific DNA and RNA bacteriophages attach, are present in cells harboring certain C and R factors and absent in others. It would appear that highly efficient conjugal transfer of extrachromosomal elements can occur even from cells which do not apparently possess structures similar to F-pili.

By the use of recombinaseless (rec^-) mutants it has also been shown that chromosomal transfer occurs in E. coli which is independent of the integration or recombination of sex factor with the chromosome.

Certain sex factors such as F, ColV2, ColV3 and Rfi^+ (222) appear to be able to recombine with the chromosome, whereas others, such as ColI, Rfi^- (R15) cannot. It therefore seems clear that certain extrachromosomal elements can be stabilized and regulated in cells without chromosomal integration. The possibility that this extends to certain temperate phages seems likely (Clowes and Moody, 1966).

The interactions of pairs of sex factors within the same cell are being investigated. In certain combinations, exclusion effects occur which are consistent with the idea that there is only one factor per nucleus. To explain the stability of these factors, a specific cell site (membrane?) attachment must be invoked (Jacob et at. 1963) and in the case of exclusion, it appears this site can be occupied by only one of a series of several alternative factors. Other pairs of factors appear to co-exist over longer periods, when there is often a repression of all the properties associated with one of the factors by the presence of the other factor. For example, a fertility inhibiting (fi^+) R factor which has been known to repress the fertility characteristics of Hfr and F^+ cells (Watanabe et al. 1964) appears to repress the colicin production of C factors, and also the activity of chromosomal genes (for example lac^+ genes determining the production of β-galactosidase) which may be incorporated as part of an F sex factor in an F prime factor.

Studies are also in progress on the activity of acridine orange in the elimination of extrachromosomal factors in bacteria. From a study of seven independent sex factors, a correlation is observed between those factors which are both susceptible to acridine orange curing and which also appear to be able to recombine with the chromosome (5 factors) and other factors which are insensitive to curing and which also do not appear to be able to recombine (2 factors). This conforms with the recent idea of Sesnowitz et al. (1966) that the mutagenic and curing effect of acridine orange may depend upon the ability of the dye to intercalate within the DNA, and this intercalation may occur only during the recombination but not during the replication processes.

Brinton, C.C., P. Gemski and J. Carnahan, Proc. Natl. Acad. Sci. U.S. 52, 776 (1964).
Clowes, R.C. and E.E.M. Moody, Genetics 53, 717 (1966).
Jacob, F., S. Brenner and F. Cuzin, Cold Spr. Harb. Symp. Quant. Biol. 28, 329 (1963).
Sesnowitz, S., E.A. Adelberg and H. Boyer, Bact. Proc. 26 (1966).
Watanabe, T. et al., J. Bact. 88, 716 (1964).

M. L. COVAL

Structure and Activity of Enzymes

The enzyme, thyroid peroxidase, catalyzes the iodination of tyrosine residues of proteins in the presence of peroxide. It is therefore of importance to the understanding of thyroid physiology. The properties of this enzyme and, in particular, the presence of a heme group at the active site are in dispute, largely due to the crude preparations so far available. In collaboration with Dr. Alvin Taurog of the University of Texas Southwestern Medical School, this enzyme has been extracted from hog thyroids and a thousand-fold purification has been obtained in several preparations. Currently, the purest preparations have an optical density ratio at 410:280 $m\mu$ of only about one fifth that expected on the assumption of a molecular weight of 63,000 which has been determined by gel-filtration studies. Current studies involve further purification and the investigation of the properties of this enzyme.

A phosphatase has been purified several hundred-fold from frog ovaries. Initially thought to be a phosphoprotein phosphatase, it is now found to be specific for high-energy phosphate monoesters. Its molecular weight has been determined as 56,000 and analytical sedimentation indicates only one sedimenting boundary (Coval and Martinek 1966) but electrophoresis has shown two or three remaining protein bands in addition to the active band. This enzyme has also been shown to be activated by reducing agents and inactivated by oxidizing agents, both reversibly. The kinetic measurements of K_m vary over a fifty-fold range (7×10^{-5} to 3.3×10^{-3} M) and the V_{max} values over a two-fold range (4.9 to 9.7) for a number of different substrates. Current studies involve the labelling of the active site by ^{32}P-phosphate followed by the examination of its properties.

Lately, an investigation of the levels of specific enzymes which can be measured in the tissue of cells and in certain teratomas derived from these cells has been initiated. Mouse teratomas which spontaneously develop in the testes of mice have been studied. When grown subcutaneously, these produce organized and differentiated structures, whereas when grown intraperitoneally, they resemble five or six day-old mouse embryos. It has been shown that the levels and isozyme patterns of lactic dehydrogenase and acid phosphatase both in the embryoid bodies and in the original non-tumorous testicular tissue are related. Three different teratomas, a pleomorphic, a myoblastic, and a neural form, are being maintained as solid tumors. A known ascitic form of the pleomorphic tumor is also being maintained and the ascitic forms of the two other tumors have been derived with the intent of studying the enzymic complements of the two forms of each tumor.

Coval, M.L. and J. Martinek, Fed. Proc. 25, 342 (1966).

A faculty office (Dr. Gutz)

H. GUTZ

Molecular Genetics of Yeast

This work has involved the investigation of genetic fine structure, allelic complementation, gene conversion, and haploidization in yeast.

1) Gene fine structure and allelic complementation: In the ad-6 locus of Schizosaccharomyces pombe, 40 further mutants have been isolated and mapped. A total of 374 mutants of independent origin are now mapped in this locus, being located on 120 different sites.

The majority of these mutants show allelic complementation in certain diploid combinations. The complementation pattern of the ad-6 locus is complex, and several hundred crosses have been performed to extend our experimental results. In collaboration with Dr. K. Haefner, a mathematical analysis of the complementation data is in progress.

2) Studies on gene conversion: One ad-6 mutant (M26) gives, in crosses with other ad-6 mutants, conversion frequencies to prototrophy which are 10-16 times higher than those normally obtained in the ad-6 locus. The conversion event in different crosses with M26 has been studied by tetrad analysis. So far about 2700 tetrads have been analyzed (Gutz, 1966 a, b).

3) Haploidization experiments: Experiments with two diploid strains of S. pombe have shown that effective haploidization can be achieved by treatment with p-fluorophenylalanine (Gutz, 1966b). These strains are now being used to map genes in hitherto unknown locations.

Gutz, H., Microbial Genet. Bull. No. 24, 8 (1966a).
Gutz, H., Genetics 54, 338 (1966b).
Gutz, H., J. Bact. 92, 1567 (1966c).

K. HAEFNER

Pedigree Analysis of Irradiated Yeast and Bacterial Cells

This program investigates the fate of individually-irradiated yeast and bacterial cells and of their descendents, by micromanipulation of individual cells, with emphasis on the mechanisms responsible for "lethal sectoring" ("Lethal sectoring" describes the appearance of a complex pattern of inactive and active cells within the progeny of an irradiated cell.)

In synchronized Schizosaccharomyces pombe (haploid), the UV dose-dependence of lethal sectoring has been studied extensively for doses which gave survivals of more than 1% (Haefner, 1966a). About 1200 pedigrees derived from UV irradiated cells with 20% survival have been analyzed for mutations from prototrophy to auxotrophy. Mutations in twelve pedigrees were found permitting inferences of mutational segregation in S. pombe (Haefner, 1966b). An analysis correlating the mutation-segregation pattern (from a larger number of pedigrees, in which mutations had occurred) with the pattern of lethal sectoring is now in progress.

In S. pombe a lack of photoreactivation was found after UV irradiation. In cooperation with C. S. Rupert, it was demonstrated that a photoreactivating enzyme is absent in these strains.

A detailed study of synchronized Saccharomyces (haploid) showed that for X-ray doses less than 12 krad (survival at these doses > 1%) no lethal sectoring occurs.

To exploit the consequences of a specific model which has been proposed to explain lethal sectoring (Haefner, 1965), pedigree analyses have been performed with Escherichia coli B/r and B_{s-1} after irradiation with X rays and UV. For E. coli B/r the picture is similar to haploid Saccharomyces: lethal sectoring occurs after UV irradiation and is lacking after X rays. E. coli B_{s-1} shows extensive lethal sectoring after both kinds of irradiation.

A modified replica plating technique has been developed to select UV-sensitive mutations in Schizosaccharomyces pombe. Four UV mutants have been found and characterized (Haefner and Howrey, 1966). Pedigrees derived from these sensitive strains after UV irradiation show an increase in lethal sectoring over that found with the wild type at equal doses.

Haefner, K., Photochem. Photobiol. 5, 587 (1966a).
Haefner, K., 4th Int. Cong. Rad Res., Cortina, Italy (1966b).
Haefner, K., Intern. J. Rad. Biol. 9, 545 (1965).
Haefner, K. and Lucy Howrey, Mutation Res. (in press, 1966).

Dr. Haefner monitoring micromanipulation via TV

W. HARM

Dark Recovery from Ultraviolet Lethal Damage

An attempt is being made to determine the role played by the excision-resynthesis repair (ERR) mechanism in various UV dark-recovery phenomena in E. coli. These studies may help explain differences in the survival curves of various strains.

Liquid-holding recovery (LHR). LHR, the increased survival observed when UV-irradiated cells are stored in a liquid for up to several hours prior to plating, is probably caused by a stimulation of ERR. This is suggested by the following results, derived partly from data obtained in this laboratory (Harm, 1966): (a) LHR occurs in strains with ERR activity but slightly or not at all in those with little or no ERR activity. (b) LHR is inhibited if the holding liquid contains caffeine or acriflavine, substances known to inhibit the ERR mechanism (see

below). (c) Lysogenic strains, carrying an extra UV sensitivity due to prophage induction, show much more LHR than their non-lysogenic derivatives. (In lysogenic strains it is assumed that, without LHR, the ERR is very incomplete because of the rather limited time available before the prophage-inducing UV lesions become effective.) E. coli B, carrying an extra UV sensitivity (compared to B/r), which is expressed as filament formation, likewise shows a larger LHR effect than B/r.

Excision-resynthesis repair (ERR). Caffeine and acriflavine are known to decrease the UV survival of E. coli and phage T1, supposedly by inhibiting the ERR mechanism. Comparison of various strains of E. coli and of phage T1 shows a correlation of ERR with LHR in the sense that ERR inhibition by caffeine and acriflavine is largest where the LHR effect is largest (i.e., lysogenic strains and strain B). The UV survival of mutant strains with little or no ERR repair shows much less decrease in the presence of these inhibitors.

Harm, W., Photochem. Photobiol. 5, 747 (1966).

D. L. HARRIS

Size and Shape of the Active Site of an Enzyme

Certain properties of a phosphoanhydride hydrolase (Harris et al, 1966) have been further investigated. A systematic study has been made of the inhibition of this phosphatase by phosphate and its structural analogs. Subject to certain systematic restrictions, anions of all oxy-acids derived from elements in groups V, VI, and VII of the Periodic Table are inhibitors. Both trigonal and tetragonal oxy-acids are inhibitors, provided they have at least three oxygens attached to the central element and carry at least one negative charge at the ambient pH. The effectiveness of inhibition (pK_I) increases with increasing size of anion up to a certain limit and then drops very sharply.

These data are consistent with the view that the active site is a pit of limited diameter on the enzyme surface from which large anions (e.g. ReO_4) are effectively excluded but in which small anions (e.g. NO_3, SO_4) fit loosely. Anions of intermediate size (e.g. MoO_4, VO_4) fit snugly and are accordingly tightly bound. The quantitative relation between size and pK_I suggests that the free energy of binding is related to ion-ion interaction over short distances. Preliminary calculations suggest that this pit is approximately 6-7 Å in diameter.

Harris, D., S. J. Pilkis, and B. J. Mizock, J. Biol. Chem. 241, 707 (1966).

R. L. HAUSMANN, B. GOMEZ

Mutual Exclusion Between Phages T3 and T7

This work is directed towards clarification of mutual exclusion between bacteriophages T3 and T7, when infecting E. coli host cells. After simultaneous infection with both phages, only a few percent of the cells produce phages of both types (double yielders). It has been found that this percentage is largely independent of the total and relative multiplicities of infection of the two phages, but that it can be varied within a 20-fold range by changes in growth conditions of the host. Resting cells and cells growing in minimal medium give rise to the lowest percentages of double yielders (1-2%), while for broth-grown cells infected in late lag phase, this value is 20%. The significance of these findings is still obscure, but the importance of other factors besides the viral genomes for an understanding of mutual exclusion is indicated.

In order to investigate whether this phenomenon acts at an early stage during the latent period, measurements of a T3-directed "early" enzyme, SAMase, were made under conditions of simultaneous infection with phage T7. As control, host cells were mixedly infected with T3 and a SAMase-deficient mutant of this phage. It was found that the amount of enzyme made by mixedly-infected cells was related to the input ratio of enzymatically-competent genomes to incompetent genomes. T7 inhibits enzyme production less than SAMase-deficient T3; no effect of mutual exclusion upon T3-specific enzyme synthesis was detected.

Dr. Hausmann operating scintillation counter

In a further search for functional incompatibilities between T3 and T7, conditionally-lethal mutants of these phages were found to be most useful. Several hundred independent amber and temperature-sensitive mutants have now been isolated. Preliminary crosses show that these mutants are distributed over a much wider map region than the linkage group of previously available morphological markers. A minimum map length of 80 and 100 recombination units has been established for T3 and T7 respectively. Complementation studies have led to the identification of 22 cistrons in T3 and 23 in T7, respectively. Many amber mutants cannot be allocated unequivocally to one specific cistron; whether this is due to technical difficulties or whether it reflects a real biological phenomenon, such as polarity, is now under investigation. Representatives of each amber cistron are being analyzed for their ability to lyse the restrictive host cells, to promote phage-specific DNA synthesis and to give rise to serum-blocking power. So far, 7 out of the 22 cistrons of T3 have been tentatively classified as controlling early functions.

After this preliminary characterization, cross-complementation between T3 and T7 amber mutants was investigated. In general, heterologous complementation was rather poor, most amber cistrons giving negative results with all heterologous cistrons. A minority gave positive tests with only a few, whereas mutants in the remaining three or four amber cistrons of both T3 and T7 complemented almost half the heterologous cistrons. Whenever heterologous complementation was found, it was much less efficient than homologous. For example, the burst size of restrictive host cells infected with two complementing heterologous amber mutants was only 2-10% normal, while complementing homologous mutants generally gave normal burst sizes. Recombinants between certain amber mutants of T3 and T7 were found among the progeny of mixed-infected host cells. The maximum frequency of these hybrids is about 2%; their genetic characteristics are now being analyzed.

The low levels of heterologous complementation may be a consequence of mutual exclusion between T3 and T7, for example if the gene products of the excluded phage were too low. An alternative explanation would be that mutual exclusion may not operate at the level of transcription and translation of the excluded genomes but rather that the heterologous gene products are not utilizable. In view of the implications of these findings for mechanisms of gene expression, future work will be directed towards investigating these points.

Euplotes crassus

K. HECKMANN

Mating Type Changes and Killer Traits in the Ciliate Euplotes

In Euplotes crassus, each of the five different mating types is determined by a different allele of the mating type locus. Among these alleles, complete dominance prevails so that only one of any pair of alleles is phenotypically expressed (Heckmann 1964). It has been recently observed that in clones which originally expressed only one mating type, cells may arise after approximately 500 consecutive fissions which express another mating type. It thus appears that the dominance relationship among the mating type alleles of Euplotes crassus may be abolished at a stage late in the life history of a clone. In this stage, cells heterozygous for mating type alleles might express either one or the other allele and hence be of either one or the other mating type. These cells are no longer stable with respect to their mating type, but switch back and forth from one type to the other within a few fissions. This phenomenon provides a mechanism which (in a manner similar to autogamy in other ciliates) may assure rejuvenescence, in the start of a new life cycle for cells which have failed to find interclonal mates. A few exceptional clones have been found which are not able to enter this life-cycle stage. They are genetically related to each other and appear to result from a genetic block which prevents them from entering the stage of mating-type instability.

An earlier paper on the inheritance of autogamy and the killer traits in Euplotes minuta (Siegel & Heckmann, 1966) claimed that the killer trait was under control of a cytoplasmic factor, but microscopic identification of the factor was not achieved. In collaboration with Dr. J.R. Preer, Jr. University of Pennsylvania, 1—2.5μ long bacteria-like particles have now been found in the cytoplasm of cells belonging to killer stocks. These particles resemble "non-bright" kappa particles originally described by Preer (1950) for killer stocks of Paramecium aurelia. The killing activity has been found to be associated with large particles which readily sediment in the centrifuge at 1500 g, and may be identical with the observed particles. The killing activity is inactivated by heat, certain proteolytic enzymes, and by rupture with the French press.

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W. HEUMANN

Conjugation in Rhizobium and Pseudomonas

Cells of certain strains of Pseudomonas and Rhizobium aggregate to form star-like rosettes. This has been shown to represent a novel type of conjugation and the genetics and the conjugation mechanism of such star-forming strains are being studied. Previous work has shown genetic transfer in one star-forming strain, Ps. echinoides, and established genetic linkage relationships between certain markers (Heumann and Marx, 1962, Marx and Heumann, 1962).

During the last year genetic investigation of Rh. lupini has been studied from which a series of auxotrophic and pigmentation mutants had previously been isolated. Preliminary two-point crosses were consistent with the idea of a circular chromosomal map, all the pigmentation markers being concentrated in one region. This result has been confirmed by a series of three and four-point genetic crosses. Further genetic analysis of the recombinants suggests a lack of sexual differentiation in Rh. lupini, each cell behaving simultaneously as donor and recipient. From kinetic studies of the rate of appearance of recombinant colonies, together with the incidence of mixed colonies showing a series of segregant types, it appears possible that in an active conjugating pair, chromosomal transfer, replication, and recombination are continuous, to give rise to a series of recombinants of various types. Methods are now being developed to further investigate this conclusion by the separation by micromanipulation of individual segregants from a mating pair as they are seen to separate. In this way a clearer analysis of the conjugation events should be possible.

In previous work with Pseudomonas it has been shown that rosette formation depends upon polarly-inserted fimbriae (pili) which connect the cells and maintain contact. We have now shown that a similar type of fimbria is involved in star formation in Rhizobium. This work is now being followed in greater detail by electron microscopy in collaboration with D. Lang. The distribution of fimbriae in Rhizobium, in contrast to Pseudomonas, appears to be at one of a number of points around the cell, in any one cell only a few fimbriae in the form of a tuft being visible.

The biological role of the pigments (carotenoids) in Rhizobium and Pseudomonas is now being studied. These pigments appear to protect cells against damage by visible light, white mutants which lack the pigment being more sensitive to irradiation than the yellow, wild-type strains. These experiments are being continued after pre-sensitization of the cells by toluidine blue. In collaboration with Dr. F.Z. Czygan (Erlangen, Germany) the chemical nature of these carotenoids is being studied. Recently a new carotenoid, 3,3',5-trioxi-5,6-dihydro-β-carotene has been identified.

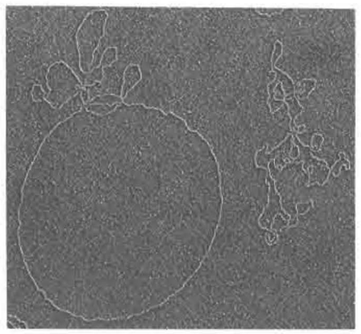
Heumann, W. and R. Marx, Z. Vererbungslehre 93, 441 (1962).
Marx, R. and W. Heumann, Arch. Mikrobiol. 43, 245 (1962).

H. HIROKAWA

Intracellular Recombination Investigated by Transformation in Protoplasts

We continue our study of the fate of transforming DNA in recipient cells by biochemical and biophysical methods. It is now well established that transforming DNA is directly integrated into recipient DNA of Pneumococcus, Hemophilus and Bacillus. However, several points are still obscure, e.g.: the site of synapsis on both DNAs; and whether a minor level of DNA synthesis occurs, as in the repair synthesis of UV irradiated DNA.

DNA recombination in protoplasts of Bacillus subtilis is being studied as an approach to a cell-free system of genetic recombination. In these studies, it has been shown that protoplasts of B. subtilis have an increased capacity for the uptake of exogenous DNA, compared with intact competent cells, and that intracellular genetic recombination of donor and recipient DNA is more easily detected in protoplasts than in intact cells (Hirokawa, 1966a, b). Moreover, intracellular recombination between two different, exogenous DNAs has been detected in this system, recombinant molecules being formed while linkage between the corresponding markers is lost following uptake of a single DNA molecule. This suggests that genetic recombination between transforming DNA's occurs through a breakage and reunion process.



Electron micrograph of DNA from bacteriophage x. The DNA has "sticky" ends and forms a closed loop after phage extraction

It has also been shown that the transforming ability of re-extracted recombinant DNA does not decrease after RNase or trypsin treatment but remains sensitive to DNase treatment. Recent experiments show that dilution of a recombinant DNA preparation leads to a proportional decrease in the number of transformants, indicating that recombinant markers are located in a single DNA molecule. However, different responses of recombinant-type and wild-type DNA are found after heat treatment, linkage between two markers on the recombinant DNA being more sensitive than that of wild-type DNA.

The structure of the recombinant DNA molecule is unknown. Different models can be proposed, and we undertook to test some. By examination of 200 wild-type, transformant colonies produced by recombinant DNA, it has been shown that they are pure and do not segregate single mutants of either type. From this it is concluded either (a) that only a single strand of DNA is integrated or (b) that if double strands are involved, both strands carry both wild-type alleles. In other words, if recombinant molecules have "hetero-duplex" structures, these are not integrated as such. To further investigate this question, wild-type DNA was re-annealed with the DNA of a histidineless mutant, then used to transform B. subtilis cells. The transformant colonies so obtained are now being analyzed.

Other recent experiments show the appearance of nuclease activity in the medium during the process of protoplast formation by lysozyme. A more precise analysis of this endonuclease activity by biological and chemical methods is now under way.

H. Hirokawa, J. Bact. 91: 125 (1966).
H. Hirokawa, J. Bact. 92, 455 (1966).

E. B. HOROWITZ

Galactokinase Synthesis by Defective Transducing λ Prophage (λdg)

We currently continue our study of galactokinase synthesis by λdg particles, and have recently discovered a second, previously unsuspected, species of transducing particle in UV-induced lysates of a heterogenote strain of E. coli K12 with abnormally high galactokinase activity. These new particles are more dense than those of the λ dg preparation from which they were derived. It seems possible that these particles contain a duplication of the bacterial gal region, and attempts are being made to verify this hypothesis. If a duplicate region is involved, the system is likely to prove useful in clarifying the origin of localized chromosomal duplications.

J. JAGGER, H. TAKEBE

Protection and Recovery from Ultraviolet Damage in Bacteria

1. Photoprotection

Recent work has suggested that photoprotection operates by inducing delays in growth and division, and that it induces such delays through the destruction of essential respiratory quinones (Jagger et al, 1964).

Consequently, we have begun experiments on the photochemistry of isoprenoid quinones. It has been found that iradiation of the naphthoquinone vitamin K_2 in the near-UV region causes a drastic change in the entire UV absorption spectrum, indicating a major change in the aromatic moiety of the compound. The doses required for this effect are consistent with those required for photoprotection. In addition, we have found that the near-UV action spectrum for this destruction corresponds to the action spectrum for photoprotection. These results support our hypothesis that quinones are the chromophores for photoprotection.

We have found that the benzoquinone, coenzyme Q_8, is considerably less sensitive to near UV-irradiation. It is probably a less important chromophore for photoprotection.

Studies with benzo- and naphthoquinones that lack isoprene side chains have shown that these compounds respond to near-UV destruction in a manner rather parallel to that of the isoprenoid compounds, indicating that the damage is occurring in the aromatic moiety, as would be expected.

2. Liquid-holding recovery

Based on reports of Alper and Gillies (1960), we had previously postulated that liquid-holding recovery operates by means of the induction of a growth and/or division delay, similar to the mechanism proposed for photoprotection. Recent experiments in our lab, however, show no evidence of such delays after holding in liquid. These experiments were done with both log-phase and log-phase-starved cultures and at 23° and 37°. Our results are in accord with recent findings of Harm, and indicate that liquid-holding recovery actually takes place primarily during the holding period, and not on the plates after holding, as we had previously thought.

3. Search for red:far-red effects in bacteria

Recent work (Kondo and Jagger, 1966) shows that visible light in the green-to-red range causes some photoprotection of mutation to prototrophy in E. coli. Since photoprotection is believed to operate by inducing a growth delay, this suggests that red light might induce a growth delay in bacteria. Wolff and Luippold (1965) have shown that red light induces a division delay in seeds of Vicia faba, and the reversibility of this effect makes it apparent that it is caused by the red:far-red (phytochrome) system so widely used by plants as a photomorphogenetic factor.

In view of these findings, it was felt reasonable to search for a phytochrome system in E. coli. Such systems have not so far been demonstrated in bacteria. Extensive studies, using narrow bands of light at 6670 A and 7360 A, and doses up to 10^7 erg mm^{-2}, showed (1) no effect on growth, (2) a slight delay, under certain conditions only, of division by both wavelengths, and (3) no photoprotection from UV killing by pretreatment with red light (6670 A). Although more experiments are required before we can reach an unequivocal conclusion, it now seems likely that E. coli does not possess a phytochrome system.

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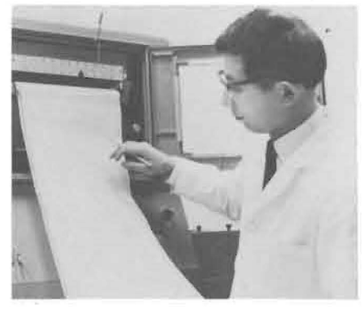
Jagger, J., W.C. Wise, and R.S. Stafford, Photochem. Photobiol. 3, 11 (1964).
Kondo, S. and J. Jagger, Photochem. Photobiol. 5, 189 (1966).
Wolff, S. and H.E. Luippold, Photochem. Photobiol. 4, 439 (1965).



Dr. Takebe examining Cary recording spectrophotometer data

W. KRONE, H. BRUNSCHEDE

Biochemical Studies of Human G_1-trisomic Cells in Tissue Culture

These experiments are aimed at the detection of biochemical alterations in the tissues of humans afflicted with the congenital disease called Down's Syndrom (or mongolism), which is characterized by a G_1-trisomic karyotype. Knowledge of such alterations should lead to better understanding of the nature of the disease, as well as contributing to our knowledge of gene action.

Karyotypes of eleven G_1-trisomic boys were determined by the blood culture method; one of them was a G_1/G-type translocation trisomy. For experimental purposes body-skin biopsies were taken from five of these patients and cultures were made. An additional culture was established using the foreskin of a ten-day-old G_1-trisomic boy as the starting tissue. Control fibroblast cultures were derived from seven foreskins of normal newborn infants and from three body-skin biopsies of normal children.

UDPG-4-Epimerase Activity in Human Fibroblasts

The enzyme UDPG-4-epimerase, which converts uridine diphosphate galactose to uridine diphosphate glucose (Leloir, 1964), operates in microorganisms as well as in mammals and the conversion is a prerequisite for cell growth.

Our studies have shown that the epimerase activity in human fibroblasts changes markedly with growth phase of the culture, but reaches a plateau after 72 hours. Cultures derived from foreskins of newborn infants exhibit considerably higher specific epimerase activities (+52.7%) during the plateau period than cultures derived from body-skin biopsies of boys between 8 and 13 years of age.

However, we find that there is essentially no difference in the specific epimerase activity of cultures derived from normal and from G_1-trisomic boys within the age group of 8 - 13 years.

In contrast to cells with normal karyotype, G_1-trisomic cells do not lose an appreciable percentage of their specific epimerase activity when galactose replaces glucose in the growth medium. This was shown in two experiments using foreskin-derived cells. (Krone and Brunschede, 1966).

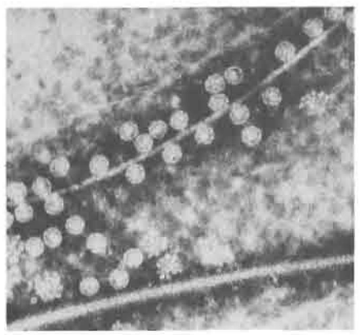
Nucleoli

No significant difference between the average number of nucleoli per cell as well as in the frequency distribution of the nucleoli of G_1-trisomic and normal cells was found in four out of five consecutive experiments.

The swelling of nucleoli with thioacetamide, known to occur in liver cells, was conspicuous in the fibroblasts. Besides this, giant cells, high ploidy in mitoses, and an occasional micronucleus were observed. The effects of thioacetamide on tissue-culture cells are per se interesting enough to be investigated in a separate program, biochemically as well as cytologically. However, as a reagent causing differential effects in G_1-trisomic and normal cells, thioacetamide seems not to be suitable.

Actinomycin D caused the expected decrease in staining and shrinkage of the nucleoli reported in the literature. This effect was observable even after a two hour treatment with 1μ g/ml. The maximum of the frequency distribution was concomitantly shifted to the cells with lower numbers of nucleoli. Short-term treatment with actinomycin D might be a means for better differentiation between the capabilities of normal and of G_1-trisomic cells in forming nucleoli.

Krone, W. and H. Brunschede, Humangenetik 2, 192 (1966)
Leloir, L.I., Biochem. J. 91, 1 (1964).



μ-2 phages, attaching to sex-fimbria of E. coli. (The thick white line is a flagellum.) Some collapsed phage particles visible, revealing capsomeres

D. LANG, H. Bujard

Electron Microscopy Studies

Molecular studies

The idea that electron microscopy of individual molecules may complement and extend the usual physical-chemical data on flexible linear or circular macromolecules is substantiated by our recent work.

Intact, complete genomes of viral DNA were isolated from bacteriophage T1, bacteriophage T3, and bovine papilloma virus, and found to consist of single pieces of DNA of molecular weight 31 x 10^6, 23 x 10^6, and 5.0 x 10^6, respectively, as determined by molecular length. Analysis of errors showed the natural length variability of the intact DNA to be less than ±2% (sample standard deviation). At ionic strengths below 0.1, a linear expansion (up to 20%) of DNA was observed, indicating a deviation from the Watson-Crick structure at these ionic strengths.

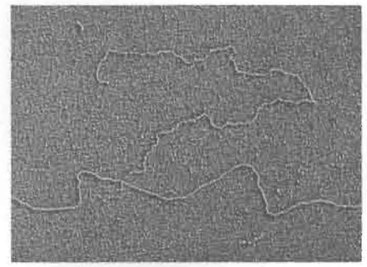
It was demonstrated that our preparation technique [in which DNA in solution (5 x 10^{-8} g/ml) diffuses to a protein monolayer where it becomes irreversibly adsorbed] results in a direct projection of the molecule into two dimensions in a manner that can be predicted mathematically. This finding permitted for the first time determination of the "shape" of DNA molecules by measuring directly the distribution of end-to-end distances and their mean values. DNA in high-ionic-strength solution was found to be a (stiff) random coil. Such knowledge is necessary for reliable interpretation of the hydrodynamic behavior of DNA.

Actinomycin was found to cross-link DNA molecules internally.

Encouraged by the precision and internal consistency of these results, we are now attempting a complete physical-chemical description, by ultracentrifugation, viscometry, and electron microscopy, of the circular DNA of bovine papilloma virus.

Morphological studies

Cooperation with other groups in the Division involved electron microscopic studies of male-specific sex fimbriae of several bacterial strains (Clowes), bundles of fimbriae believed to be conjugation tubes in star-forming bacteria (Heumann), flagellae of various other bacteria (Heumann, Mitani), as well as studies of aggregation in vaccinia virus (Bresch, Wuesthoff).



Single-stranded DNA (top) in electron micrograph can be distinguished from double-stranded DNA (bottom) by presence of more "kinks" and less contrast

A.D. McLAREN (Visiting Professor, 6 months)

Uptake of Enzymes and Histones by Plant Roots

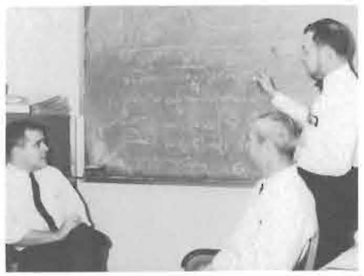
It has been suggested by E. Stedman, J. Bonner, and others that histones are active in cells as regulators of gene action, so an effort was made (McLaren and Bradfute, 1966) to see if there was a specific inhibition of plant root elongation by histones from plant and animal sources. Other basic polymers were also tested.

Polycations, including salmine, lysozyme, ribonuclease, wheat germ histone, thymus histone, and polylysine, inhibit root elongation of barley and wheat. Polyglutamate and lysylglycine at comparable weight concentrations are not inhibitory. No difference in the efficacy of the plant and the animal histones could be found with either plant, which suggests that the action is non-specific and is probably confined to the external cell membrane.

Growth of roots inhibited by histone or lysozyme can resume after removal of the polycation. Inhibition by ribonuclease is more nearly permanent. A protein which does not readily penetrate a root, such as bovine plasma albumin, is not inhibitory.

The mechanism whereby polycations influence root growth is not known, but it is clear that the polymeric state of ionic functional groups is of paramount importance in the binding of the polycations to cell surfaces.

A. D. McLaren and O. E. Bradfute, Physiol. Plantarum 19, 1094 (1966)



Photobiology discussion (left to right, Drs. Patrick, Werbin, and Rupert)

C.S. RUPERT

Ultraviolet Photochemistry and Photobiology of DNA

UV photoproducts in DNA

In collaboration with Drs. Shih Yi Wang, M.H. Patrick, and George Varghese of Johns Hopkins University, an investigation of the mechanism of thymine photoproduct formation in DNA was undertaken. The first results of this study strongly suggest that the products currently believed to be cyclobutane-type pyrimidine dimers (T=T, C=T) (Setlow, 1966) are not formed directly in DNA, but exist as an intermediate compound before reaching the form in which they are usually studied (in acid hydrolysates).

The formation of thymine photoproducts in denatured E. coli DNA containing radioactive thymine was observed as a function of the pH during irradiation. The two products detected chromatographically after acid hydrolysis were both greatly, but differently, affected by the pH during irradiation. (Controls showed that, for DNA irradiated at neutral pH, acid or alkali pretreatment or posttreatment had no effect.) Production of the substance commonly supposed to be thymine homodimer (T=T) decreased with the irradiation pH and approached a zero value over a range (approximately pH 4-2) in which the extinction of thymine does not change, and in which this base has no titrating groups. Therefore, the pH effect must be exerted on some intermediate step of the reaction, possibly through an ionic mechanism.

UV survival kinetics of transforming DNA

Earlier studies showed that the survival of transforming activity in UV-irradiated DNA followed the equation $T/T_o=1/(1+cD)^2$ for a variety of genetic markers, T_o being the number of transformants produced by unirradiated DNA, T the number produced after irradiation by a dose D, and c a constant characteristic of the genetic marker (Rupert and Goodgal, 1960). The law can be derived from a simple theoretical picture resembling that for marker rescue in bacteriophage. We have also observed that a linked double marker did not follow this law. Two cases of linked double markers were shown to follow the law $T/T_o=1/(1+cD)^4$ for both UV and nitrous-acid inactivation (the single markers follow the inverse-square law under nitrous acid treatment). The same laws hold when host-cell reactivation of UV-irradiated transforming DNA is inhibited by acriflavine. (We have found that there is no host-cell reactivation of nitrous-acid damage to DNA.) The fourth-power law can be accommodated under the earlier theoretical picture if it is assumed that, on the average, the length of DNA recombined into the recipient cell genome in one transformation event is somewhat smaller than the distance between the linked markers. However, the relative sensitivity of the linked double, as compared to the single, markers is not accounted for and remains to be understood.

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C.S. Rupert and S.H. Goodgal, Nature 185, 556 (1960).
C.S. Rupert, Photochem. and Photobiol. 4, 271 (1965).

H. WERBIN

Photoreactivation in Blue-Green Algae (from July, 1966)

Although it has been known for many years that ultraviolet (UV) damage to most plant viruses can be photoreactivated after virus infection of leaves, no detailed studies have been made of a possible relationship between photoreactivation and photosynthesis. The discovery by Safferman and Morris (1964) of a virus, LPP-1, that infects the blue-green alga Plectonema boryanum stimulated our search for photoreactivation in these microorganisms and study of its relation to photosynthesis. (Begun with Drs. J.H. Wu and R.A. Lewin at the Scripps Institution of Oceanography).

The alga

If the infected algae are held in the dark for several hours after UV before placement for several days in white light (the latter treatment being necessary because the alga is an obligate autotroph), the survival is considerably lower than for zero time in the dark. This result suggested that photoreactivation was occurring when there was no holding in the dark after UV, but that several hours of holding in the dark allowed the photoreactivability to decay, so that subsequent placement in the light produced no reactivation. Confirmation of the existence of this photoreactivation was obtained when light- and dark-survival curves were plotted as a function of the time of UV irradiation.

Further experiments showed that the uninfected algae alone could be photoreactivated with either near ultraviolet (360 mμ) or blue light, but not with red light (above 580 mμ), behavior typical of photoreactivable organisms.

The virus

The virus also could not be photoreactivated with red light. This shows that photosynthesis, which is active in red light, does not produce the photoreactivation we observed with either the host cell or the virus, unless the nature of photosynthesis is different in the blue and red regions.

Unlike the host cell, the virus is not photoreactivated by near ultraviolet light, a result not understood, expecially in view of the observation (Jagger and Latarjet, 1956) that E. coli B/r and one of it phages show nearly identical action spectra for photoreactivation.

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(Names of members of our academic staff are capitalized. Some of the publications refer to work carried out at previous locations)

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Division volleyball

Invited Seminar Speakers

J. F. Albright, Oak Ridge National Laboratory
Immunological competence of cells as revealed by studies of antigen competition

P. Day, Conn. Agricultural Experimental Station
Genetic studies with *Coprinus*

S. Dick, Indiana University
Heterokaryosis in the higher fungi

A. H. Doermann, University of Washington
Studies on genetically-deficient T4 phages

M. Elkind, National Institutes of Health
Radiation repair in mammalian cells

A. H. Ellingboe, Michigan State University
Sexual incompatibility and somatic recombination in *Schizophyllum commune*

S. Fogel, City University of New York
Gene conversion in yeast

J. Frankel, Iowa State University
The maintenance of development in ciliates

H. W. Goedde, University of Freiburg
Biochemical genetics of human pseudocholine-esterase and INH-acetylase polymorphisms

C. W. Haidle, University of Texas
Control of morphogenesis in *Mucor rouxii*

P. H. Hofschneider, Max-Planck Institute (Munich)
The formation and properties of the replicative form of the RNA phage M12

S. J. Karakashian, Rockefeller University
Specificity in the endocellular symbiosis of *Chlorella* in *Paramecium bursaria*

Grete Kellenberger, University of Geneva and Kansas State University
Head formation in T4 phage

B. J. Kilbey, Oak Ridge National Laboratory
Photoreactivation and UV-induced forward mutation in *Neurospora crassa*

A. K. Kleinschmidt, New York University
Recent results in electron microscopy of nucleic acids

M. Konrad, University of California, Los Angeles
Regulation of λ RNA synthesis

L. Kozloff, University of Colorado
Chemical studies on viral morphogenesis

F. Lanni, Emory University
Physiological and genetic analysis of phage T5

Yvonne Lanni, Emory University
Some genetical and biochemical aspects of infection by phage T5

R. Z. Lockhart, Jr., University of Texas
Virus inhibition of interferon

O. Maaløe, University of California, Berkeley
DNA replication and the division cycle in *E. coli*

L. A. MacHattie, The Johns Hopkins University
Electron microscopic characterization of viral DNA molecules

M. Mandel, M. D. Anderson Hospital and Tumor Institute, Houston
Sequential RNA transcription in *B. subtilis*

T. S. Matney, M. D. Anderson Hospital and Tumor Institute, Houston
Some recent studies involving a mutation affecting recombination in *E. coli* K12.

F. Melchers, Salk Institute, San Diego
Structure and biosynthesis of the carbohydrate portion of immunoglobulins

H. Mohr, University of Freiburg
Photomorphogenesis in higher plants; Basic phenomena and phytochrome. Phytochrome and the diversity of photoresponses; The problem of differentiation. Differential gene activation as a mode of action of phytochrome. Differential gene repression as a mode of action of phytochrome.

A. Muhammed, Oak Ridge National Laboratory
Purification of photo-reactivating enzyme: a status report

A. Muhammed, Oak Ridge National Laboratory
Phage reactivation

J. R. Preer, University of Pennsylvania
Kappa and its relatives in *Paramecium*

D. R. Ritchie, The Johns Hopkins University
The gross arrangement of nucleotide sequences along phage DNA molecules

G. Sato, Brandeis University
Tissue culture of adrenal and pituitary tumors

R. Schmitt, Palo Alto Research Institute
Enzymology and genetics of α-galactosidase in *E. coli*

O. Siddiqi, Tata Institute (India)
Enzyme synthesis following conjugation and recombination in *E. coli*

R. W. Siegel, University of California, Los Angeles
The embryology of *Paramecium*

L. Simon, Institute for Cancer Research (Philadelphia)
Phage morphology and the mechanism of injection

D. Soell, University of Wisconsin
sRNA specificity and codon recognition

H. C. Spatz, Stanford University
Effect of single-strand breaks on the kinetics of denaturation of DNA

J. Speyer, Cold Spring Harbor Laboratory
Mutagenic DNA polymerase

D. Stadler, University of Washington
Genetic recombination in *Neurospora* and *Ascobolus*

T. Trautner, University of California, Berkeley
The DNA of *B. subtilis* phage SP50 (chemical and biological studies)

H. L. K. Whitehouse, University of Cambridge
The mechanism of crossing-over

N. E. Williams, Iowa State University
Problems of synthesis and assembly in development of the oral primordium in synchronized *Tetrahymena*

J. Wilson, The University of Texas Southwestern Medical School (Dallas)
Intranuclear localization of testosterone in the prostate gland

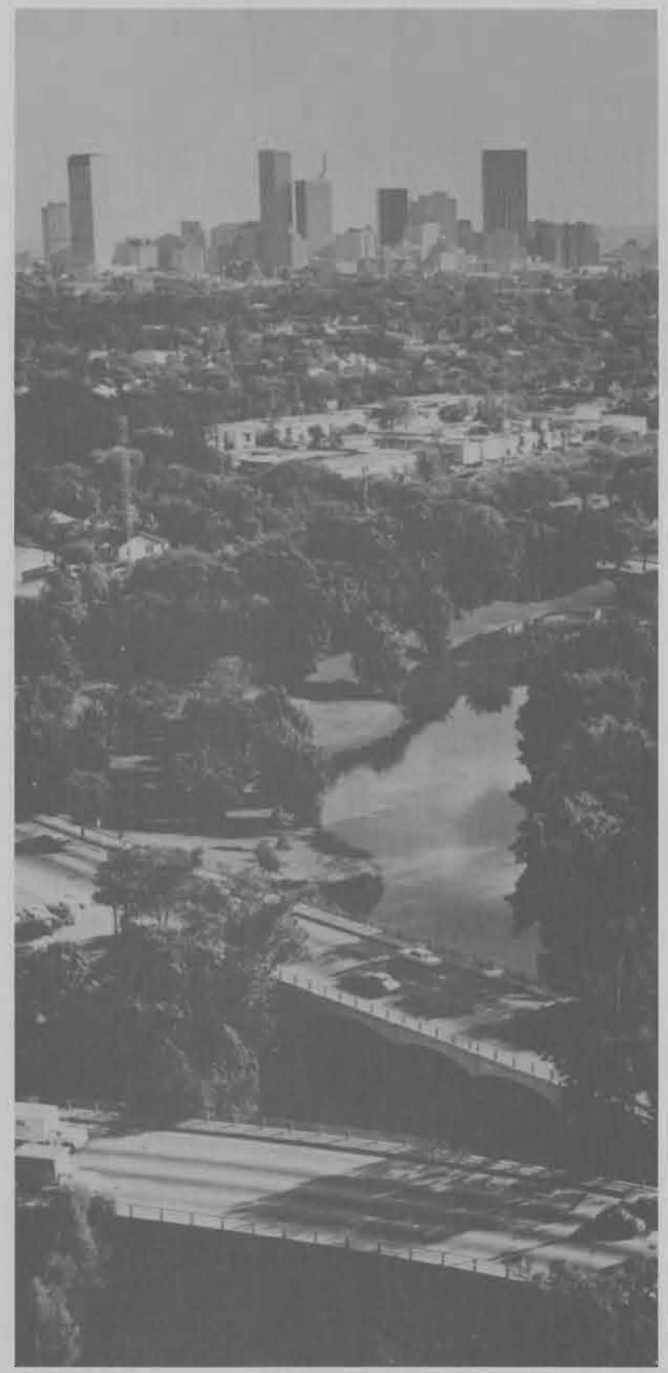
B. N. Wise, University of Chicago
Problems of morphogenesis and fine structure in *Euplotes*

P. Witonsky, Institute for Enzyme Research (Madison)
Selective binding of RNA polymerase to polynucleotides of unknown structure

U. Wolf, University of Freiburg
Sex chromosomes and dose compensation in mammals

Frances Womack, Vanderbilt University
Single burst analysis of multifactor crosses in T4

V. Woodward, Rice University
Genetics, function and location of aspartate transcarbamylase in *Neurospora*



The Dallas skyline

Kalita Humphreys Theater, designed by Frank Lloyd Wright

The Community

The Biology Division is housed in the Founders Building on the main campus of the Southwest Center for Advanced Studies. This campus is on an open tract of land some two square miles in area, situated to the north of Richardson, about 18 miles from the center of downtown Dallas. On the campus are also two smaller buildings, one housing the Materials Sciences Division and the other the Mathematics and Mathematical Physics Division and some administrative offices.

The town of Richardson (pop. 43,000) centers around two electronic establishments, Texas Industries, Inc., and Collins Radio Co. About one-third of the SCAS faculty live in this almost exclusively residential town, which is modern and well laid out, with broad streets, excellent schools, and good recreational facilities, including several well-equipped parks.

Dallas (pop. 800,000) is a rapidly growing city that is the financial and banking center of the Southwest. It is a modern city, spread out over a large area, and in general character is more similar to the large cities of the Southwest than to those of the Northeast. It has excellent highways and a striking skyline. Dallas is a clean and pleasant town, having virtually no smog and very little heavy industry.

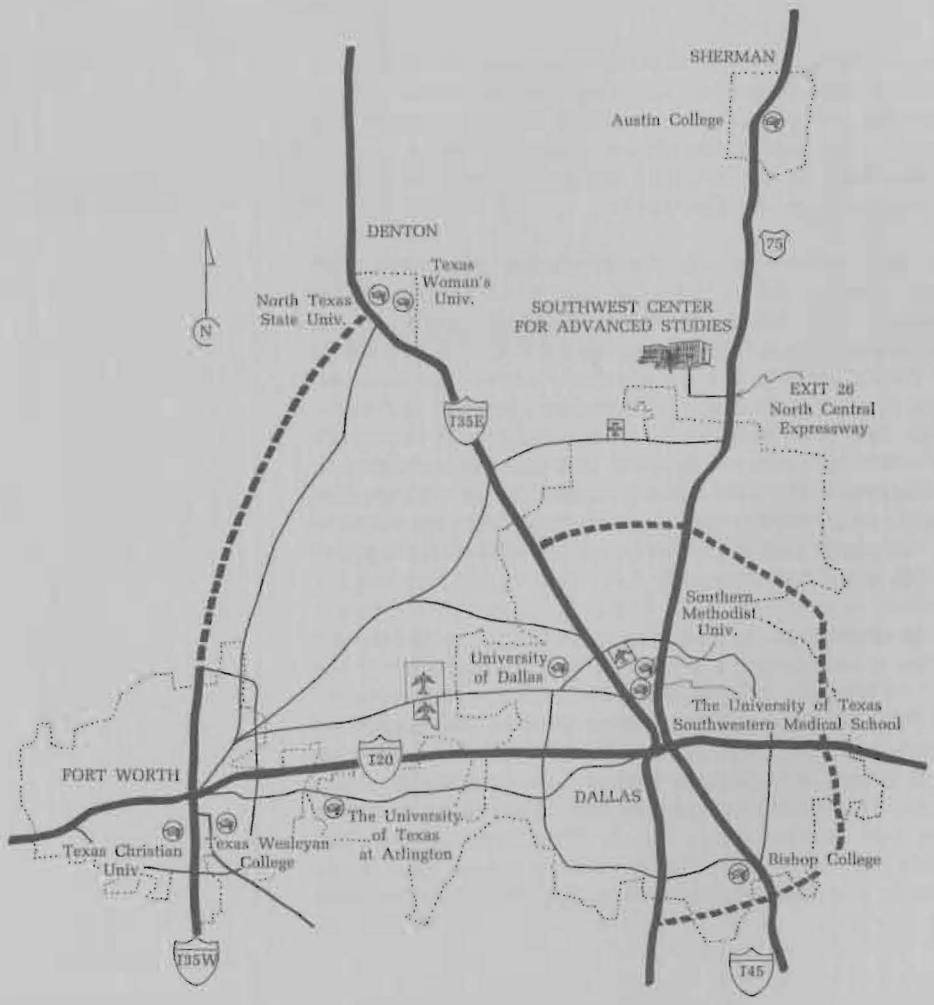
Although certainly not the equal of the large cities of the Northeast, Dallas is nevertheless a cosmopolitan city with a variety of entertainment and cultural activities. It has upwards of 100 foreign restaurants. Cultural activities are expanding rapidly, as exemplified by the existence here of the only theater designed by Frank Lloyd Wright. This Theater Center houses a permanent dramatic company and is one of the outstanding cultural attractions of the Southwest. Dallas also has a symphony orchestra, a civic opera company, a civic music association, and a ballet. A variety of musical and dramatic activities are associated with Southern Methodist University and The University of Texas at Arlington. There are several art museums, an aquarium, an excellent zoo, and a museum of natural history. Sports and recreational facilities are widely available in the city, including a large number of municipal swimming pools and tennis courts. The city is the home of the Dallas Cowboys and of the famous Cotton Bowl.

Almost everyone at the SCAS Biology Division has come from outside Texas, so it is a new experience for all of us. On the whole, people are generally pleasantly surprised by the total impact of their new lives in this community. The public educational system is good, probably being close to the best in the South or the Southwest. The cost of living in Dallas is about average for the nation.

The countryside is flat and relatively uninteresting compared with mountain and ocean areas, but it has its own attractions in being typical prairie country that often blooms with wildflowers. In the springtime, the wildflower display in Texas exceeds that of any other part of the nation. The climate is hot, as everyone knows, but because of the widespread use of air conditioning in factories, homes, and automobiles, people often find that they lead more comfortable summers in Dallas than they ever had elsewhere. The average temperature in January is 45°, in August 85°. The annual rainfall is 34 inches. The air is generally quite dry and the skies are sunny. This encourages active participation in such outdoor sports as horseback riding and sailing. Many large lakes surround the city.

One of Dallas's main attractions is that it is central to a large variety of well-known vacation areas. A day's driving brings the traveler to the exotic border towns of Mexico, the wild desert and mountain country of the Big Bend, the Pueblo Indian country of New Mexico, the mountains of Colorado, the beaches and aquatic sport areas of the Gulf or the gay night life of New Orleans. In addition, Dallas is one of the main air travel centers of the United States and has direct flights to most major cities of the nation and many foreign cities. This tends to make the city much less provincial than other Southwestern towns, and it particularly contributes to our interaction with scientists from the East and West Coasts.

White Rock Lake, in North Dallas



Location of the Southwest Center for Advanced Studies, North Texas area educational institutions, and major transportation routes