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Quenching of Chloroplast Fluorescence by Photosynthetic Phosphorylation and Electron Transport

Fluorescence in photosynthesis represents that portion of the absorbed radiant energy that is not converted into chemical energy but is reemitted as radiation. Many investigators have used fluorescence measurements to probe the mechanism of photosynthesis.

In photosynthesis by green plants, the conversion of radiant energy into chemical energy takes place in chloroplasts by way of the process of photosynthetic phosphorylation. Thus, in isolated chloroplasts, fluorescence measures a portion of absorbed radiant energy that is not used to drive photosynthetic phosphorylation. Photosynthetic phosphorylation subdivides into: (i) cyclic photophosphorylation, a photochemical reaction that produces only adenosine triphosphate (ATP), and (ii) noncyclic photophosphorylation, a photochemical reaction that produces in addition to ATP a strong reductant, reduced ferredoxin, which in turn reduces triphosphopyridine nucleotide (TPN) in a dark reaction. TPN is a well-known hydrogen carrier in cellular biosyntheses, including carbon assimilation in photosynthesis.

If cyclic and noncyclic photophosphorylation are indeed the primary photochemical energy-conversion reactions of photosynthesis, they should act as quenchers of chloroplast fluorescence, and we have evidence of this. Fluorescence of chloroplasts was quenched by placing them in a reaction mixture containing the components needed for cyclic photophosphorylation: adenosine diphosphate (ADP), inorganic phosphate (P_i), and ferredoxin, the iron-containing protein native to chloroplasts and now known to be the terminal electron acceptor in the photochemical reactions of chloroplasts. Quenching of chloroplast fluorescence was particularly marked when the widely used nonphysiological catalysts of cyclic photophosphorylation, menadione or phenazine methosulfate, were added, with or without further addition of ADP or P_i.

With respect to noncyclic photophosphorylation, chloroplast fluorescence was quenched by adding ferredoxin and TPN, with additional quenching when ADP and P_i were added. The bearing of these observations on the mechanism of energy conversion in photosynthesis will be discussed.

DANIEL I. ARNON
HARRY Y. TSUJIMOTO
BERAH D. MCSWAIN
University of California, Berkeley

Composition of the Earth's Mantle and Core

An equation of state is developed which involves the seismic ratio Φ and the quality factor Q instead of the conventional variables T and P. The equation supplies a theoretical bridge between ultrasonic and shock-wave data and seismic data.

Φ depends primarily on pressure, and Q depends primarily on temperature, but both can be written in terms of density, ρ, the important state variable. The equation of state is of the form

$$\Phi = \sum_i a_i(\rho/\rho_0)^{n_i}$$

where a_i is a function of the molecular weight and n_i can be written in terms of the Grüneisen ratio. The relation provides a consistency and homogeneity test for available density structures and provides a method for determining the molecular weight and Grüneisen ratio in the various regions of the earth if density can be determined independently. The free oscillations provide the independent source. A density structure is determined from the free oscillations excited by the Chilean and Alaskan earthquakes; this gives values for a_i and n_i in the various regions of the earth. Shock-wave and high-pressure ultrasonic data are used to convert these to molecular weight (that is, composition) and Grüneisen ratio. The latter parameter is sensitive to the degree of packing and indicates a close-packed molecular structure for the lower mantle, in agreement with Birch. The Q data are also used to estimate the defect structure of the mantle.

DON L. ANDERSON
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Stars of Anomalous Chemical Composition

The so-called A-peculiar stars comprise a class of objects of late spectral class B and early spectral class A showing spectral lines of many elements with unusual intensities. That is, they are stars roughly about 100 times as bright as the sun with surface temperatures from 8000°K up to 20,000°K or even higher. Many of them show conspicuous magnetic fields; some are classed as "magnetic variable stars." Their anomalous chemical compositions have been established by the work of many investigators, including W. W. Morgan, G. and E. M. Burbidge, W. P. Bidelman, L. Searle and W. Sargent, M. Hack, and C. Jaschek. The purpose of the present investigation was to analyze high-dispersion spectra of a number of these objects both by the "conventional curve of growth" and by the more refined "model atmosphere" methods. The limitations of each procedure are discussed. Most investigators have employed the curve of growth technique, but advances in computing technology have made it possible now to use model atmosphere methods equally easily. The difficulty lies in assigning the proper model atmosphere for a given star; one must account both for the shapes (profiles) of the strong

hydrogen lines and the energy distribution in the continuous spectrum and, in addition, for the intensities of the observable lines of other elements. A principal limitation lies in our lack of knowledge of the oscillator strengths or "*f*-values" for the lines of interesting elements such as ionized chlorine, or mercury (which may be present in some of these stars).

High-dispersion spectrograms secured at the coude focus of the 120-inch telescope are supplemented for many of the stars by photometric measurements of the continuous spectrum secured at the 60-inch reflector at Mount Wilson.

L. H. ALLER
University of California, Los Angeles

Fit of the Continents around the Atlantic

One of the earliest arguments for the possibility of continental movement was the similarity in form of the coast lines of South America and Africa. For many years this matter was treated rather casually until interest was revived by the work of Crary. In a paper by Bullard, Everett, and Smith to appear shortly in *Philosophical Transactions of the Royal Society* the best fits of the continental edges have been found by numerical methods. Slides illustrating these fits around the North and South Atlantic Oceans will be shown; they are so close that it is difficult to believe them to be fortuitous.

E. C. BULLARD
University of Cambridge

Surface of Escherichia coli

Thin sections of bacteria embedded in polyesters or methacrylate fail to show a clear picture of the surface. On the other hand, studies of the various fractions of bacterial walls after different mechanical and chemical treatments reveal structures disconnected from their proper morphological location on the surface. In order to investigate the wall without extensive pretreatment such as fixation and embedding, we have set up an ultramicrotome within a cryostat at −30°C, which permits one to cut thin sections of quick-frozen bacteria. After floating on 30-percent ethylene glycol in ammonium acetate solutions at −30°C, negatively stained sections show fragments of bacterial cells with remarkably complex surface structures. *Escherichia coli* strains C600, B, and B/4 show a system of channels, 100 to 130 Å wide, that open to the extracellular space. Fixation of bacteria before or after sectioning with either OsO_4 or various aldehydes does not destroy these structures. The layer of the wall containing the channels is removed by treatment of sectioned cells with 0.5-percent sodiumdodecylsulfate; only 5 minutes are required for strain B, while several hours are required for strain C600 to lose this layer. The portion of the cell wall resistant to sodiumdodecylsulfate is the fragment clearly depicted by Weidel, Frank, and Martin [*J. Gen. Microbiol.* **22**, 158 (1960)]. It consists of a layer of adhering balls with diameters of about 120 Å. This layer is disintegrated into the isolated balls by treatment with lysozyme. This work has been supported by NSF grant GB 982.

M. E. BAYER
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Microclimatic Contrasts within a Subalpine Valley

Most high mountain systems of the world are geologically young and precipitous. In rising, they are being sculptured by glaciers and young rivers that in time provide new habitats and contrasting exposures. Evolution is accordingly highly active.

Climatic contrasts in sites that would correspond to differences of many degrees of latitude exist on such mountains within a kilometer or two. Such situations favor crossings and selection at an accelerated pace.

The Slate Creek Valley of the Harvey Monroe Hall Natural Area, Mono County, California, ranges in altitude between 3000 and nearly 3800 m and provides such climatically contrasting sites. In the bottom of the valley at 3000 m frosts occur almost nightly during the brief growth period, mid-July to mid-September, and the daily amplitudes are about 20°C. On a steep northward slope 60 m above the valley floor frosts did not occur during the 1962 growing period, and the daily temperature range was 4° to 15°C, an amplitude of only 11°C. Opposite this site, and in full view of it on a southward slope but at 3200 m, the daily minimum-maximum temperatures ranged from 2° to 28°C, an amplitude of about 25°C.

These drastic differences between local climates within the Slate Creek Valley are reflected in great differences between the vegetations on opposite sides of the valley.

JENS CLAUSEN
Carnegie Institution of Washington, Stanford, California

Further Analysis of Sensory Coding in Electroreceptors of Electric Fish

Sternopygus sp., a gymnotid fish, maintains an incessant discharge of electric organ pulses at constant frequencies of 85 to 140 per second in different individuals. We extended the work of Hagiwara *et al.* (1963) by sampling single lateral-line nerve fibers by the method of teasing bundles. This revealed (i) tonic, low frequency units, not synchronous with the electric organ, that change frequency with "electric" stimulation (conducting or dielectric objects brought near the fish in the water; short circuit between two points in the water; weak electric currents); (ii) similar units that adapt rapidly ($1/e = 0.5$ to 2.0 seconds) and are therefore essentially movement detectors; and (iii) synchronous units that respond without adaptation.

After the electric organ was silenced, sensitivity to objects (metal, or freshly killed small fish), not attributable to mechanical disturbance, was still seen in type-ii units. Small d-c fields arising in the object, the gymnotid, or the container are suggested as a possible cause. Hence the likelihood of nonelectric fish using electroreceptors seems enhanced.

Type-iii units were found which phase-code the position of objects, that is to say, tonically alter their phase relative to other units and the electric organ, typically shifting 2 msec for a 1-cm change of object position, often without any failure of 1:1 following. Other type-iii units, as well as those just mentioned, in some cases code by altering probability of following each electric organ pulse. The skipping patterns have been analyzed and are not random. Weak extrinsic pulses from other electric fish modulate phase and

probability, for example, establishing beat frequencies. Stronger or longer currents elicit long bursts of sensory spikes.

The hospitality of the Museu Goeldi, Belem, Brazil where these experiments were conducted is gratefully acknowledged. Aided by grants from NSF, ONR, NIH, and AFOSR.

THEODORE H. BULLOCK
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Cellular Growth, Contact Inhibition, and Macromolecular Synthesis

In cultures of human diploid fibroblasts, the rates of DNA, RNA, and protein synthesis per cell decrease progressively as the culture becomes confluent, reaching levels 5 to 15 percent of those in the growing culture in the cases of DNA and RNA, and 30 to 50 percent in the case of protein. Concurrently, most of the free cytoplasmic polyribosomes disappear. These changes are completely and rapidly reversible on subdivision of the culture.

The progressive reduction in the rate of RNA synthesis primarily affects the 35 and 45*S* RNA species (the presumptive ribosomal precursors), which are the main components labeled by uridine-C^{14} within 20 minutes in growing cultures. In a fully contacted culture, ribosomal synthesis is therefore almost totally inhibited. The nature of the residual RNA synthesis is still under study.

Several of the RNA species found in diploid fibroblasts have been analyzed for their base composition. Material sedimenting within the single ribosome peak after a long pulse (ribosomal RNA) and in the polysome region after a short pulse (messenger RNA) had base compositions which agreed with published data for heteroploid human cells.

HARRY EAGLE, ELLIOT M. LEVINE
CHARLES W. BOONE
Albert Einstein College of Medicine

Technique for Assessing Variability of Perceptual Span

During the past 5 years there have been important improvements in methods for estimating the average number of elements perceivable by a human observer from a brief visual display (of the order of 0.1 second). As a next step in narrowing the field of alternative models for the perceptual process we need sensitive methods of estimating variation from trial to trial in the number of elements perceived. The detection method gives the desired estimate in a relatively simple and direct manner.

In the standard visual detection experiment the display on each trial includes noise elements and one critical element of either of two classes, *A* or *B*. From the proportion of correct detections one can estimate the mean, but not the variance, of the number of elements perceived per display by the observer. To get at the variance, we introduce redundant critical elements into the displays; for example, we may include in each display either two critical elements of type *A* or two critical elements of type *B*, plus noise elements, the observer's task still being simply to indicate after each stimulus exposure which class of critical elements was represented. It can be shown that efficiency of detection is optimum if the number of elements sampled per trial is constant. Further, a method of estimating the variance of the number of elements sampled per trial can readily be derived.

An application of this method to data for eight observers, with display sizes of 16 elements including 1, 2, or 4 redundant critical elements per display, suggests that the variance of the number of elements perceived is near zero. This result, if substantiated, will dictate revision of a recently proposed serial processing model for visual detection.

W. K. ESTES
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Electron Microscopy: Application of High-Field Superconducting Solenoid Lenses

Based on earlier work [H. Fernández-Morán, *Proc. Nat. Acad. Sci. U.S.* **53**, 445 (1965)], experiments have been carried out with different types of electron microscopes using high-field, superconducting, niobium-zirconium solenoid lenses in open-air-core, liquid-helium Dewars. Electron-microscopic images of test specimens were recorded directly on high-resolution photographic emulsions while the solenoid lenses were operating at 10, 20, and 32 kilogauss in a persistent-current mode, without pole pieces, and with pole pieces of various types. Pointed-filament sources and highly regulated accelerating potentials of 4, 6, 8, and 50 kv were used.

The experiments demonstrated the exceptional stability of the images and their relatively high quality under carefully controlled conditions. The results and observations on imaging phenomena with superconducting solenoid lenses are providing essential data for the design of new types of miniaturized electron microscopes immersed in a liquid-helium cryostat. Potential advantages of this promising approach to high-resolution electron microscopy at cryogenic temperatures, as well as present methodological limitations, will be discussed.

This work was supported by AEC contract AT (11-1) 1344, NIH grant NB 04267, NASA grant NsG 44163, and the L. Block Fund of the University of Chicago.

HUMBERTO FERNANDEZ-MORAN
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Possible Regulatory Sites in Aldolase

The catalytic activity of fructose 1,6-diphosphate aldolase can be affected by chemical modification of specific sulfhydryl groups. Treatment of the enzyme at *p*H 9.6 with chlorodinitrobenzene (ClDNB) or other sulfhydryl reagents leads to loss of catalytic activity associated with the dinitrophenylation of 4-6 sulfhydryl residues (*1*). Under other conditions [*p*H 6.0 and with fluorodinitrobenzene (FDNB)] two sulfhydryl groups react selectively and enzyme activity is increased about threefold; *p*HMB produces qualitatively similar effects. Both inactivation with ClDNB at *p*H 9.6 and activation with FDNB at *p*H 6.0 are prevented by the presence of substrate; this may be due to change in conformation of the protein induced by substrate or to steric effects.

When the activated enzyme, formed by addition of two dinitrophenyl (DNP) groups at *p*H 6.0, is exposed to mild alkaline conditions, the S-DNP group is eliminated and enzyme activity returns to the original level. Beta-elimination of dinitrothio-

phenol was demonstrated by (i) loss of radioactivity when ^{14}C-labeled FDNB was used, (ii) spectrophotometric determination of free dinitrothiophenol, and (iii) conversion of enzyme-linked dehydroalanine to pyruvate by acid hydrolysis. The desulfoenzyme (DS aldolase) shows normal activity but can no longer be activated by sulfhydryl reagents such as FDNB or *p*HMB. By addition of suitable compounds such as thioglycolic acid to the double bonds in the dehydroalanine residues, it is possible to make other enzyme derivatives and study their catalytic properties, as has been done with chymotrypsin (*2*). The desulfo-enzyme behaves like the normal enzyme in forming a Schiff-base intermediate with dihydroxyacetone phosphate (DHAP). It is possible to prepare a doubly-labeled protein by reducing the Schiff base formed with ^{14}C-DHAP and then adding thioglycolate to the double bonds; this permits study of the relation of the two reactive sulfhydryl groups to the two lysine residues involved in Schiff-base formation.

The results suggest that two sulfhydryl groups may be part of a regulatory site.

Supported by NIH grant GM 11301 and NSF grant GB 1465.

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Comparative Studies of Phosphoglucomutase

The present studies were undertaken to ascertain whether phosphoglucomutase activity, ubiquitous in living organisms, is the expression of an equally ubiquitous, homologous gene. The enzyme was isolated from rabbit muscle and liver, shark and flounder muscle, yeast, *Escherichia coli*, *Bacillus cereus*, and *Micrococcus lysodeikticus*. All such enzymes have molecular weights close to 62,000; all require glucose-1,6-diphosphate and Mg^{++} for activity.

The properties of enzyme from rabbit liver, shark and flounder muscle, and from yeast closely resemble those of the rabbit-muscle enzyme that was shown by Najjar and Harshman to contain two seryl residues which accept phosphate from substrate. The "active site" sequences have not yet all been established. The *E. coli* enzyme contains only one reactive seryl which, however, is in a pentapeptide identical with that in rabbit enzyme. Those from the other two bacteria fail to react with substrate to yield labeled enzyme; they also fail to exhibit "shuttle" kinetics and are neither inhibited by Be^{++} nor stimulated by preincubation with Mg^{++} and imidazole. The *E. coli* enzyme, which does exhibit shuttle kinetics, is slowly inhibited by Be^{++}, but is not stimulated by Mg^{++} and imidazole, may represent a transitional form; alternately the bacterial enzymes may be genetically unrelated to the others.

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Total Social Isolation: Effects on Macaque Monkey Behavior

Sixteen rhesus monkeys were separated from their mothers at birth. Twelve were placed in isolation chambers for 3, 6, or 12 months, during which period they saw no human being or animal. Four monkeys were raised in semi-isolation for 6 months and then in isolation for 6 months; in semi-isolation they were given extensive social experience in a playroom, in pairs with a pair of equal-aged, semi-isolated, control monkeys, to examine their social behavior.

The 3-month isolates, after recovery from initial shock, made effective social contacts with controls and with each other. The 6-month isolates failed to adjust to the controls and were enormously impaired in play with each other. The 12-month isolates failed completely with controls and with each other. The impairment of the 6- and 12-month groups appears to be permanent.

The monkeys isolated for 6 months (after semi-isolation from birth to 6 months) reacted effectively with controls and with each other in a relatively short time, but showed excessive aggression.

Isolates and controls were also tested for learning ability on an extensive battery of problems. The differences slightly favored the control animals, although in no instance were differences statistically significant.

Results indicate that monkeys can withstand at least 3 months of total isolation starting at birth, or 6 months of total isolation starting at 6 months of age, but their social potentialities are destroyed if isolation from birth persists for 6 or 12 months. Learning ability is apparently not impaired by isolation.

HARRY F. HARLOW
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Structural Relations among Proteolytic Enzymes

Considerable experimental evidence has been recently obtained on the chemical structure of proteolytic enzymes and on structural elements involved in their biological function. Foremost among these are bovine chymotrypsinogen (Hartley, 1964) and bovine trypsinogen (Walsh *et al.*, 1964) whose linear amino acid sequence has been reported. Partial sequences of other "serine" proteases which are believed to operate by a common mechanism have been elucidated by Hartley and Smillie (1964) for chymotrypsin *B*, and elastase. From these and other data it has been concluded that these enzymes share elements of structure that are necessary for catalytic function (Walsh and Neurath, 1964).

The comparison can be extended to other enzymes which, on the basis of their substrate specificities, can be considered to belong to the class of chymotrypsins; these include bovine chymotrypsin *B* and its precursor (Kassel and Laskowski, 1963), fraction II of bovine procarboxypeptidase and the product of its activation (Brown *et al.*, 1963), and the analogous enzymes and zymogens of the porcine pancreas (Desnuelle and Rovery, 1963; Folk, 1965). All these enzymes show similarities in amino acid composition, molecular weight, reactivity toward acylating and phosphorylating agents, and in the chemical events accompanying their formation from the corresponding zymogens. More recently, chymotrypsinogen and procarboxypeptidase *B* have been iso-

lated from a more primitive species, the salt-water fish *Squalus acanthias*, commonly known as the Pacific spiny dogfish (Prahl and Neurath, 1962; and to be published). These proteins share many structural and functional features of the corresponding enzymes of the bovine or porcine gland, including composition, mechanism of activation, and specificity toward peptide and ester substrates.

HANS NEURATH
University of Washington

Seismic Source as Revealed by Static Fields of Displacement, Strain, and Tilt

Propagating waves and free oscillations of the earth have been used by many investigators to recover fault length and orientation, slip direction, and rupture velocity. Static displacement fields have been used in the immediate vicinity of faults to estimate fault depth. It is shown in this paper that static displacement, strain, and tilt fields are measurable with modern instruments to distances of several thousand kilometers from major earthquakes.

The patterns of the static fields are diagnostic of the source. Vertical and horizontal dimensions of the source can be recovered. Faulting sources can be discriminated from volume-change sources. Examples are presented from the Alaskan earthquake of 1964, the Hebgen Lake, Montana, earthquake of 1959, and volcanic earthquakes associated with the current eruption on Hawaii.

FRANK PRESS
California Institute of Technology

Hydrolysis of Gamma-Phenylpropyl Di- and Triphosphates

This report describes the hydrolytic cleavage of the nucleotide analogs

$$\begin{matrix} O\ O \\ ROPOPO^{-3} \\ O\ O \end{matrix} \quad \text{and} \quad \begin{matrix} O\ O\ O \\ ROPOPOPO^{-4} \\ O\ O\ O \end{matrix}$$

where R is γ-phenylpropyl. The organic part of the molecules was chosen so that the compounds would exhibit the properties of singly substituted polyphosphates without any of the possible contributory properties of the nucleoside. The monophosphate, diphosphate, and triphosphate were prepared by the methods of Cramer and of Moffatt and Khorana.

The hydrolyses were studied at 95° and 99°C over a *p*H range from 0.3 to 13. The observed rate constants for the diphosphate obey the relation

$$k_{obs} = \left[k_1 + k_2\frac{(H^+)}{K}\right] \Big/ \left[1 + (H^+)/K\right]$$

at from *p*H 3 to 13, where K is the third dissociation constant of the triacid. The values for the constants k_1 and k_2 (1.4 and 20 $\times$ 10^{-5} sec^{-1} at 99°C) are nearly identical with those for adenosine diphosphate. The rate of hydrolysis of the triphosphate is only five times faster than that of the diphosphate. These hydrolyses are interpreted as those of the unusual monoprotonated forms, such as $R—O—PO_2H—OPO_3^{--}$ (Westheimer, 1962).

Hydrolysis of the triphosphate is catalyzed by the enzymes potato apyrase, by rabbit myosin, and by yeast inorganic pyrophosphatase plus zinc ion. The rates and Michealis constants are similar to those found for adenosine triphosphate; the triphosphate gives no response with firefly luciferase.

This work was supported by NSF grant G15819.

DAVID L. MILLER
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Reversible G2 Lag of X-irradiated Mammalian Cells

Three effects are exhibited by all organisms exposed to ionizing radiation: mutation, death of cell reproduction, and reversible mitotic lag. It has been postulated that in mammalian cells the last of these effects also resembles the first two in reflecting chromosomal damage. This hypothesis was tested by life-cycle analysis of mammalian cells exposed to various types of chemical agents with specific actions on cell constituents. Collection functions were determined for cell populations treated with various agents in order to determine whether any consistent pattern exists differentiating those which do from those which do not resemble ionizing radiation in producing a specific lag in the $G2$ period.

Vinblastine and vincristine, agents which block cells in mitosis, and an excess of thymidine which blocks DNA synthesis, had no effect on $G2$. Similarly, various concentrations of fluorophenyl alanine, which prevents protein synthesis or produces defective proteins, displayed marked inhibition of the $G1$ and S periods but had no effect on $G2$. The following agents all produced a large $G2$ delay without affecting any other part of the cell cycle: (i) H^3-thymidine, but only when incorporated into DNA in a concentration great enough to irradiate the nucleus significantly; (ii) BUDR, but only when incorporated into cellular DNA and also illuminated with visible light; and (iii) Streptonigrin, an agent known to produce chromosome breakage. This specificity of action strongly supports the hypothesis that the reversible, radiation-produced $G2$ lag reflects chromosomal damage which presumably is being repaired.

THEODORE T. PUCK
University of Colorado Medical Center

Proposed Biological Exploration of Mars between 1969 and 1973

The theoretical importance of discovering life elsewhere in the solar system is outlined, and the possibility that life exists on Mars is evaluated in the light of all available evidence. The judgment is reached that the biological exploration of Mars now is justified. Plans for the Voyager missions to Mars between 1969 and 1973 are outlined. They include orbiter and lander missions whose scientific purposes and possible payloads are discussed.

COLIN S. PITTENDRIGH
Princeton University

Research toward a High-Efficiency Voice Communication System

A speech analyzer and synthesizer with a capacity of 200 speech elements has been developed with the object of evaluating its performance. The analyzer, consisting of normalizers, syllable and syblet segmenters, sound recognizers, sequencers, and memories, converts the speech elements into a code. The synthesizer converts the

code to speech by reproducing prerecorded speech elements. Advantages of the voice-communication system employing the analyzer and synthesizer are high intelligibility and the low transmission rate of only 23 bits per second.

HARRY F. OLSON, HERBERT BELAR
EDWARD S. ROGERS
RCA Laboratories

Reconstruction of a Sub-Mitochondrial Ion Translocating System

By controlled fragmentation of heavy beef-heart mitochondria, a submitochondrial preparation that accumulates massive amounts of calcium can be obtained. Translocation is thus not dependent on an intact membrane but is the property of mitochondrial subunits. Fresh mitochondria allowed to swell for a week yield a submitochondrial fraction after freezing. The frozen particles retain translocating activity for months. The translocation requires both adenosine triphosphate (ATP) and oxidizable substrate (routinely succinate). To obtain maximum initial rates, the aged particles must be activated by preincubation with ATP, succinate, or both.

It is already evident that this preparation behaves quite differently from that studied by Vasington. We have further succeeded in extracting two essential cofactors for calcium translocation by a high concentration of dibasic phosphate. After extraction, cytochrome *c* restores a large part of the translocase activity but has a proportionately very much smaller effect on the rate of oxygen uptake. The second cofactor, as yet unidentified, restores the remainder of the translocase activity without further increasing oxygen uptake. The effect of cytochrome *c* indicates that it is required to form a common intermediate, capable of driving phosphorylation or translocation. However, this requirement is above and beyond the requirements for electron transport. This example of a resolution of the functions of cytochrome *c* indicates the power of this approach to the clarification of mitochondrial coupling.

JOHN T. PENNISTON
HAROLD VANDE ZANDE
DAVID E. GREEN
University of Wisconsin

Mutations at the Incompatibility Loci in Schizophyllum

Mating competence and heterokaryosis in many of the higher fungi are determined by two incompatibility factors, *A* and *B*, each of which is constituted of two distinct loci. Haploid strains carrying different *A* and *B* factors interact to establish the fertile heterokaryon, the dikaryon, whereas strains carrying common factors, either *A* or *B*, interact to form characteristic infertile heterokaryons. Mutations at the loci of either factor, *A* or *B*, lead to self-compatibility (in the matter of the affected factor) and closely mimic the effects elicited in heterokaryosis by pairs of normal but different wild factors. Thus a homokaryon carrying a mutant *B* mimics the common-*A* heterokaryon; a mutant *A*, the common-*B* heterokaryon; and both mutant *A* and mutant *B* mimic the dikaryon.

These mutations appear to result in loss of factor-function and suggest the regulatory role of wild factors to be inhibitory or repressive for the stages in the morphogenetic sequence of dikaryosis. Secondary mutations in mutant-*B* factors restore self-incompatibility and endow the doubly mutant factor with a new specificity, distinct from that of the factor whence it came by two successive mutations.

JOHN R. RAPER
Harvard University

Pharmacological Support for Men on Long Space Flights

A small medicine chest was available to Mercury astronauts for emergencies, but the only indications of need arose when Cooper manifested inattention before his last orbit and orthostatic hypotension on ambulation after landing. Such deteriorations during 34 hours in space justify systematic planning of medicine chests appropriate to the Gemini, MOL, Apollo, and subsequent space missions lasting weeks or months. The problem is unusual because the anticipated indications of need for drugs are due to environmental abnormalities; only by simulating such conditions can the favorable or unfavorable effects of drugs be ascertained.

Examples of the possibilities include: during the preflight period, antibacterial and antiviral agents and drugs that diminish secretions in the respiratory tract; during acceleration into orbit or space, drugs that combat bronchospasm and oppose pulmonary hypertension; during space flight, antibacterial and antiviral agents, drugs to relieve minor pains, combat allergies, motion sickness, digestive disorders, somnolence, or insomnia, and preparations to cleanse and preserve the skin, protect the eyes, exercise the circulatory baroreceptors, and for protection from toxic radiation; during deceleration, as for acceleration but more imperative; on disembarkation, drugs to combat circulatory deconditioning, residual respiratory abnormalities, fatigue, trauma, and endemic diseases in case of landing in inhospitable country.

If the precedent of the Mercury flights (of pretesting each drug on each astronaut) is to be followed, drugs for the medicine chests for the Gemini and MOL flights should be available now during the training period.

CARL F. SCHMIDT
U.S. Naval Air Development Center, Johnsville, Pennsylvania

Erratum. The Ionic Centrifuge and Fusion Nuclear Power

In one of my first papers before the Academy I showed by six drawings the operation of an ionic centrifuge in two types of discharge. The first three drawings were shown correctly with negatively polarized end-plates and insulated cylinder. The last three were shown incorrectly, with the end-plates voltage marked positive. In fig. 5 the current flowing to the upper plate was shown to be equivalent to a positive ion current flowing down from the upper electrode or an electron current flowing up to the same electrode.

In an actual case we would get the course of the current flow shown in fig. 5, if we made the voltages shown at the left of fig. 5 −100 v, −90 v, and so on, instead of +100 v, + 90 v, and so on. The voltage of the end-plates should have been negative and not positive. With the course of the current reversed by reversing the potential on the end-plates, a strong effect of the space-charge of the electrons on the nearly pure electron flow adjacent to the end-plates will be introduced and will greatly reduce the

current density of pure electrons taken from the glow to the end-plates. The great reduction in the current density to the end-plates is important for the opportunity of using the space-charge voltage as a countereffect in determining the current density at the electrodes.

JOSEPH SLEPIAN
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Geotropic Response in Starch-Free Coleoptiles

The statolith theory of georeception in plants, first put forward in 1900, has never been rigidly proved. Proof, or disproof, requires that the plants be freed from starch under conditions in which their growth remains essentially normal. Such destarching has now been achieved. Isolated young *Triticum* coleoptiles were incubated with gibberellic acid and kinetin for 34 hours at 30°C; after this period starch-disappearance was complete. The isolated coleoptiles still showed moderate growth rate. Their geotropic response, although slower than that of freshly excised plants, was nevertheless still strong, and average curvatures as large as 45 deg were obtained within 8 hours. The ratio of curvature rate to growth rate was the same for depleted plants and controls containing starch.

KENNETH V. THIMANN
Harvard University
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Harvard University and
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Secular Changes of Natural Radiocarbon and Their Interpretation

Changes of the order of several percent in the radiocarbon activity, produced by cosmic rays, of the carbon belonging to the atmospheric reservoir can be observed by precise measurements of the carbon-14 content of wood of which the years of growth are known from study of its rings. Changes occurring on a time scale of the order of 100 years are almost certainly due to changes in the production rate $\overline{Q}$ of radiocarbon caused by changes in the cosmic-ray intensity. This can be seen from correlation between average sunspot numbers and results of some 150 radiocarbon measurements carried out during 1964 in La Jolla, Calif. Periods of low solar activity in the 15th and 17th centuries A.D. coincide with periods of rising carbon-14 activity of the atmospheric carbon. The same periods coincide with periods of cold climate of the "little ice age". Amplitude of the C^{14} variations is determined by the magnitude of change of $\overline{Q}$ and by the rate of exchange of atmospheric CO^2 with the bicarbonate of the oceans. Changes in $\overline{Q}$ of the order of 30 percent must be assumed to have occurred in order to account for the observed C^{14} variations of this type.

Changes with a time constant of more than 1000 years involve the total radiocarbon inventory on Earth's surface; such changes in $\overline{Q}$ could be attributed to changes in the magnetic field of Earth. Radiocarbon measurements in wood dated dendrochronologically promise to lead to extremely important information bearing on solar activity, climate, and perhaps other geophysical constants going back in time as far as tree-ring records are available.

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Individuality in Nutrition: The Genetotrophic Principle

Heterogeneous populations of rats from four inbred strains were fed diets varying in degree and in type of deficiency; the growth and condition of each rat on each diet were periodically recorded. About 280 rats were tested.

The results show clearly that enormous differences in the nutritional needs of "normal" young rats occur, in that individual reactions to a given deficient diet vary widely; in the case of diets with different concentrations of vitamin A, the responses show extremely wide individual variation.

Since the animals were chosen to simulate a young human population, it seems safe to infer that similar large differences would occur within a group of children in responses to both highly deficient starchy diets and diets low in vitamin A. It may be expected that one normal child may be seriously impaired by a diet which yields relatively satisfactory results in another.

These striking animal data indicate the urgency of further exploration of genetotrophic diseases—those that are generated because the individual nutritional needs of the afflicted individual are not fully satisfied. Diseases which may have genetotrophic roots include rheumatoid arthritis, gout, atherosclerosis, carious teeth, alcoholism, epilepsy, cataract, acne, mental retardation, schizophrenia, and mental depression.

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Synthesis and Flow of Neuroplasm: A Progress Report

The mature neuron regenerates neuroplasm continuously in its cell body and then conveys it into the nerve fiber as a cohesive column advancing at a daily rate of about 1 mm ("axonal flow").

Three years ago, before this Academy, the earlier evidence from "damming" of flow in constricted nerve (Weiss and Hiscoe, 1948) was supplemented by electron-microscopic and cinemicrographic data (Weiss, Taylor, and Pillai, 1962). Tracing the flow by radioactive P^{32} and C^{14} confirmed, but did not significantly elucidate, the process [see Weiss, in *Regional Neurochemistry,* Kety and Elkes, Eds. (1961)] until Droz and Leblond (1962) applied higher-resolution autoradiography with H^3 as marker and demonstrated the localized incorporation of tritiated leucine into nerve cell protein, followed by progressive cellulifugal displacement at the predicted rate (rat sciatic nerve).

Our recent experiments have introduced further refinement by (i) administering labeled amino acid topically to circumscribed neuron groups, instead of systemically, and (ii) by twin administrations to symmetrical nerve sources in the same animal. We chose the retinal ganglion cells of the optic nerve. Minute amounts of H^3-leucine (2 μc) injected into both orbits of young mice became promptly incorporated into the retinal cell proteins, with some endoneurial escape into the optic nerve that was soon fixed in glia cells. Superimposed upon this background, daily autoradiographs (1 to 6 days) revealed the expected traveling crest of labeled protein advancing in the optic fibers at about 1 mm/day; progressive broadening of

the crest indicated differential spreading rates in different fibers.

The opportunity to use both eyes, one for test, the other as control, opens the way for quantitative studies of factors (drugs, stimulation, disuse, and such) affecting the rate of synthesis and flow of neuroplasm.

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A Serotonin Synergist, Probably a Ganglioside, in Blood of Schizophrenics

Blood sera taken from schizophrenic patients contained a substance that markedly increased the sensitivity of isolated rat uteri to serotonin. Doses of serotonin, which by themselves caused no contraction, induced maximum contractions in concert with this material. The substance by itself did not cause contraction of the muscle. Purified preparations of the synergist also increased sensitivity to acetylcholine and to bradykinin, but it is not yet clear whether a single chemical compound or a mixture was responsible for these effects.

The synergist was not found in normal sera. It was nondialyzable, stable to boiling, and soluble in chloroform-methanol; thus it is not a protein. It was destroyed by highly purified neuraminidase. In the bioassay for serotonin receptors based on neuraminidase-treated stomach-strips, the purified synergist showed high potency.

These facts suggest that in schizophrenia a substance is present which increases the sensitivity of certain tissues to serotonin, thereby in essence increasing the effective concentration of this hormone. The substance seems to be a ganglioside with activity as the serotonin receptor. The possibility of its being causally related to the disease is being considered.

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DNA Synthesis and Hormonal Control of Insect Metamorphosis

The epidermal tissues of diapausing pupae show no detectible DNA synthesis even after months of exposure to tritiated thymidine. By contrast, very rapid synthesis occurs in synchrony with the termination of diapause and the initiation of adult development. The synthesis of DNA is, therefore, one of the most impressive biochemical changes associated with the action of the prothoracic gland hormone (ecdysone) in terminating the pupal diapause.

DNA synthesis is known to require formation of the triphosphate derivatives of all four deoxyribonucleosides. Consequently, by virtue of its inhibition of the enzyme thymidylate synthetase, the compound 5′-fluoro-2′-deoxyuridine (FUDR) is recognized as a potent inhibitor of DNA synthesis.

Diapausing pupae of the cecropia silkworm show almost unparalleled resistance to FUDR, and are seemingly undisturbed by injection of 5 mg into 5-g pupae. However, the drug singles out and apparently destroys all spindle-shaped hemocytes—one of the few cell types which normally continues to synthesize DNA during diapause; it also blocks the healing of integumentary wounds.

When ecdysone is secreted to provoke the termination of diapause and the initiation of adult development, the insect becomes extremely sensitive to FUDR: a single injection of 1 to 2 mg is capable of blocking the termination of diapause without killing. Moreover, administration of the drug at any stage during the first few days of adult development causes cessation of further development.

These findings suggest a close connection between the synthesis of DNA and the action of ecdysone.

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