National Academy of Sciences

Abstracts of papers presented at the autumn meeting, Durham, North Carolina, 17–19 October 1966.

Genetic Control of Cell Division in Bacteria

We are studying two mutants of *Escherichia coli* K-12 that may help us understand the mechanisms controlling cell division in this organism. The first mutant is characterized by its failure to complete cytokinesis, particularly after exposure to radiation. Cell growth and nuclear division are unimpaired, and long, non-septate, multinucleate filaments are formed. These filaments do not divide unless they are treated with cytokinesis-stimulating agents. One of these agents is an extract of normal *E. coli* cells. This extract is being characterized, for we suspect that it contains some part of the normal cell division mechanism. At present we recognize three factors that, when added to filaments, affect cytokinesis. One of these is apparently a polypeptide that stimulates cytokinesis, a second is a fraction, of low molecular weight, that inhibits cytokinesis, and a third seems to be a protein associated with 100*S* ribosomes in some way balances the activities of the stimulator and inhibitor.

The second mutant is characterized by the fact that many cells, in addition to undergoing normal binary fission, produce small cell-like structures from their poles. These miniature cells are approximately 1/10 the volume of the parental cells and may be isolated by sucrose density-gradient centrifugation. The miniature cells are surrounded by a wall and membrane and contain proteins and ribonucleic acid in the same ratios as parental cells. They do not, however, contain any detectable deoxyribonucleic acid. They are apparently formed by an impaired cytokinesis mechanism that does not distribute nuclear material to both sides of the cross septum.

H. I. Adler, W. D. Fisher
G. E. Stapleton
Oak Ridge National Laboratory

Formation and Properties of Thin Lipid Membranes from Sheep Red Cell Lipids

A lipid extract was prepared from high potassium (HK) and low potassium (LK) sheep erythrocytes in isopropanol-chloroform; it contained cholesterol and phospholipid in a molar ratio of approximately 0.8 to 1.0. Optically black bimolecular membranes were formed from an erythrocyte lipid–hydrocarbon solution in aqueous media and had a d-c resistance of approximately 10^8 ohm-cm^2 and a capacitance of 0.3 to 0.4 μfarad/cm^2. Membrane thickness was estimated to be 40 to 130 Å, depending on the dielectric constant.

Although the membranes were subject to hydrostatic bulging, they could be restored to an optically plane state with constant resistance and capacitance. With a flow system which permits rapid changes of solution in one of the two chambers surrounding the medium, membrane potentials were recorded in the presence of NaCl or KCl ionic concentration gradients, or both, and ionic transference numbers (T_{ion}) were computed. T_{Na} or T_K were 0.80 to 0.85 with reference to T_{Cl}, indicating a high degree of membrane cation selectivity, and T_K was slightly greater than T_{Na}. Membrane voltage was a function of the ionic activity ratio of the solutions bathing the membrane and was independent of hydrostatic or osmotic gradients, or ionic strength. Cation selectivity was attributed to the negatively charged phospholipids (phosphatidyl ethanolamine and phosphatidyl serine) present in the membrane, and cholesterol-depleted membranes had the same cation selectivity. Membranes formed from the neutral phospholipid lecithin had minimal cation selectivity.

Thomas Andreoli
Duke University

Evidence for the Association of a Phenylalanine Transfer tRNA with Neurospora Mitochondria

It has been demonstrated that *Neurospora* contains two aminoacyl-RNA synthetases for both phenylalanine and aspartic acid. In addition, it has been shown that distinct species of transfer RNA (tRNA) are aminoacylated by each of these enzymes. Attempts to investigate the role of these multiple synthetase–multiple tRNA systems have led to the observation that the minor phenylalanine tRNA component of *Neurospora* appears to be associated with the mitochondrial cellular fraction, rather than with the nonparticulate or "soluble" fraction.

Sponsored by the AEC under contract with Union Carbide Corporation.

W. Edgar Barnett, D. H. Brown
Oak Ridge National Laboratory

Effects of Nicotine Absorption in Rats during Pregnancy

Retrospective and prospective clinical studies on the effect of heavy smoking by women during pregnancy indicate an increase in abortions and in premature and underweight young. Population and treatment variables are not easy to control in such human studies.

When controlled populations of pregnant rats were injected twice daily with nicotine (0.5 or 1.0 mg/kg) throughout pregnancy, gestation times, birth weights, survival, and developmental status differed no whit from these same parameters in saline-injected controls. These daily doses were within range of those absorbed by heavy smokers, and they constituted the lower ranges of dose levels administered.

With 3 or 5 mg of nicotine, given twice daily, pregnant rats consumed far less food and gained far less weight than control mothers. Delivery dates were prolonged beyond term by 2 to 4 days or more. Young were underweight and fetal in appearance. Stillbirth and infant mortality rates were high. There were no abortions and no premature young. The fetal characteristics of the young were compared with those of post-mature young whose delivery was purposefully delayed by administration of chorionic gonadotropin.

Another group of pregnant rats imbibed nicotine in the drinking water; the drug severely cut down on their food and water intake. A control group

received the same reduction in food and water throughout pregnancy so that the effects of severe starvation and dehydration could be contrasted with the effects of nicotine.

R. FREDERICK BECKER, J. EDWARD KING
Duke University

Behavioral Modification by Injection of Brain Extract Prepared from a Trained Donor

Several recent reports have indicated that the injection of brain-derived fractions prepared from trained donors result in significant behavioral changes in the injected animals. In contrast to the inconsistent results with the extraction procedures designed to contain nucleic acid-like materials, those efforts which involved minimal or alternative prefractionation procedures have continued to yield evidence in support of the phenomenon of interanimal transfer of information via brain extracts.

Our initial goal was to obtain reproducible data by automatic testing procedures where the behavioral modification was quantitatively large enough to permit statistical significance with small groups of animals. Tests with reinforcement, as compared with tests without reinforcement, appear to be a more sensitive means of detecting behavioral modification(s) which are consistent with transfer of information among animals via brain extracts.

Tests with reinforcement demonstrated that intraperitoneal injection of brain extracts prepared from donors trained in bar-pressing shortened the time required to learn the process. The reproducibility of the enhancement of learning has been established in a series of experiments which include changes in the "strain" and sex of the rats and changes in the personnel. The enhancement phenomenon is consistent interanimal transfer of information, but the specificity and fundamental significance of the enhancement of learning by injection of brain extracts derived from a trained donor remains to be determined. Preliminary results with a modified training and testing procedure indicate that bar preference in a two-bar Skinner box, left versus right, might also be transferred.

Work supported in part by the AEC.

WILLIAM L. BYRNE
Duke University
DAVID SAMUEL
Weizmann Institute

Combinatorial Approach to State Vectors in U_n

The explicit expression of the orthonormal states of irreducible U_3 representations are obtained entirely by combinatorial methods, a procedure that admits of generalization to all U_n. This combinatorial enumeration problem, though now explicitly formulated, increases rapidly in complexity with increasing n; it is essential for the general problem to make use of the recent techniques in combinatorial analysis systematized by Rota, [*Z. Wahrscheinlichkeitztheor.* **2,** 340–368, (1964)]. By such methods we have obtained the expressions for the states of U_n with maximal quantum numbers, as well as of those whose U_{n-1} quantum numbers are maximal, directly from the partial order of the invariants of these states. These methods reveal the combinatorial content of the representations of SU_n which use the invariants of the group for labels of the basis vectors. Such methods also admit a natural generalization of the ordinary hypergeometric functions (which appear in formulating the states of U_3). A "tableaux calculus" will be presented, which represents states of U_n in terms of "generalized standard skew tableaux."

Applications of this work in nuclear and elementary particle physics will be briefly discussed.

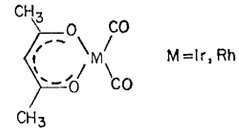
Work supported in part by the U.S. Army Research Office (Durham) and NSF.

M. CIFTAN
L. C. BIEDENHARN
Duke University

Anisotropic Electron Transport through Metal-Metal Bonds

Catenation of metals through the formation of covalent metal-metal bonds is now feasible. It is our belief that polymeric chains of metal-metal bonds may exhibit some of the properties of a metal. In particular, a one-dimensional polymeric arrangement of metal atoms might display novel solid-state properties, such as anisotropic conductivity and photoconductivity.

Seeking models to test this hypothesis, we have investigated electrical transport in crystals of planar d^8 group VIII complexes. X-ray analyses of single crystals show a one-dimensional metal-metal interaction along the needle axis of crystals of dicarbonylacetylacetonatoiridium and its rhodium analogue, which have the molecular structure.



Both a-c and d-c measurements over a range of temperatures reveal linear current-voltage relationships and the temperature dependence of an intrinsic semiconductor. The ratio of conductivities parallel and perpendicular to the needle axis is at least 500 for the iridium complex (σ parallel at 25° is 10^{-5} ohm^{-1}cm^{-1}, with an activation energy of 0.27 ev). These materials exhibit photoconductivity.

JAMES P. COLLMAN
C. G. PITT
L. K. MONTEITH
University of North Carolina, Chapel Hill, and Research Triangle Institute, Durham

Occurrence of Virus Particles in Sieve Tubes and its Relation to Virus Movement in the Plant

With regard to the establishment of systemic infection by plant viruses, the prevalent concept is that viruses are transported in the phloem through the sieve tubes and that this transport parallels the translocation of organic substances. The slow, local spread of infection is assumed to be a cell-to-cell movement through plasmodesmata. In the minor veins of leaves of *Beta vulgaris* L. complete particles of beet yellows virus were recognized in mature sieve elements and in the associated parenchyma cells. They were present also in the channels in the walls that connect sieve elements with one another, sieve elements with parenchyma cells, and parenchyma cells with one another. The distribution of virus particles in the phloem of the beet indicates that the virus moves from cell to cell and within the translocation stream in the food conduit as complete particles. It also supports the postulate that the virus is carried passively with the organic solute in accordance with the concept of mass-flow mechanism of translocation in the phloem.

K. ESAU, J. CRONSHAW
L. L. HOEFERT
University of California, Santa Barbara

Basic Biophysics of Blood Clotting

With appropriate logic, two basic biophysical principles apply to blood clotting, specifically in key reactions of the enzymic conversion of prothrombin to thrombin [*Fed. Proc.* **23,** 762 (1964)]. Thrombin yields are measured as clotting times for a standard fibrinogen, and are followed to the equilibrium end points by testing successive samples of incubated mixtures of prothrombin and activators (here nanograms of thrombokinase, with phospholipid [cephalin], cation [Ca^{++}, Sr^{++}, or Ba^{++}], and factor V). Colloidal aspects of enzyme action obey an "inverse law" (O'Sullivan and Tompson, 1890), from which a log-log rectilinear relationship is derived. This holds for plots of end-point clotting times (on ordinate), against factor concentration variable (on abscissa), when this is prothrombin or phospholipid but only to a limited extent when this is factor V. Classical enzyme kinetics, in the form of (modified) Lineweaver-Burk (1934) double-reciprocal rectilinear plots, hold for end-point clotting times ($\sim 1/v$, on ordinate), versus "substrate" concentration variable ($\sim 1/c$, on abscissa), when this is thrombokinase (needing cation to yield enzymic product), specific cation cofactor (for example, Ca^{++}), or factor V (not an enzyme in its own right). Applying these analyses to "successive" suboptimal factor additions provides further evidence for the key reactions: (i) prothrombin + phospholipid → *micellar* substrate complex and (ii) thrombokinase + cation → *true* prothrombin-convertor enzyme, (as well as evidence that) factor V acts in both, like an "amboceptor." This basic approach offers a new and fundamental explanation of blood clotting.

JOHN H. FERGUSON
University of North Carolina

Evidence Concerning Violation of Charge Conjugation Invariance in Decay of the Eta Meson

One hypothesis invoked to explain the decay, $K_L^0 \rightarrow \pi^+ + \pi^-$, which apparently violates CP invariance, is that the electromagnetic interaction of the hadrons violates charge conjugation invariance. One possible consequence of this hypothesis is that an asymmetry in the division of available energy between the positively and negatively charged particles in $\eta \rightarrow \pi^+\pi^-\pi^0$ decay would occur. If such an asymmetry is observed, either the η must not be a pure state or C must be violated in its decay. The reaction $K^- + p \rightarrow \Lambda + \eta$ has been used to produce a sample of etas decaying to three pions. Seven-hundred-and-forty MeV/c K^- particles emerging from the Brookhaven National Laboratory's Alternating Gradient Synchrotron are separated from other particles with static, crossed, electric and magnetic fields and focused on the 30-inch hydrogen bubble chamber. The resulting 275,000 triads of stereophotographs have been scanned and measured at Duke University and have yielded about 650 identified $\eta \rightarrow \pi^+\pi^-\pi^0$ events. It is already established that the amount of asymmetry is smaller than 10 percent. Results from this experiment deviate from symmetry in the same direction ($T_+ > T_-$) and roughly the same amount (about 5 percent) as other published results. Many more events are needed and are being sought to settle this question.

L. R. FORTNEY, J. W. CHAPMAN
S. DAGAN, E. C. FOWLER
Duke University

Genetic Control of Xanthine Dehydrogenase from Drosophila melanogaster

In *Drosophila melanogaster,* three loci affect xanthine dehydrogenase (XDH). The *ma-l* and *ry* mutants contain traces, if any, of this enzyme, while the *lxd* mutant has about 20 percent normal activity. Antigenic cross-reacting material to XDH is present in normal amounts in *lxd* and *ma-l* but is almost totally lacking in *ry*. This indicates that *ry* is a structural gene for xanthine dehydrogenase, a conclusion supported by the existence of four electrophoretic variants which map at the *ry* locus, and which are designated ry^{el-F}, ry^{el-I}, ry^{el-SI} and ry^{e-S}. It is not clear, however, what role $ma\text{-}l^+$ and lxd^+ play in the formation of the enzyme. Another enzyme, pyridoxal oxidase, is also deficient in *ma-l* and *lxd,* but is present in normal amounts in *ry* flies. In addition, extracts of *ma-l* and *ry* flies can undergo complementation in vitro since incubation of a mixture of extracts from these flies produces xanthine dehydrogenase activity, indicating the presence of an ry^+ complementation factor in *ma-l* and an $ma\text{-}l^+$ complementation factor in *ry* flies. The molecular sizes of both these factors exceeds 200,000 as does the molecular size of xanthine dehydrogenase, the cross-reacting material, and pyridoxal oxidase. The $ma\text{-}l^+$ complementation factor is also missing in *lxd* flies, indicating again a similarity between underlying chemical defects in these flies. Thus, both *ma-l* and *lxd* mutants contain normal amounts of cross-reacting material, and both lack pyridoxal oxidase and the $ma\text{-}l^+$ complementation factor. Two likely hypotheses to account for the effects of *ma-l* and *lxd* are that these loci control (i) production of a polypeptide chain or (ii) production of a cofactor which is shared by xanthine dehydrogenase and pyridoxal oxidase. Immunological and other data indicate that the shared cofactor hypothesis is more likely to be correct.

EDWARD GLASSMAN
University of North Carolina

Fractionation of Complementary Strands of Bacterial DNA's

Complementary strands of a number of bacterial DNA's fractionate on equilibrium banding in alkaline CsCl (Guild and Robison, 1963, and new data), but those of others do not (*Bacillus subtilis, Escherichia coli,* T–4 phage, for example). Szybalski *et al.* (1964, 1966) found that guanine-rich ribopolymers, particularly polyG, bound preferentially to one of the strands of *B. subtilis* DNA, increasing the buoyant density in neutral CsCl and allowing fractionation. PolyG does *not* fractionate pneumococcal DNA, but polyGU does. Since the alkaline separation is due to a bias in the distribution, between strands, of guanine (G) and thymine (T), which titrate in alkali and bind an extra Cs^+, the correlation of polyGU with the GT alkali bias is probably significant. Preparative fractionation of denatured pneumococcal DNA with polyGU yields *heavy* fractions which are *light* in alkaline CsCl, and vice versa. Differences in phenotypic expression time of cells newly transformed by these DNA fractions have been found for four drug resistance markers (novobiocin, bryamycin, erythromycin, and streptomycin). The combined results with alkaline CsCl and various ribopolymers indicate that each species has a different optimum pattern of fractionation. Since in both our studies and those of others, fractionation correlates with transcription into message RNA,

the next question is whether a specific local sequence of bases controls both phenomena.

Study aided by NIH grant GM-10965-06.

WALTER R. GUILD, ELIZABETH GREEN
JOHN M. PETERSON
Duke University

Zero Magnetic Field and Experiments on Time-Reversal Invariance

Experiments on quantized flux have indicated the possibility of obtaining a macroscopic region inside a superconducting shield with truly zero magnetic field. If a hollow superconducting shield is cooled in a field corresponding to less than half a flux unit through the cross section of the shield, experiments show that there will be no trapped flux inside the shield. [B. S. Deaver, Jr., and W. M. Fairbank, *Phys. Rev. Letters* **7**, 43 (1961)]. We are constructing an apparatus for producing such low fields by the successive expansion of superconducting bladders. This apparatus is designed to have an accessible room-temperature region, 75 cm in diameter, in which the magnetic field is less then 10^{-10} gauss. In such a field a hollow superconducting spherical shield 28 cm in diameter should trap no flux when cooled through its transition temperature. Aligned He^3 nuclei placed at the center of such a spherical shield should remain oriented, given sufficiently long relaxation times and perfect spherical symmetry. An electric field applied to the He^3 would cause an observable precession if the nuclei have a permanent electric dipole moment larger than 10^{-23} *e* cm. Experiments designed to measure a dipole moment would be an indication of a breakdown of time-reversal invariance. Indications of a possible breakdown of time-reversal invariance have recently been reported. [J. H. Christenson, J. W. Cronin, V. L. Fitch, R. Turlay, *Phys. Rev. Letters* **13**, 138 (1963)]. The orientation of the He^3 nuclei will be detected by a very sensitive superconducting magnetometer. This magnetometer and its application to other experiments in low-temperature physics will be discussed.

Work supported by Air Force Office of Scientific Research, Avionics Laboratory and Aerospace Research Laboratory.

WILLIAM O. HAMILTON
WILLIAM M. FAIRBANK
Stanford University

Interaction of Hydrogen Atoms and Hydroxyl Radicals with 5-Halogen Uracils

Electron spin resonance was used to study products of interaction of gaseous sprays of hydrogen atoms and hydroxyl radicals with powdered 5-halogen uracils. With the exception of 5-Cl-uracil at room temperature, the electron spin resonance spectra for the bombarded compounds at room temperature and at 77°K are the same as those for similarly bombarded uracil. These results indicate that halogen atoms are first replaced by hydrogen atoms and that the uracil molecules thus formed interact with additional H atoms or OH radicals in a way already known [*Science* **153**, 1649 (1966); *Proc. Natl. Acad. Sci. U.S.* **54**, 1287 (1965)]. The mechanism of replacement for 5-F-uracil is different from that for 5-Br-uracil and for 5-I-uracil. In the former, F atoms are abstracted by H atoms, forming stable HF molecules and uracil radicals. The uracil radicals thus produced are transformed into stable uracil molecules by reaction with additional H atoms. In the Br- and I-uracils, the halogen atoms are directly replaced by hydrogen atoms. In 5-Cl-uracil at room temperature, H atoms add directly to carbon No. 6 to form an addition radical; at 77°K the Cl is first replaced by H, and then addition radicals are formed with the resulting uracil. When exposed to the discharge products of H_2O or H_2O_2, the halogen in all the compounds, except 5-Cl-uracil, is replaced, and uracil-like OH addition radicals are formed. The spin densities calculated in accord with molecular orbital theory for the OH addition radicals agree with the observed values.

Study supported by the U.S. Air Force Office of Scientific Research, grant AF-AFOSR-493-66, and by the Army Research Office (Durham), grant DA-ARO-D-31-124-G731.

JANKO HERAK, WALTER GORDY
Duke University

Experimental Determination of Free Energy Changes in Helix-Coil Transitions of Polypeptides and Denaturation of Proteins

With various optical methods it is possible to determine helix-coil equilibria and protein denaturation equilibria as a function of *p*H and temperature. The difficulty lies in converting these data to molar equilibrium constants or free energies. One can only say that $\Delta F^\circ_{den} = 0$ at the midpoint of the transition. The calorimetric determination of molar enthalpies of denaturation will allow the calculation of ΔF°_{den} at other points. Another method, pointed out by Zimm and Rice, consists of measuring ΔV°_{den}, the number of protons bound upon denaturation, and using $\Delta F^\circ_{den} = RT\int \Delta V^\circ_{den}\, d\ln a_{H^+}$. We have applied this last method to obtain ΔF°_{den} for poly-L-glutamic acid as a function of temperature, urea concentration, and ethanol concentration. It is found that $\Delta F^\circ_{den} = -100$ cal/mole and $\Delta H^\circ = -1000$ cal/mole of monomer unit at 25°. Urea decreases ΔF°_{den} at low concentrations as required by Schellman's binding theory, but little change of ΔF°_{den} occurs between 3 and 8*M* urea concentration. Ethanol increases ΔF°_{den} appreciably. The results on the thermal transition of polyglutamic acid in 8*M* urea are in good agreement with the theory of the helix-coil transition. In contrast, results obtained studying the denaturation of spermwhale myoglobin as a function of temperature and *p*H indicate that the reversible denaturation can be described as an equilibrium between only native and fully denatured molecules. For this reaction at *p*H 9, $\Delta F^\circ_{den} = 16$ kcal/mole at 25° and ΔH°_{den} varies from 20 kcal/mole at 0° to 150 kcal/mole at 80°, the transition temperature.

JAN HERMANS, JR.
University of North Carolina

Evolutionary Origins of the Immunoglobulins

The three major classes of immunoglobulins, IgA, IgG and IgM, are composed of two different types of polypeptide chains. The K and λ type chains, or light chains (molecular weight 25,000), are found in all major classes, whereas each class possesses a unique heavy chain (molecular weight 50,000). We have recently established the sequence of 161 residues from the COOH-terminus of the heavy chain derived from rabbit IgG. This sequence has been compared with that established by others for the Bence-Jones proteins, which are considered to be one type of human light chain. This comparison shows a considerable homology in sequence between light and heavy chains. On the basis of this homology it is now possible to formulate a scheme for the evolutionary origins

of the immunoglobulins. It appears that the gene for the light and heavy chains of the three major classes of immunoglobulins possess a common ancestor. This ancestor, which is a gene that controlled the sequence of a chain containing 110 to 120 residues, gave rise to light chains by a process of duplication so as to form a light chain (K and λ type) of some 212 to 215 residues. These chains in turn, again by duplication, gave rise to the heavy chains, which are recognized in each of the major immunoglobulin classes. This scheme explains many of the structural variations as well as similarities among the immunoglobulins; it may generally account for the evolutionary origins of other proteins which are structurally analogous to the immunoglobulins.

R. L. HILL, H. E. LEBOVITZ
R. E. FELLOWS, JR., R. DELANEY
Duke University

Standard Errors of Correlation Coefficients from Non-Normal Populations

The only standard error of the correlation coefficient r widely known or used is $(1 - \rho^2)/n^{1/2}$, where ρ is the correlation in a hypothetical infinite population. This is an approximation, the first term of a convergent series of powers of $n^{-1/2}$. Since ρ is usually not known, the sample value r is often used instead, introducing a bias. The bias is however largely offset by using R. A. Fisher's z, the inverse hyperbolic tangent of r with standard error approximately $n^{-1/2}$.

The accuracy of these and related methods depends among other things on a correctly assumed basic bivariate normal (that is, "Gaussian") population. Where the distribution is not really normal, the errors of the correlation coefficient may be considerable. Thus if the true probability distribution has a constant positive density within an ellipse, with zero probability outside it, the square of the standard error formula above needs to be multiplied by $\frac{2}{3}$ even for very large factors.

HAROLD HOTELLING
University of North Carolina

Nuclear Relaxation in Solid Helium-3

We have carried out measurements of the nuclear longitudinal relaxation time, T_1, in solid He^3 between 0.03 and 0.5°K, using pulse techniques. For all the densities investigated, we have found two temperature regions in which the dominant relaxation processes are very different. In the first region, down to about 0.3° to 0.2°K, the recovery of the magnetization after saturation by radio-frequency pulses is exponential with time and strongly dependent on temperature. This is the region where relaxation takes place by means of lattice vibrations (phonons). In the second region, at lower temperatures, the recovery is no longer exponential and is practically independent of temperature. This is consistent with relaxation measurements down to 0.12°K by Richards, Hatton, Gifford [*Phys. Rev.* **139**, A91 (1965)]. The characteristic time constant in this region decreases appreciably with the size of the helium sample, as was shown by experiments with different cavities. The absence of temperature dependence of this relaxation process makes its interpretation in terms of phonon processes difficult. The possibility of spin diffusion processes must therefore be considered. In this process, an excited spin state diffuses to a boundary where relaxation takes place. The results will be presented and interpretations of such processes will be discussed and compared with the experiment.

E. R. HUNT, R. C. RICHARDSON
J. THOMPSON, R. GUYER, H. MEYER
Duke University

Binding of Pyrimidine Nucleotides to Ribonuclease-A and to Derivatives of This Enzyme

The binding of phosphate ions and pyrimidine nucleotides to ribonuclease-A (RNase-A) and to 41-DNP-RNase-A, carboxymethyl RNase-A, and S-protein has been evaluated by spectrophotometry and by equilibrium dialysis. In each case the binding number n was approximately one ligand per molecule of protein. For RNase-A the intrinsic binding constant k was: for phosphate, 5.2×10^3; for 2′-CMP, 2.5×10^4; for 3′-CMP, 2.7×10^4. In the case of 41-DNP-RNase-A the values of k were: for 2′-CMP, 1.6×10^4; for 3′-CMP, 1.1×10^4; for 2′,3′-cyclic CMP, 1.3×10^4; for 2′,3′-cyclic UMP, 1.3×10^4. In the case of S-protein the values of k were: for 2′-CMP, 1.5×10^3; for 3′-CMP, 1.1×10^3; for 2′,3′-cyclic CMP, 0.9×10^3; for 2′,3′-cyclic UMP, 2.1×10^3. Binding of pyrimidine nucleotides to carboxymethyl RNase-A was too weak for quantitative determination. The binding data will be used to support a proposed modification of the mechanisms of RNase-A action described by Witzel and by Findlay *et al.* According to this modified mechanism, the imidazole groups of histidine-119 and histidine-12 are involved in enzyme binding of the phosphate group of substrates while the ε-NH_2 group of lysine-41 is a catalytic group possibly involved in "activation" of water.

J. H. IM
J. LOGAN IRVIN
University of North Carolina

Interaction of Phenazinium Radicals with DNA

The free radical of N^9-methylphenazinium methyl sulfate was prepared by sodium borohydride reduction in aqueous acetate buffer. The observed electron paramagnetic resonance spectrum consists of nine peaks, which can be separated into about 120 hyperfine components under high resolution. The spectrum of the N^9-trideuteromethyl homologue was studied in the same manner, and the analysis of these spectra leads to the conclusion that the radical species observed was the monopositive ion of N^9-methyl-10-protophenazine, taking into account the results of molecular orbital calculations.

Strong quenching of the hyperfine lines was observed when a solution of salmon sperm DNA (0.001*M* NaCl) was added to the solution containing the radical. At high DNA concentration only a single broad line was observed, because of complex formation between DNA and the radical. The optical spectrum of the radical was also studied. A 6-mμ red shift observed in the visible region was brought about by the interaction between the radical and DNA. Complex formation was strongly inhibited by the presence of alkali or alkaline earth cations, and the effect of the latter was about ten times larger than that of the former. From these experiments it may be assumed that the phosphate groups of DNA play an important role in binding the radicals. Delocalization of the unpaired electron into the DNA molecule may increase the stability of the radical and enhance binding of the radical to DNA.

K. ISHIZU
H. H. DEARMAN
M. T. HUANG
J. R. WHITE
University of North Carolina

Masking the Effects of Insect Sex Attractants

Insect sex attractants are of great value as survey tools to determine the location and size of natural infestations of destructive insects. Their potential use in insect control, either alone or in conjunction with attractive light traps, insecticides, or chemical sterilants, makes the sex attractants even more interesting to investigators in the pesticide field. It has, however, become increasingly evident that the attractiveness of insect pheromones may sometimes be masked by their admixture with certain contaminants. The outstanding example of this phenomenon has been found with the synthetic sex attractant for the gypsy moth (gyplure); as little as 3 percent ricinoleyl alcohol is sufficient to mask attractiveness completely. Natural masking agents have been found to occur with the sex attractants of the gypsy moth, introduced pine sawfly, bollworm, tobacco budworm, American cockroach, and cynthia moth; masking of food attractants has been shown for the common vinegar fly (*Drosophila*). Although masking of attractants may be a significant problem in attempting to demonstrate the presence of a sex attractant in a particular insect species, as well as in the practical use of attractants for insect survey and control, these findings offer the possibility of suppressing insect populations by releasing masking agents for natural attractants.

MARTIN JACOBSON
U.S. Agricultural Research Service, Beltsville, Maryland

Status of Research on Surtsey, Iceland's Volcanic Island

Surtsey, a permanent volcanic upthrust off southern Iceland, is a unique site for biological, physical, and chemical research. Since its origin in 1963, geochemists and geophysicists have investigated atmospheric and aquatic changes resulting from volcanic activity. Biological studies, particularly as they relate to colonization and succession, are prominent in the fields of bacteriology, mycology, entomology, lichenology, and ecology of vascular plants. Studies in these areas, conducted largely by foreign scientists, are in initial stages and are coordinated by the international Surtsey Research Society.

T. W. JOHNSON, JR.
Duke University

Source of Carbamyl Phosphate for Pyrimidine Biosynthesis in Mouse Ehrlich Ascites Cells and Rat Liver

Several laboratories have failed to demonstrate an enzyme synthesizing carbamyl phosphate in Ehrlich's ascites cells or in the liver of mammalian fetuses, despite the fact that these tissues synthesize nucleic acids rapidly. Recently we [*J. Biol. Chem.* **240**, 4556 (1965)] studied pyrimidine synthesis in intact ascites cells and our data suggested the existence in these cells of a carbamyl phosphate synthetase which requires glutamine or ammonia (as the nitrogen source) and bicarbonate (as the carbon source) for biosynthesis of carbamyl phosphate.

We have now been able to extract a carbamyl phosphate synthetase which occurs in the soluble fraction of the ascites or fetal rat liver cell. ATP, bicarbonate, and glutamine ($K_m \leqslant 10^{-5}M$) or ammonium ion ($K_m = 5 \times 10^{-3}M$) are required for synthesis of carbamyl phosphate. Glutamine analogs inhibit this enzyme.

The enzyme is extremely unstable, and all activity is lost at 4°C during the time interval required to separate the homogenate into a heavy particulate (nuclear), mitochondrial, microsomal, and soluble fractions unless magnesium and ATP are added to the fresh homogenate.

The localization of this glutamine–carbamyl phosphate synthetase and aspartate transcarbamylase in the soluble portion of the liver cell while the acetylglutamate-requiring carbamyl phosphate synthetase (which utilizes only ammonia as a nitrogen source) and ornithine transcarbamylase are located in the mitochondria suggests that each synthetase provides carbamyl phosphate for only one biosynthetic pathway—either arginine or pyrimidine biosynthesis.

MARY ELLEN JONES
SALLY E. HAGER
Brandeis University

Comparison of Repair and Fixation Processes with Radiation and Chemical Mutagens

Previous work with *Paramecium aurelia* with x-rays has shown that two main classes of mutational processes can be distinguished: (i) those in which conversion of premutational damage to final mutation (fixation) occurs at or near the normal DNA synthetic period and (ii) those in which conversion occurs at other times in the cell cycle. The rate of repair of damage responsible for class ii, and possibly class i, processes varies during the cell cycle. The alkylating agent, triethylene melamine (TEM), produces mutations almost if not entirely by class i processes, and the rate of repair is essentially constant through the cell cycle. With ultraviolet light, there is some evidence that mutations arise through a class i process, but the premutational damage becomes irreversible by photoreactivation independently of DNA synthesis. Finally there is some evidence that the chemical mutagen, *N*-methyl-*N'*-nitro, *N*-nitrosoguanidine, produces initially reparable premutational damage. It is expected that some of the properties of fixation and repair will be worked out for this mutagen before the paper is given.

Research sponsored jointly by NIH and AEC under contract with Union Carbide Corporation.

R. F. KIMBALL
Oak Ridge National Laboratory

Metabolic Requirements for Secretion from the Adrenal Medulla

Recent studies on the secretion of catecholamines from the isolated perfused adrenal gland have shown that during stimulation a specific protein contained in the catecholamine storage vesicles was released, together with the catecholamines, in relative amounts similar to those present in the intact gland. It was also shown that the rate of release of catecholamines and vesicle protein at 33° was two to three times the rate at 23°, indicating the chemical nature of the secretory process. Further studies indicate a metabolic requirement for secretion. When potassium cyanide or iodoacetic acid alone was added to Locke's solution perfusing an isolated bovine adrenal gland, stimulation of secretion, evoked by acetylcholine, nicotine, or Ba^{++}, was not inhibited. However, when both cyanide and iodoacetate, each at $10^{-4}M$ concentration, were present in the perfusion medium, stimulation by acetylcholine, nicotine, or Ba^{++} was completely inhibited. The inhibitory effect of cyanide could be reversed by flushing the gland with Locke's solution; the inhibition due to iodoacetate was irreversible. Antimycin or oligomycin by themselves did not inhibit secretion, but either one in combination with iodoacetate com-

pletely and irreversibly inhibited secretion evoked by acetylcholine.

Supported by PHS grant AM-05427.

N. KIRSHNER, W. J. SMITH
Duke University

Small-Angle X-Ray Study of Transfer RNA

Small-angle, x-ray diffraction measurements were performed upon alanine transfer RNA (tRNA) and bulk yeast tRNA in 0.1*M* tri(hydroxymethyl)aminomethane hydrochloride buffer at *p*H = 7.0. The radius of gyration obtained for each type of molecule was 23.3 and 23.9 Å. Addition of 0.1*M* $MgCl_2$ to the buffer caused aggregation (possibly dimerization), as shown by upward curvature in the inner region of the Guinier plots. This was less pronounced for alanine tRNA. The scattering curves of both types of tRNA can be matched to the curve of a prolate ellipsoid having axial ratio 3.98:1. The equivalent ellipsoid for alanine tRNA has semi-axes 49.0 and 12.3 Å (overall dimensions 98 by 24.6 Å), while that of bulk yeast tRNA has semi-axes 50.3 and 12.5 Å. The volume of the scattering particle, as obtained from the invariant, is 34,300 $Å^3$ for alanine tRNA, which corresponds to 0.21 g of H_2O per gram of tRNA. The volume for bulk yeast tRNA is 35,600 $Å^3$. The surface-volume ratio obtained from the invariant is 0.293 $Å^{-1}$ for alanine tRNA, and 0.300 $Å^{-1}$ for bulk yeast tRNA. These ratios are considerably larger than that expected for a smooth prolate ellipsoid (0.204 $Å^{-1}$ for the ellipsoid equivalent to alanine tRNA), probably because of the nucleotides that loop out of the helical regions.

The tRNA model of Brown and Zubay appears to best fit the small-angle results. Our data provide further evidence for size differences among the various specific tRNA molecules. Finally, it is of interest that there is a similarity between the dimensions we have obtained for molecules of tRNA, those deduced by Timascheff, Witz and Luzzati for the individual helical segments of high polymer RNA, and those obtained by Spencer, Fuller, Wilkins, and Brown for partially degraded high-polymer RNA. This suggests the possibility that the individual specific tRNA molecules may be formed by multiple cleavage of high polymer RNA in the nonhelical regions.

W. R. KRIGBAUM, R. W. GODWIN
Duke University

Anti-Bacterial Action of a New Series of Positively Charged Steroidal Compounds: Spectra of Activity, Structure Activity Relationships, Mechanism of Action

A new series of steroid (bile acid) compounds containing a positively charged quarternary nitrogen group in the side chain was prepared and found to inhibit bacterial growth. These compounds are effective against *Pseudomonas aeruginosa, Staphylococcus aureous, Bacillus subtilis* and *Streptococcus hemolyticus*. The effective concentrations, which varied with the analogue and bacterial species, ranged between 3×10^{-4} to 2×10^{-5} *M*. Studies of the mechanism of action of these compounds suggest two loci of action: (i) these compounds affect the structure of the bacterial membranes, as evidenced by their enhancement of bacterial swelling; and (ii) they affect protein synthesis, as indicated by their ability to inhibit enzyme and permease induction. These two events are apparently separate. Thus, negatively charged analogues can affect bacterial swelling (membrane phenomenon) but they have no adverse effect on induction. In addition, one can vary the ionic strength of the test media and elicit separately either bacterial swelling or induction inhibition by the positively charged compounds. Structure-activity studies demonstrate that optimum activity requires α-hydroxyl substitutions at the 3 and 7 positions of the cholanic acid moiety.

LEON LACK, FREDERICK BERNHEIM
Duke University

A Proposed Energetic Mechanism for Photophosphorylation

Protons, AsO_4^{--}, and NH_4^+, have been shown to be competitive inhibitors of P_i in cyclic or noncyclic phosphorylation in chloroplasts. Likewise, P_i, AsO_4^{--}, NH_4^+, and other monovalent cations are competitive inhibitors of protons in the light-induced cation/H^+ exchange reaction of chloroplasts. Since it appears from the above observations that protons compete for the same electron-activated fixed anionic sites in chloroplasts, removal of a proton from bound magnesium P_i by the passage of an electron through a semiconducting anionic lattice, possibly acidic lipids, within chloroplasts, should result in esterification of P_i. In the absence of P_i, the passage of electrons through this lattice nonspecifically increases the affinity of this lattice for cations, with the result that marked cation exchanges occur both in the light and in the dark. The proposed mechanism of oxidative phosphorylation in chloroplasts is probably operative in mitochondria as well, since the same qualitative relationships that have been shown to exist between cation exchanges and oxidative phosphorylation also exist in mitochondria.

WILLIAM S. LYNN
Duke University

Antibody Synthesis Several Days After Exposure of Functional Spleen Cells to 10,000 Roentgens

In view of recent advances in genetics of antibody molecules, there is a need for systematic biochemical studies of antibody synthesis at the intracellular level. Unfortunately, progress in this field has been curtailed to a large extent because the vast majority (95 to 99 percent) of the cells of any lymphatic tissue of an individual undergoing maximum antibody response are not synthesizing the specific antibody, and various physical attempts (centrifugation, electrophoresis, and chromatography) to selectively separate antibody-forming cells from cells not synthesizing antibody have not been very successful; that is, a purification factor of greater than 10 has not been achieved. Previous cytokinetic studies on antibody formation indicated to us that ionizing radiation might be used as the selective agent to separate specific antibody-synthesizing from other lymphoid cells. Spleen cell cultures were therefore exposed to 10,000 r at the end of the logarithmic phase of secondary antibody response and then cultured for an additional 3 days. The total number of cells decreased drastically. Most of the cells were killed, but the survivors, of which up to 80 percent of the free cells were plasma cells, synthesized as much specific antibody as unirradiated control cultures, as judged in terms of antibody titer and incorporation of ^{14}C-amino acid into γG globulins.

Research sponsored by AEC under contract with Union Carbide Corporation.

T. MAKINODAN, PAUL NETTESHEIM
TOSHITERU MORITA
CAROL J. CHADWICK
Oak Ridge National Laboratory

New Staining Technique for Ultrastructure Research

For electron microscopy of biological and organic materials of low electron density, a novel method of introducing contrast has been developed. It consists of injection of atoms directly into the structure and is essentially independent of chemical bonds. This has been accomplished with cesium atoms with the aid of a special low-voltage (10 to 500 electron volts) accelerator. Several atoms per square angstrom can be injected in reasonable exposure time. Good contrast is obtained at quite low concentration. The energy is deliberately kept low to avoid radiation damage. In distinction to conventional chemical stains, the atoms which provide contrast detail are preferentially accepted in structural holes of atomic dimensions or regions of low binding of the specimen. Furthermore, since penetration of several atomic layers, depending on local bond strength, can be obtained, more detail is revealed than by shadowing with heavy metals.

The method is illustrated with new results on the structure of collagen and examples of the appearance of other biological materials as well as some organic films.

Work performed under the auspices of AEC.

J. H. MANLEY
Los Alamos Scientific Laboratory

Behavior of Low-Voltage Gas-Focused Electron Streams

Steadily running, noise-free, gas-focused electron streams have been produced, and measurements of the beam energy profile and noise pattern have been made with a grided collector. The beam source used was an electron gun with an oxide-coated filament. By suitable electrode geometry and appropriate electrode potentials, it has been found that long-lived filaments may be operated in pressures of 1 micron or less of mercury vapor.

The stream is injected into a cylindrical glass tube, the inside walls of which have been made electrically conductive by a transparent coating. Grided "wall" probes have been used to measure the ion and electron energy distributions of the background particles. Examination of the data suggests that the radial ion motion is largely free flight but that a small fraction of the ions, in particular the low-energy components, are dominated by collision in their radial motion. Measurements of the potential drop supported by the beam itself and also of the total potential existing between the beam and the wall have been made with the "wall" probes for various beam voltages, currents, and background pressures. These measurements were made when gun and collector voltages were chosen to produce a uniform beam potential in the axial direction.

R. A. MCCORKLE, W. H. BENNETT
North Carolina State University

Contributions of Inner Shell Electrons to the Ionization Energy Loss of Protons

Recent improvements in the accuracy of measurements of the ionization energy loss of charged particles, especially protons, traversing matter have encouraged us to take a new look at the theory of the stopping process. The availability of computers for algebraic as well as numerical work has enabled us to make detailed calculations of the interaction between incident protons in the nonrelativistic energy range (but above a few hundred thousand electron volts) and the tightly bound atomic electrons in the K, L, and M shells. By treating the various subshells separately, we have obtained improved inner shell corrections to the Bethe theory of energy loss. The accuracy of these calculations is still limited by the neglect of Coulomb distortions of the proton orbit and by the use of hydrogenic wave functions. Preliminary results of calculations by G. Basbas, which avoid these simplifications, will also be discussed.

Supported in part by the AEC.

EUGEN MERZBACHER
GOVIND S. KHANDELWAL
University of North Carolina

Basidiospore Discharge: A Generalized Concept Based on Cell Biology

To explain the apparent anomaly of how a bursting lateral gas vesicle can project a basidiospore along the axis of the sterigma, Olive and Savile [*Science* **148**, 533 (1965)] have suggested additional or alternate mechanisms. However, it is felt that electron micrographs by Wells [*Mycologia* **57**, 236 (1965)] can provide the basis for resolving this enigma. These outstanding figures show the initiation of the basidiospore as an enlarging swelling from the sterigma tip. Later stages show the nucleated basidiospore before discharge with a parahilar, subspore area and the beginnings of the gas vesicle beneath an extension of the sterigma wall around the adaxial face of the maturing basidiospore. It is postulated that after a nucleus passes through the sterigma, there is a cytokinetic centripetal plasma membrane invagination that separates the new spore protoplast from that of the basidium, and that subsequent respective contractions of the severed cytoplasmic isthmus establish a cylindrical chamber, within the sterigma apex beneath the spore base, that is continuous with the developing lateral gas reservoir. It is immaterial where the gas is stored but only significant where its pressure is applied when it is released. Therefore, it seems probable that when the pressure becomes sufficient to rupture the parahilum—the juncture of sterigma and spore—the force of the gas will be directed against the top and bottom of the intercellular chamber. Since the base of this chamber is the immobile sterigma, the total thrust will perforce be applied to the spore propelling it outward from the hymenium in the manner reported by Buller (*Researches on Fungi*, vol. 1, 1909).

ROYALL T. MOORE
North Carolina State University

Cytoplasmic and Intra-Nuclear Microtubules in Relation to Development, Chromosome Morphology, and Motility of an Aflagellate Spermatozoon

The sperm of the Homopteran insect *Steatococcus tuberculatus* (n=2) is a pencil-like structure consisting of only a chromosomal core and thin cytoplasmic sheath. Its bizarre development from a papilla on the spermatid, originally described by Hughes-Schrader [*J. Morphol.* **78**, 43, (1946)], raises fundamental questions about the mechanostructural basis for: (i) elongation and rigidity of the simple, filamentous sperm; (ii) migration in tandem of the two chromosomes in predictable sequence from the spermatid nucleus into the sperm tube; and (iii) motility in the absence of a flagellar apparatus.

Morphological and cytochemical observations with the electron microscope suggest that the following process takes place. Cytoplasmic microtubules originate as anlage adjacent to the nuclear

envelope coincident with the formation of a papilla. By addition of new material at their basal ends, the anlage extend outward, carrying a cytoplasmic sheath with them. They progressively acquired characteristic 250-Å-diameter microtubular morphology. Proximally, microtubule anlage contact the nuclear envelope; as they elongate, the associated envelope appears to be pulled out into a tube, which is extended and held rigid by them. Chromatin microfibrils 15 to 20 Å in diameter align against the envelope in the vicinity of the papilla and are drawn out with it to form the chromosomal core as it extends. This apparently constitutes the mechanism of chromosome migration. The microfibrils contain DNA and aggregate to form microtubules 75 Å in diameter. As many as 60 may be counted in a chromosomal cross section, an observation difficult to reconcile with a unineme model of chromosome structure. Further coalescence leads to an absence of visible substructure in the two chromosomes of the mature sperm. Approximately 80 cytoplasmic microtubules are tightly packed in two or three concentric rings around the chromosome core in the mature spermatozoon. Cytochemical evidence of ATPase activity associated with the microtubules suggests their participation in an energy-releasing system concerned with motility of the sperm.

Supported by PHS grant (GM-06753) and The American Cancer Society (E-213).

MONTROSE J. MOSES
Duke University

Further Purification of Pig Thyrocalcitonin

Thyrocalcitonin is the hypocalcemic, hypophosphatemic polypeptide of mammalian thyroid gland discovered by Hirsch, Gauthier, and Munson in 1963 (*Endocrinology* **73**, 244). A considerable degree of purification of thyrocalcitonin from pig thyroid extracts has been achieved independently in several laboratories. The method of Tenenhouse, Arnaud, and Rasmussen [*Proc. Nat. Acad. Sci. U.S.* **53**, 818 (1965)], using gel filtration on Sephadex G–75, was of special interest because it resulted in the isolation of a homogeneous polypeptide which was biologically active. We have repeated this work and obtained an active product with the same amino acid composition as reported by Tenenhouse *et al.* However, the yield of activity from starting material was low. Quantitative biological assay of neighboring eluates revealed a more retarded fraction possessing about ten times the specific biological activity of the previously isolated peptide and accounting for the major portion of activity in the starting material. The amino acid composition of this more active fraction was so different from that of the peptide isolated by Tenenhouse *et al.* that the likelihood that it represents degradation products seems remote. The highly active fraction is heterogeneous, showing five bands on analytical polyacrylamide gel electrophoresis. However, they appear to be separable on the preparative gel column.

Supported in part by NIH grants AM–8261 and AM–10558.

PAUL L. MUNSON
University of North Carolina
JOHN T. POTTS, JR., RALPH A. REISFELD
National Institutes of Health
CARY W. COOPER, EDWARD F. VOELKEL
Harvard School of Dental Medicine

Specific Action of Tetrodotoxin Derivatives on Nerve

Tetrodotoxin, the active ingredient of the puffer fish poison, blocks nerve excitation in a very specific way, namely by inhibiting the increase in membrane sodium conductance, which is directly responsible for excitation, without affecting other membrane properties, such as the mechanism for potassium conductance and the resting potential. It is for this reason that tetrodotoxin is now widely used as a tool for electrophysiological study. In order to gain some insight at the molecular level into the mechanism of the sodium conductance increase which normally occurs upon stimulation, the ability of four derivatives of tetrodotoxin to block excitation, as well as their mechanism of blocking action were compared, using giant axons of lobster and squid. Three of them blocked excitation in the same way as tetrodotoxin itself, although the threshold concentrations were higher. Since the tetrodotoxin molecule contains a guanidinium group, and since guanidine can pass through the nerve membrane as sodium does, the blockage by tetrodotoxin might be visualized by assuming that the guanidinium group is plugged in the sodium channel, thereby preventing the movements of sodium ions. However, the present results suggest that the presence of a guanidinium group is not the sufficient condition for the molecule to become potent, because one of the tetrodotoxin derivatives lacked the blocking ability despite the presence of a guanidinium group.

TOSHIO NARAHASHI, JOHN W. MOORE
ROBIN N. POSTON
Duke University

Synthesis and Degradation of Canavanine, an Analog of Arginine in Canavalia ensiformis (L) DC

Because of its structural similarity to arginine, canavanine acts as an inhibitor of protein synthesis. However, jack beans, which synthesize canavanine in larger quantities than does any other species, do not appear to be affected by the substance and do not incorporate it into their protein. In the dry seed, where more canavanine is present than in any other part of the plant, most of the amino acid is in the cotyledons, some is in the principal parts of the embryo, and essentially none is in the seed coat. During germination, the level of canavanine in the cotyledons declines, while that in the epicotyl, hypocotyl and radicle rapidly increases. Vegetative growth is accompanied by some canavanine synthesis in the apical bud. As flower buds develop and grow to maturity, their canavanine content rapidly increases. In freshly opened flowers, approximately half of the canavanine is found in the petals. While the concentration of canavanine is declining in the petals, there is a corresponding increase of concentration in the pistil. Canavanine content of the seeds increases steadily during pod development. At least in the early stages of seed development, canavanine is synthesized in the wall of the pod and translocated to the seeds. In general, the curve for canavanine content of the developing seed parallels the curves for protein content and fresh weight.

AUBREY W. NAYLOR
Duke University

World Geochemical Inferences from Recent Studies of Ecosystems

Recent measurements of photosynthesis and total system metabolism of small pieces of the earth's biosphere provide additional insight on the operation of the geochemical cycles of the worldwide ecological system.

1) From studies of species diversity

and organic content of marine waters in Texas and Puerto Rico, the efficiency of community consumption is related to network complexity. It is proposed that a species diversity in fossil assemblages, less than five species per thousand individuals, indicates an oil-forming strata, a principle useful in prospecting.

2) From measurements of forest floor microcosms from the rain forest, terrestrial microcosms are found to balance gaseously in 48 hours and remain balanced even with 25,000 r γ-radiation. This is evidence for the multiple species principle for supporting man in space, a principle neglected in NASA programs. Since the systems balance at varying CO_2 levels, depending on their biological composition, it is suggested that evolution controls climate as much as climate controls evolution.

3) From studies with a giant plastic cylinder in the El Verde Rain Forest in Puerto Rico, direct evidence has been obtained for the high respiratory work and concurrent production of tropical forests in spite of a very small rate of storage. The Schrodinger energy cost of maintaining low entropy states of maximum complexity are thus obtained.

Studies aided by the Rain Forest Project of the AEC Division of Biology and Medicine through the Puerto Rico Nuclear Center.

HOWARD T. ODUM
University of North Carolina

Endomitosis and Polyribosome Formation

In preparation for secretory activity, the cells of the royal jelly gland of the bee undergo a number of endomitotic division cycles. During each cycle the chromosomes closely follow, up to a point, the synthetic and morphological changes of normal mitosis; also, there is a cyclic nucleolar fragmentation. An electron microscopic study has been made of nurse cells and salivary gland cells of *Drosophila virilis* to determine if other endopolyploid cells show similar behavior. The primary function of nurse cells in the egg follicle is to synthesize ribosomes and various proteins, which pass directly into the cytoplasm of oocytes by way of protoplasmic bridges. Salivary glands secrete proteins and the cells have a well-developed endoplasmic reticulum.

During the endomitotic interphase, nurse cells have one or several large nucleoli. In the prophase stage the nucleoli fragment and release vast numbers of ribosomes into the cytoplasm. There may be as many as six or eight cycles, and as a result the cytoplasm of nurse cells is crowded with ribosome associations, although few membranes of the endoplasmic reticulum are evident. In salivary gland cells, the nucleolus at interphase is very large and is differentiated into a fringe of tassel-like processes containing numerous ribosomes and a diaphanous felt-like interior. At prophase, the tassels break away and small aggregates of ribosomes are set free in the nuclear sap and then pass into the cytoplasm. As the ribosomes emerge from the pores of the nuclear envelope, they appear to be organized into polyribosomes.

This study sheds new light on the role of endomitosis in organisms and explains why, with pulse-labeling, there may be a great deal of variation in the uptake of isotope by the same band in different salivary gland cells of an individual.

Supported by NIH grants HD-01016 and 5-K6-CA-18, 366.

THEOPHILUS S. PAINTER
JOHN J. BIESELE
University of Texas

Folded Chain Concept of Fiber Structure

Natural fibers, like cotton and wool, and man-made fibers exhibit an extreme anisotropy of physical properties, such as elastic modulus, tensile strength, refractive index and so on, with a nearly perfect orientation of the macromolecular chains in the fiber axis. The high strength in the fiber axis and the much smaller value perpendicular to it is in good agreement with the fringed micelle model. This model assumes that the aligned chains pass through alternating crystalline and amorphous regions. Therefore, along the fiber axis strong covalent forces are involved, whereas perpendicular to it are the smaller van der Waals forces. The arrangement of crystallites in the fiber, however, shows a surprisingly high regularity. By electron microscopy of stained or etched fibers and by small-angle x-ray scattering, a period is obtained which is much larger than the dimensions of the unit cell of the crystal lattice and of the same order of magnitude as that observed with single crystals. From experiments with fiber drawing from single crystals and bulk samples under special conditions, I conclude that the fibers contain folded chain crystals with a great many tie molecules interconnecting adjacent crystals and contributing to the high tensile strength in the fiber axis. In cold drawn samples the tie molecules are under high strain and far from thermodynamic equilibrium, so that entropy, heat content, sorption and diffusion are much below the values of relaxed amorphous polymer.

A. PETERLIN
Research Triangle Institute, Durham

Fructification in a Unique Cellular Slime Mold

An undescribed cellular slime mold, isolated in 1962 from mesquite-scrub forest soil, differs from other species of *Dictyostelium* in a number of ways, particularly in the pattern of its mature fructifications (sorocarps). These structures consist of erect or inclined cellular stalks (sorophores) several millimeters in length which bear numerous small sessile spore masses (sori) along their entire length, in addition to terminal globose or citriform sori. The lateral sori develop from small masses of myxamoebae that are periodically abstricted from the posterior region of the ascending cell masses (sorogens). In sorocarps formed in an optimal environment, such sori tend to be fairly constant in size and more or less uniformly distributed along the central axes. Satisfactory growth occurs under conditions approximating those favorable for many other species—for example, in association with *Escherichia coli* on lactose- (0.1 percent) peptone (0.1 percent) agar of *p*H 6.0 at 20 to 25°C. Cell aggregation, however, appears to proceed in two distinct stages and in a manner clearly different from that of any species previously known. Sorocarp construction is apparently dependent upon the dilution or removal from the culture vessel of some self-generated gaseous inhibitor of undetermined composition. Sorocarps are not infrequently branched in a dichotomous manner and, unlike most species of *Dictyostelium,* the spores are spherical rather than capsular in form. A technical description of the new species, to be designated *Dictyostelium rosarium* because of the beaded nature of its sorocarps, is now being prepared for publication.

KENNETH B. RAPER
University of Wisconsin
JAMES C. CAVENDER
Wabash College

A Hitherto Unrecognized Difference Between Man and Other Primates

Man's discovery of fire 300,000 to 400,000 years ago caused cultural changes which still play important roles in his life. Fire gave man heat and light throughout the night, resulting in great changes in his daily routine. He no longer had to go to bed at dusk nor did he have to follow alternating periods of light and dark. Evidence indicates that this new routine resulted in inherited functional changes in man that separate him from other primates. Other organisms, particularly primates, have a 24-hour clock, by which they alternate periods of activity and inactivity and other forms of behavior. In normal man this clock no longer manifests itself by sharp alternating 12-hour periods of activity and inactivity. Through the process of evolution the clock has become submerged. It may reappear only under pathological conditions—such as after head trauma, severe shock, high fever, or stress from constant light. In primates, survival depends on ability to adjust to the alternating 12-hour period of light and darkness; in man, survival depends on his ability to free himself from the 24-hour routine in order to function at more even levels for longer periods throughout the entire 24 hours.

CURT P. RICHTER
Johns Hopkins Medical School

Experimental Studies of the Relativistic Pinch

A 30-nanosecond, 30,000-ampere pulse of 3.5 million-electron-volt electrons has been extracted from the electrode space of a pulsed x-ray machine developed by the Physics International Company of San Leandro, California. This beam of relativistic electrons was projected into an ionized medium framed by a conventional linear pinch in argon. The beam of relativistic electrons was allowed to traverse the ionized medium for distances of 9, 18, 24, and 36 inches before being stopped by a target which was one of the electrodes of the linear pinch apparatus. The damage produced at the target indictates that the total energy of the relativistic-electron beam was delivered at these distances in a manner free of any instability capable of destroying the beam. The target material (brass) was such that the damage produced by the relativistic beam makes it difficult to determine the beam diameter. These experiments are being continued to determine the beam diameter and its behavior with distance. The effectiveness of the magnetic field of the conventional linear pinch as a guide for the relativistic beam will also be investigated.

THOMAS G. ROBERTS
U.S. Army Missile Command, Redstone Arsenal, Alabama
WILLARD H. BENNETT
North Carolina State University

Biophysical Studies of Unit Membrane Architecture

All cellular membranes so far studied have been shown to be generally organized as a core of lipid arranged in a smetic bilayer with the polar heads directed outward and covered by monolayers of nonlipid material. A chemical difference exists between the inside and outside surfaces, but the exact composition and state of the nonlipid components is unknown. This formulation, called the unit membrane concept, was derived from biophysical studies of myelin and of lipid and protein model systems. The general concept has been widely tested and generally accepted. Present efforts are directed toward refinements with a more precise definition of molecular architecture. The current status of the concept will be described in the context of recent work suggesting that definite patterns of subunit structure are present. These are of two kinds, one designated "globular" and the other "granulo-fibrillar." The former is thought to involve a micellar phase transformation in the lipid core. The latter involves mainly nonlipid components. Recent work by electron microscopy and x-ray diffraction, primarily on retinal rod outer segments, will be described. This shows that there probably is present in many membranes, and perhaps in all, a pattern of hexagonal subunits greater than 100 Å in diameter, involving mainly the outer nonlipid monolayers. This is thought to be responsible for the "granulo-fibrillar" substructure. The electron optical evidence of "globular" substructure, insofar as it involves micellar phase transformations in the lipid core, seems best explained as an optical artifact, resulting from the "granulo-fibrillar" substructure.

Research supported by NIH grant GB 02665 and NSF grant GB 3128.

J. DAVID ROBERTSON
Duke University

Determination of g_I/g_J Ratios in Free Rubidium and Caesium Atoms

Measurement of the frequencies of the $\Delta F=0$, $|\Delta m_F| = 1$ Zeeman transitions in the ground electronic state of an alkali atom allows calculation of the g_I/g_J ratio through use of the Breit-Rabi formula. Optical pumping measurements have been made in magnetic fields ≤ 50 gauss using evacuated sample cells whose walls were coated with a long-chain paraffine to inhibit atom-wall interaction. Our result for g_I/g_J of Rb^{85} lies 1.9 parts in 10^4 below an atomic beam experiment value [S. Penselin, T. Moran, V. W. Cohen, G. Winkler, *Phys. Rev.* **127**, 517 (1962)]. This represents a discrepancy of three times the assessed error. Our value for g_I/g_J of Rb^{87} lies 2.0 parts in 10^4 below the value obtained by forming the product

$$[g_I(Rb^{85})/g_J(Rb^{85})] \times [g_I(Rb^{87})/g_I(Rb^{85})] \times [g_J(Rb^{85})/g_J(Rb^{87})].$$

Here the first ratio is that of Penselin *et al.*, the second is taken from nuclear-molecular-resonance data [I. Lindgren, *Arkiv Fysik* **29**, 553 (1965)], and the last is taken to be unity. The discrepancy in this case is ten times the assessed error. Our value for g_I $(Rb^{87})/g_I$ (Rb^{85}) agrees with that derived from Lindgren. Dependences on sample size, wall preparation, light intensity and polarization, and magnetic field gradients are being investigated. The measured ratios are as follows: for Rb^{87}, $g_I/g_J = -4.96984(10) \times 10^{-4}$; for Rb^{85}, $g_I/g_J = -1.46648(10) \times 10^{-4}$; for Cs^{133}, $g_I/g_J = -1.99173(10) \times 10^{-4}$; and $g_J(Cs^{133})/g_J(Rb^{87}) = 1.0001044(3)$. Work supported in part by NSF, Army Research Office, Durham, and NASA through the Sustaining University Program.

H. G. ROBINSON, CLARK W. WHITE
GEORGE S. HAYNE
Duke University

Mutation Frequency in Female Mice Exposed to High-Intensity X-Irradiation Delivered in Small Fractions

Preliminary results were recently reported showing that the mutation frequency in female mice exposed to a small x-ray dose (50 r) at high dose rate was lower than expected, assuming a straight-line relation with the mutation frequencies from large doses. This finding has now been checked in an experiment designed to avoid the vast

numbers of animals required to establish a reliable mutation frequency with a dose as low as 50 r. In this new experiment a dose of 400 r of 90 r/min x-irradiation was given to female mice in eight fractions of 50 r spaced 75 minutes apart. Mutations were scored by our standard specific-locus method. The data reported are restricted to conceptions occurring within the first three weeks after irradiation. This makes possible a rigorous comparison with the data from single 400 r exposures which were similarly restricted. The observed number of mutations, 13 in 23,387 offspring, is significantly ($p = 0.005$) below the approximately 34 that would have been expected on the basis of the frequency obtained from single 400 r exposures (21 mutations in 14,591 offspring). Thus the fractionation experiment confirms the finding from the 50 r single-dose experiment. The results indicate that some repair of premutational damage can occur at low doses as well as at low dose rates. In estimating human genetic hazards it would now appear that the risk from small doses of acute irradiation may be lower than had been calculated on the basis of large doses.

Sponsored by the AEC under contract with the Union Carbide Corporation.

W. L. RUSSELL, ELIZABETH M. KELLY
Oak Ridge National Laboratory

An ESR Study of Radicals Resulting from Ionizing Irradiation of Some Urea Compounds

There is an interesting variation in the effects of ionizing irradiation on urea compounds. For a given amount of x-irradiation, ESR measurements show that the number of stabilized radicals at room temperature in hydroxyurea is approximately 100 times the number stabilized in urea or thiourea. The electron-spin-resonance spectra show that the electrons of urea and thiourea unpaired by irradiation are localized on the oxygen and sulfur atoms, respectively. In hydroxyurea, 41 percent of the unpaired electron spin density is localized on a nitrogen atom. An appreciable unpaired spin density on a nitrogen atom in hydroxyurea is quite different from the results obtained for methyl- and ethylurea, where the unpaired spin density was stabilized on the substitutional group, with no detectable localization on a nitrogen [T. S. Jaseja and R. S. Anderson, *J. Chem. Phys.* **35**, 2192 (1961)]. The radicals trapped in x-irradiated hydroxyurea are identified as $-\dot{N}-H$. This radical is of particular interest because of its structural similarity to the

$$>\dot{C}-H$$

radical which is frequently found in irradiated organic solids. Principal values for the hyperfine interactions in the –N–H radical are in gauss -21.2, -13.5, and -1.5 for hydrogen; and $+22.5$, $+1.5$, and $+1.2$ for nitrogen. If the unpaired electron were in a pure p orbital, the induced isotropic hyperfine interactions would be in gauss -31.0 for hydrogen and $+20.2$ for nitrogen. The principal g values for the ESR spectra are 2.0027, 2.0062, and 2.0108. Analysis of the ESR data indicates that –N–H is a planar radical.

HOWARD SHIELDS, PHILLIP J. HAMRICK
Wake Forest College

Possible Transfer of Metallurgical and Astronomical Approaches to Problem of Environment versus Ethnic Heredity

The analogy proposed here makes impure "chemically pure" elemental metals correspond to impure "genetically pure" populations and makes a radioactive tracer atom correspond to a tracer gene G_i—for example, blood type or taste discrimination—that occurs in different fractions a_i and b_i of individuals in "elemental populations" P_A and P_B. A large group of G's can be used to measure, for each individual studied (rather than for populations,[1]), the "ethnic composition fractions" f_A and f_B of the individual's genes that come from P_A and P_B ancestors. Measurements of f become potentially highly reliable as advances in biochemistry and genetics identify additional G's. A proposed ethnic composition index is

$$H = \Sigma\,(a_i - b_i)\,(2\delta_i - a_i - b_i) / \Sigma\,(a_i - b_i)^2$$

where $\delta_i = 1$ or 0 if the individual has or has not G_i and the sum extends over all identifiable traits. For random G selections, expected values of H vary from $+1$ for pure P_A to -1 for pure P_B or generally equal $2f_A - 1$. (H should be modified for correlated G's.) That the expectation value of an individual's performance is independent of ethnic composition (that is, ethnic heredity is trivial compared with environment) should be possible to establish or to reject to a high level of statistical significance by observations on siblings of mixed $P_A - P_B$ ancestry. Data about such individuals, whose prior development is uninfluenced by the study, are analogous to data of pre-sputnik astronomy. Like selected sequences of stars, siblings provide one controlled variable (same family home environment) and one observable varying feature (values of H should differ significantly for siblings, especially for offspring of first generation P_A-P_B hybrids). For example, correlation of physical, mental, and social measurements with one another and with values of H might give scientific support for Washburn's proposal (that is, for equal environments American Negroes might surpass whites,[2]) by showing a positive correlation of performance with fraction of Negro genes. Data on correlations of G's would be genetically significant and relevant to I.Q. and other polygenic traits.

W. SHOCKLEY
Stanford University

References

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Defect Energies and Electrically Charged Surfaces and Dislocations in Silver Chloride

Because the surface of an ionic crystal is in equilibrium with both positively and negatively charged lattice imperfections—defects which generally require differing energies for their formation—surface charges and surface electric fields appear. Such effects in silver halides are significant not only for their intrinsic interest, but also because of their relation to the photographic process. This paper describes two experiments on AgCl to determine the energy of formation of the silver ion vacancy, a critical parameter in the surface charge phenomenon. Instead of the crystal surface, it is experimentally more convenient to work with crystal lattice dislocations, which may be considered to be equivalent to one-dimensional internal surfaces. Dislocations (with their electrical charges) can readily be moved by a mechanical stress, thereby producing an electric current in an external circuit. In one experiment, W. McGowan studied the voltages produced by pulsed deformation as a function of temperature and concentration of divalent impurity. For each specimen there is an "isoelectric temperature," such that the dislocation

is positively charged above it and negatively below. From the dependence of the isoelectric temperature on impurity content, the vacancy formation energy is calculated. In the second experiment, J. S. Kim determined the isoelectric temperature from measurements of the internal friction due to oscillating dislocations. Both experiments give the vacancy formation energy to be 0.6 electron volts. Also, this result, taken with ionic conductivity data of others, gives the energy of formation of the interstitial ion to be 0.8 electron volts.

Supported in part by Air Force Office of Scientific Research.

LAWRENCE SLIFKIN
University of North Carolina

Mechanism of Killing by Decay of Radioactive Phosphorus in Bacteriophage T4

When ^{32}P is incorporated into the DNA of viruses and bacteria, the decay of this radioactive isotope results in the death of the organism, with an efficiency of about 0.1 per disintegration. This is the result of the transmutation process itself, that is, it is not due to the beta radiation associated with the decay. Various theories have been proposed to account for the observed efficiency. The most reasonable of these postulates that killing is caused by the recoil of the ^{32}S, which often breaks its own polynucleotide strand and breaks the opposite strand with 0.1 efficiency. This hypothesis predicts that a phosphorus isotope emitting a lower-energy beta particle would have a drastically lowered killing efficiency.

In addition to ^{32}P, which has a beta radiation energy of 1.71 Mev and a half-life of 14 days, there is another isotope of phosphorus, ^{33}P, which has a beta energy of 0.25 Mev and a half-life of 26 days. We have shown that it is feasible to produce this isotope from sulfur enriched in ^{33}S by exposure to thermal neutrons. In experiments with the bacteriophage T4, it was possible to demonstrate that the killing efficiency is less than 0.02 for ^{33}P incorporated in the bacteriophage.

C. M. STEINBERG, R. C. VON BORSTEL
Oak Ridge National Laboratory

Positron Annihilation in Liquefied Gases

When an electron and positron annihilate by means of the two-photon process, the departure of the angle between the two γ-rays from 180° is proportional to the total momentum of the annihilating pair. If the positron is "thermalized" before annihilation, its momentum will be negligible compared with that of most electrons in matter. Thus the angular correlation between the two γ-rays resulting from the annihilation of positrons in matter is a direct measure of the momentum distribution of the electrons in the material.

Our apparatus uses the so-called long slit detector technique, in which the coincidence counting rate of two long NaI/Tl γ-ray detectors is measured as a function of the angle, θ, between imaginary planes joining the small specimen and the two long counters. It can be shown that the electron momentum distribution $n(k)$ equals

$$(\text{const})\, k_z \frac{dN(k_z)}{dz}$$

where $N(k_z)$ is the experimentally measured coincidence counting rate and

$$k_z = \frac{mc}{h}(180° - \theta).$$

In this paper we report measurements of the function $N(k_z)$ for the liquefied gases He, Ne, Ar, H_2, N_2, and O_2. The data for all of these liquids except O_2 clearly show the presence of a narrow component, implying the existence of very low momentum singlet positronium. The shape and width of this narrow component can be accounted for reasonably well by assuming that the positronium is contained in a bubble formed by the repulsion between a positronium atom and the atoms or molecules of the liquid. The broader components of $N(k_z)$ resemble the momentum distributions expected for the electrons of these atoms or molecules if the perturbing influence of the positron is properly taken into account.

Supported in part by the NSF and the Advanced Research Projects Agency.

A. T. STEWART, C. V. BRISCOE
University of North Carolina

Cell Wall Studies in the Actinoplanaceae

A study was undertaken to compare the morphological taxonomic grouping of some actinoplanaceae containing carotinoid with the grouping obtained by utilizing the chemical composition of the cell walls. Forty-eight isolates, previously assigned to ten species, and the four genera, *Actinoplanes, Ampullariella, Amorphosporangium,* and *Pilimelia,* were investigated. Cell walls were obtained from vegetative hyphae of all strains by mechanical disruption. Following duponal treatment and ribonuclease, and pepsin and trypsin digestion, acid hydrolysates were prepared. The hydrolysates were subjected to chromatographic analysis for the identification of amino acids, amino sugars, and sugars.

All the strains contained the components associated with bacterial mucopeptide: glucosamine, muramic acid, alanine, glycine, glutamic acid, and either 2,6-diaminopimelic acid (DAP) alone or with a major amount of 2,6-diamino-3-hydroxypimelic acid (hydroxy-DAP). Isomeric separations of 2,6-DAP showed that meso- or DD-DAP, or both, occurred in higher concentrations, and that only those strains not having hydroxy-DAP had more than a trace amount of LL-DAP. Nine other compounds often associated with bacterial cell wall polysaccharides were detected. Galactose was the sugar which occurred most frequently in the hydrolysates. In addition, galactosamine, glucose, mannose, arabinose, xylose, a deoxy sugar tentatively identified as 6-deoxy-L-glucose, and two unidentified compounds were often present. The combinations and relative amounts of these components allowed a grouping of the isolets which corresponded, in most instances, to their original morphological assignments.

Supported in part by a grant from Eli Lilly Company.

P. J. SZANISZLO, H. GOODER
University of North Carolina

An Improved Theory of Bordered Pit Membrane Differentiation

Work on mature and differentiating pit membrane structures in Longleaf Pine, with improved specimen treatment and replicating procedures, has produced evidence which calls for a modification of the current concept regarding the differentiation of the longitudinal tracheid-bordered pit membrane. A solvent exchange drying technique, which consisted of passing green wood specimens through methanol, acetone and pentane before drying the specimens at 60°C, prevented aspiration of the pit membrane. Freeze-drying procedures, with sublimation of the water under vacuum, also prevent-

ed pit aspiration. Electron micrographs of nonaspirated pit membranes revealed a much finer network of microfibrils within the margo than could be detected in aspirated pits, and thus portrayed a more realistic structure. Based on the evidence accumulated from these drying procedures and pertinent information from the literature, the proposed theory of pit membrane differentiation can be summarized as: (i) the delineation of a primary pit field, the membrane of which consists of two primary walls and the intercellular substance; (it is not certain whether or not at this stage of differentiation, the membrane is perforated); (ii) a secondary apposition of large radiating microfibrils on the existing primary wall; (iii) beginning of torus formation with the deposition of circular oriented microfibrils in the central region of the pit membrane; and (iv) enzymatic removal of the bulk of the primary wall and the intercellular substance within the margo region. If in the normal differentiation pattern the membrane perforations are formed during formation of the primary wall, the enzymatic removal is not required.

RICHARD J. THOMAS
North Carolina State University

Aerodynamic Properties of Drosophila Wings

As part of a study of flight with *Drosophila*, wings were tested under steady-state conditions in a small wind tunnel. Test conditions were established by prior evaluation of the performance of intact fruit flies. For these wings, maximum lift-to-drag ratios were low (1 to 2) and occurred at high angles of attack (15° to 25°)—consequences of the high skin-friction encountered at such low Reynolds numbers (75 to 250). Camber increased the maxima of both lift and lift-to-drag ratios, suggesting that lift is generated by the conventional mechanism even in these small insects.

A thick boundary layer should obscure the surface details of fly wings; indeed, the presence of such a boundary layer was verified by direct mapping of air velocities. Yet the complex architecture of the wing surfaces is clearly of importance, since the upper and lower surfaces proved aerodynamically nonequivalent even in the absence of camber. Moreover, the properties of smooth "mylar" plates of the same shape as fly wings were remarkably different from those of the natural product and, for flapping flight, were much inferior. In particular, smooth plates stall at angles of attack above about 20°, whereas fly wings do not appear to stall in the usual sense at all. These observations demonstrate that caution must be exercised in the use of simplified models for studying the aerodynamics of animal flight.

STEVEN VOGEL
Duke University

Nature of $tr_{1\text{-}1}$ Super-Suppressors in Saccharomyces

Super-suppressors are capable of suppressing any genetic locus, but they affect only a limited number of the mutant sites in a locus. We previously showed that super-suppressors have an enhanced mutation rate during meiosis over that of mitosis, while the super-suppressible sites do not [G. E. Magni, R. C. von Borstel, C. M. Steinberg, *J. Mol. Biol.*, **16**, 568 (1966)]. The enhanced mutation rate at meiosis is attributable to mutations of the addition-deletion type, and lack of enhancement is attributable to mutations of the base-substitution type. These facts are consistent with the hypothesis that the super-suppressible mutations are nonsense mutations and that many super-suppressor mutations occur at loci responsible for transcribing sRNA. The $tr_{1\text{-}1}$ super-suppressors belong to a special class that overlaps only rarely with other super-suppressors. In searching for super-suppressors which do not affect sRNA, the $tr_{1\text{-}1}$ super-suppressors seemed to be obvious candidates. It was found, however, that the super-suppressors of $tr_{1\text{-}1}$ exhibit an enhanced spontaneous mutation rate of approximately 15 times during meiosis, whereas the $tr_{1\text{-}1}$ mutant itself is essentially unchanged. The enhanced reversion rate at meiosis and the dominance of the super-suppressors are not consistent with the hypothesis that these are mutants of genes encoding proteins.

R. C. VON BORSTEL
PAUL D. PANUM
C. M. STEINBERG
Oak Ridge National Laboratory

Studies on the Enzymes of Fatty Acid Synthesis

The recent recognition in extracts of *Escherichia coli* of a new protein-like coenzyme participating in the buildup of the carbon chain in fatty acid synthesis has led to an understanding of the mechanism of fatty acid biosynthesis. The coenzyme, referred to as acyl carrier protein (ACP), contains 4′-phosphopantetheine as a prosthetic group to which various acyl intermediates are bound in a thioester linkage. The intermediate reactions of fatty acid synthesis and the various enzymes involved have been investigated. From crude extracts of *E. coli* at least nine different enzymes have been isolated and characterized that participate in the conversion of acetyl CoA and malonyl CoA to palmitate and *cis*-vaccenate. The coenzyme β-hydroxydecanoyl ACP was isolated from the reaction mixture and shown to be an intermediate in the synthesis of both saturated and mono-unsaturated fatty acids. Three different β-hydrodyacyl ACP dehydrases were isolated from extracts of *E. coli:* one is specific for C_4 to C_8 β-hydroxyacyl ACP, the second is specific for β-hydroxydecanoyl ACP, and the third is specific for the longer chain β-hydroxyacyl ACP.

The fatty acid synthesizing system of *E. coli* is stimulated by various salts, including phosphate and phosphorylated sugars. At least two enzymes of this system, the β-ketoacyl ACP reductase and the acetyl CoA-ACP transacylase show complete dependence on these salts for enzymic activity. This and other properties of the fatty acid synthesizing system will be discussed.

SALIH J. WAKIL
Duke University