National Academy of Sciences

Abstracts of Papers Presented at the Annual Meeting, Washington, D.C., 22–24 April 1963

Role of Ferredoxin in the Energy Conversion Process of Photosynthesis

Under special experimental conditions, reduced ferredoxin has been shown to accumulate as the product of the primary photochemical event in photosynthesis, in which radiant energy, trapped by chlorophyll, is converted into chemical energy that becomes available for cellular work [Whatley, Tagawa, and Arnon, *Proc. Natl. Acad. Sci. U.S.* **49**, 266 (1963)]. Photoreduced ferredoxin is formed prior to reduced triphosphopyridine nucleotide ($TPNH_2$), which until now has been known as the first stable, chemically isolated reductant of photosynthesis. It became important, therefore, to investigate also the role of ferredoxin in the reactions leading to the formation of adenosine triphosphate (ATP), the second component of the "assimilatory power" in photosynthesis .

The light and dark component reactions in the formation of $TPNH_2$ by illuminated chloroplasts have been chemically separated and the necessary flavoprotein enzyme, ferredoxin–TPN reductase, has been crystallized [Shin, Tagawa, and Arnon, *Federation Proc.* **22** (1963)]. Parallel experiments have been carried out to trace the role of ferredoxin in ATP formation, especially in the anaerobic cyclic photophosphorylation which is a common denominator of the strictly anaerobic photosynthesis in bacteria and the oxygen-evolving photosynthesis in chloroplasts. Evidence for an endogenous ferredoxin-catalyzed cyclic photophosphorylation in chloroplasts, which proceeds anaerobically without the aid of other cofactors, was obtained after the experimental conditions under which this type of photophosphorylation can be observed had been established. These experimental conditions include an inhibition of oxygen evolution with *p*-chlorophenyldimethyl urea (CMU) and control of light with respect to both intensity and wavelength. Light of wavelength favorable to absorption by chlorophyll *b* interfered with cyclic photophosphorylation under anaerobic conditions. The significance of these findings for the mechanism of the energy conversion process in photosynthesis will be discussed.

DANIEL I. ARNON, K. TAGAWA
H. Y. TSUJIMOTO
University of California, Berkeley

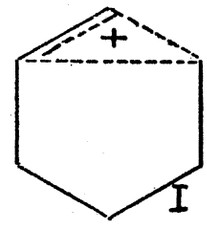
Effects of Melatonin, a Pineal Substance, on the Rat Ovary

Previous work has indicated that the mammalian pineal gland exerts an inhibitory effect on female gonad weight and function. These effects, as well as the morphology of the pineal gland, are modified by exposure to light. Melatonin (*N*-acetyl-5-methoxytryptamine), a potent lightening agent for melanocytes, has been found to be highly localized in the pineal gland of mammals. The enzyme required for its formation has been found only in this structure. All of these findings suggest that melatonin might be the substance responsible for the action of the pineal gland on gonads. The repeated daily administration of microgram amounts of melatonin to immature rats delayed vaginal opening and produced a highly significant inhibition of estrus and a decrease in ovary weight. Serotonin, a precursor of melatonin, did not have these effects. Melatonin acutely reversed the persistent estrus induced by exposure to constant light. Circulating tritiated-melatonin was found to be preferentially concentrated in the pineal gland and the ovary. Other endocrine organs as well as nervous tissue also concentrated this substance. Chronic exposure to light reduced the uptake of circulating melatonin in the ovary and the pineal gland. Since the enzyme for synthesis of melatonin has been found only in the pineal gland, the previously established presence of melatonin in peripheral tissues suggests that it is secreted by the pineal gland into the blood; this secretion may be dependent on light. The selective uptake of melatonin by the ovary and the estrus-inhibitory effects of the pure substance suggest a direct physiologic effect, but indirect effects cannot be ruled out.

JULIUS AXELROD
RICHARD J. WURTMAN
ELIZABETH W. CHU
National Institute of Mental Health

Bridged Carbonium Ions from Different Sources

Cyclohexene and cyclohexyl acetate are among the products of acetolysis of (i) cyclohexyl, (ii) cyclopentylmethyl, and (iii) 5-hexenyl, arylsulfonates. According to current theories of solvolysis [for example, S. Winstein, E. Clippinger, A. H. Fainberg, R. Heck, G. C. Robinson, *J. Am. Chem. Soc.* **78**, 328 (1956)], (I) may occur in the third stage in the reaction sequence from compounds (ii) and (iii). The ion pairs involved in the first and second stages may not be identical in cases (ii) and (iii) because of different locations of the anion.

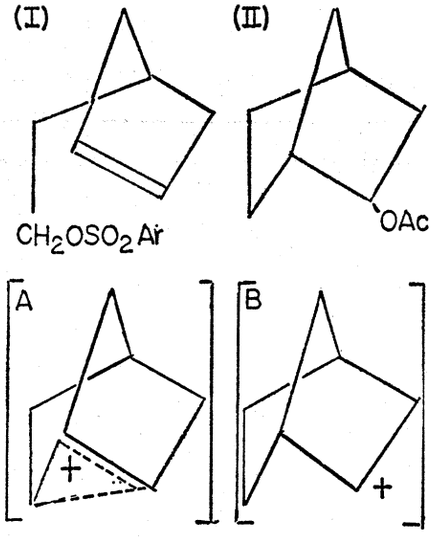


Under identical conditions leading to irreversible acetolysis and elimination, the three starting materials lead to widely differing proportions of cyclohexene and cyclohexyl acetate. On reviewing the evidence for the occurrence of (I), we conclude that these differences in behavior indicate a branching of the reaction paths at the stage of the ion pair.

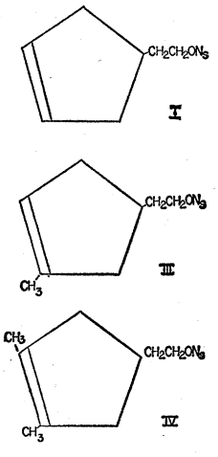
PAUL D. BARTLETT
WILLIAM D. CLOSSON
THOMAS J. COGDELL
Harvard University

Nature of Intramolecularly Assisted Ionization: Methyl Substitution on a 5,6-Double Bond

Carbon-carbon double bonds, suitably located, may appear as strong internal nucleophiles promoting the ionization of organic sulfonates.



Displacement of $ArSO_3^-$ by the π-electron pair of the double bond in (I) without localization would yield the symmetrical intermediate (A), a structure favored by reaction rate evidence and by the configuration (all *exo*) of the product (II). Localization of the electron pair, as when attacked by a proton, would give an unsymmetrical intermediate (B). A comparison of the rates of acetolysis of (I), (III), and (IV)



(which are in the ratio 1 : 7 : 38) in the light of model reactions confirms the preference for the symmetrical structure (A).

PAUL D. BARTLETT
GEORGE DANN SARGENT
Harvard University

Electron Microscopy of Chromosomal Transformations in Mitosis in Salamanders

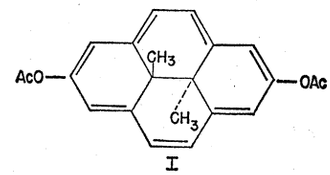
In seeking structures which might reflect the exquisitely meticulous organization of chromosomes, I have found in cells fixed chemically or physically repetitive hierarchies of complex patterns of focalized chromosomal filaments. These are visible throughout the mitotic cycle, but are less prominent in the most dispersed and condensed stages. In prophase, filaments of dispersed interphase chromosomes begin to condense around minute bodies (550 to 750 Å). These become surrounded by progressively larger, confluent aggregations of filamentous structures (1350 to 6500 Å). Nucleoli apparently become included in a few chromosomes. Numerous smaller, nucleolus-like structures disappear into many of the chromosomes. In telophase, along the line of fusion of adjacent chromatids, bundles of filaments form a row of "zipper-like" formations (1500 to 2200 Å). After nuclear membrane formation, the fused mass of chromatids swells quickly and separates into large, irregular rods. As these disperse, nucleoli develop, numerous small nucleolus-like bodies appear transiently, the filaments of the larger focalized aggregations separate, and the interphase appearance is achieved. There is some evidence in this material that the various foci consist largely of structural protein with attached nucleoproteins. It may be assumed that they contain enzymes, some of which function as organizing centers for condensation of dispersed chromosomes and others which participate in the separation, fusion, and subsequent dispersion of chromatids. This work was aided by a grant from the U.S. Public Health Service.

WILLIAM BLOOM
University of Chicago

Aromatic Molecules with Functional Groups Internal to the π-Electron Cloud

This investigation was undertaken in an attempt to answer two classical problems in the chemistry of aromatic compounds: (i) Would a suitably designed monocyclic polyene larger than benzene be aromatic? (ii) Would it be possible to insert functional groups within the cavity of such an aromatic π-electron cloud? The synthesis of 2,7-diacetoxy-*trans*-15,16-dimethyl-15,16-dihydropyrene (I), a molecule fulfilling both of these requirements, has now been accomplished.

The nuclear magnetic resonance spectrum of (I) shows evidence of a very strong ring current, which is a clear indication of aromaticity. Also, the signal for the "internal" methyls appears at very high field as would be expected theoretically for methyls placed within the cavity of the π-electron cloud. Further, the chemical properties of I, insofar as they have been studied, indicate that it is "aromatic" in the nonthermodynamic, classical sense of its reactivity and its stability toward light, heat, and air.



We thank the National Science Foundation for financial support.

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Electron Transport in the Oxysome

The reaction sequence of Keilin's cytochromes (*1, 2*) has been determined by "crossover points" for inhibitors (*3*), time sequence of reaction kinetics (*4*), and reconstruction of electron transport from its components (*5, 6*): $\rightarrow b \rightarrow c_1 \rightarrow c \rightarrow a \rightarrow a_3 \rightarrow O_2$. One-to-one stoichiometry is measured in mitochondria (*3*) and a functional unit (*7*) for electron transfer and oxidative phosphorylation totals about 2×10^6 in molecular weight (*6*). We term a unit of such capabilities an "oxysome" by analogy with the "quantasome" of photosynthesis (*8*). Mechanisms of electron transport must be consistent with the binding of electron carriers to the mitochondrial structure. Electron conduction is unlikely because of the high stability at 77°K of pairs of adjacent electron carriers, for example oxidized c_1 and reduced b (*9*). Electron transfer may operate between juxtaposed hemes by means of heme-linked imidizole (*10, 11*) but not between non-adjacent hemes (*3, 9*). Electron transport by rotation or translation is required to bring an electron-bearing

hematin into contact with the active center of the next hematin (*2, 3*). Inhibitory effects of glycerol (*9*) and D_2O (*12*) confirm that some carrier mobility is required. Electron micrographs (*13, 14*) show small particles (75 Å) attached by 35 by 50 Å stems to cristae at spacings of 100 Å. Although the nature of this fine structure is not fully established, it is proposed (*13, 15*) that these particles represent the 170 Å "oxysome." But we identify the particles with electron carriers and their oxidative phosphorylation coupling factors in a structure and spacing affording a satisfactory dynamic model for both electron transfer and transport processes. This hypothesis does not require that "elementary particles" (*6, 15*) exist as a physical entity in mitochondria and indicates that mechanical disruption of the cristae may lead to rearrangements of stoichiometry and mechanism of electron transport (*2*).

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Tree Lines and Germ Plasma: A Study in Evolutionary Limitation

The limit for tree growth is determined not only by latitude and altitude, but even more by the germ plasm. Each species has its characteristic tree line; beyond this limit some tree species evolve elfinwood, and others do not.

Pines have higher tree lines than oaks, and oaks are much more tolerant than myrtles and eucalypts. For example, the cloud forests above 3000 feet in the Organ Mountains of southern Brazil at 22° South latitude contain 308 tree species, including 82 myrtles. However, trees cease to exist at 6000 feet, where during winter the temperature occasionally drops slightly below freezing. The relatives of these tree species occupy all continents but only at low latitudes.

In contrast, in the Sierra Nevada of California at 38° North latitude a subalpine forest of three species of conifers thrives at 10,000 feet in a climate which has 8 months of freezing temperatures, some as low as −30°C.

The trees that occupy the highest altitudes at the highest latitudes are limited to only 12 circumboreal species complexes. Six belong to the pine family; one is a juniper; and the others are birches, alders, aspens, willows, and mountain ashes. All are noted for frequent interspecific hybridizations and have exceptionally large gene pools. The same is true with oaks, the next most tolerant group.

All of these very hardy tree groups are absent south of the equator where the tree limits are lower. From this it is clear that previous concepts of tree lines have been greatly oversimplified.

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Dialysis Behavior of Peptides and Proteins Which Associate

With the thin film dialysis technique [*Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1961), vol. 10, p. 175] it has been well established that a pure ideal solute will give a linear escape plot over a wide range of concentration. A deviation from linearity is an indication (i) of impurity, (ii) of a solute which associates or (iii) of one whose conformation is a function of concentration. These possibilities can be distinguished by redialysis, under appropriate conditions, in membranes of different porosity.

A number of peptides and proteins known to associate strongly (tyrocidine A, subtilin, ACTH, insulin, hemoglobin) were chosen for study. In all cases the *p*H and ionic strength of the solution were particularly important. Tyrocidine A showed a dialysis rate much too slow for the known size of the monomer. Subtilin showed a rate consistent with the size of its monomer except when salt was added. The behavior of ACTH was indicative of a conformational shift rather than of association. The presence of the monomer of insulin was indicated at low concentration, low *p*H, and low ionic strength. The behavior of hemoglobin was consistent with the published data relating to its dissociation except that a degree of dissociation from the tetramer was indicated even under conditions most favoring association. Dissociation to monomeric α and β chains was indicated at high dilution, low ionic strength, and low *p*H.

Since the method has a kinetic basis, it probably emphasizes the relative amount of the smallest diffusional size. The data supplement those from the ultracentrifuge in a useful way.

LYMAN C. CRAIG, GUIDO GUIDOTTI
Rockefeller Institute

Regulation of Gastric Secretion

The previous generation of physiologists was concerned with the mechanisms which bring about an augmented secretion of gastric juice during mealtaking and while food is present in the stomach. Recently interest has developed in the mechanisms which bring about a cessation of gastric secretion when a sufficient amount of gastric juice has been secreted to digest the food and before the acid and pepsin concentration of the gastric content becomes great enough to damage the mucous membrane. Experiments have demonstrated that when food is applied to the mucous membrane of the antrum of the stomach a hormone, gastrin, is released into the blood stream and stimulates the gastric glands. When the food becomes acid in reaction, this stimulation ceases. Our experiments indicate that this cessation is due to inhibition of the release of gastrin rather than to the production of an inhibitory hormone from the antrum. When acid food enters the duodenum, the secretion of gastric juice is inhibited. This is due in part at least to a humoral agent since the inhibition is exhibited by a gastric pouch that has been deprived of vagus innervation. Our experiments suggest that this inhibitory agent is pancreatic secretin.

LESTER R. DRAGSTEDT
University of Florida
School of Medicine

Subunit Organization of Mitochondrial Membranes

In 1960–61 we detected a repeating particulate structure in the membranes of all types of mitochondria examined by electron microscopy with improved negative staining techniques. This entity, designated by us as the "elementary particle" (EP), has since been observed by others. In preparations of both *in situ* and isolated mitochondria, large numbers of the particles could be seen disposed in highly regular fashion in the cristae and outer limiting membranes. These particles show characteristic dense substructure and consist of two recognizable parts, a polyhedral head (80 to 100 Å) and a cylindrical stem (about 50 Å long and 40 Å wide) that links the head piece to the central layer of the membranes. By actual count in whole membrane mounts it is estimated that there are 10^4 to 10^5 elementary particles per mitochondrion. The fact that the elementary particle and associated membrane structures can be demonstrated reproducibly by a variety of techniques would indicate that these are actual components of the native mitochondrion. Recognition of this new structural constituent led to the isolation and reconstitution of a particulate unit in homogeneous state (molecular weight 1.4×10^6, diameter 140 to 160 Å) that contains the complete electron transfer chain and closely resembles native EP. Extensive electron microscope and biochemical evidence now available is consistent with the assumption that the isolated or reconstituted particles from beef heart mitochondria correspond essentially to the native elementary particle seen by electron microscopy. Correlated ultrastructural and biochemical studies carried out jointly with Green, Blair, and Oda *et al.* support the concept that this highly compact multienzyme unit is the ultimate seat of electron transfer in the respiratory chain. Research supported by the U.S. Atomic Energy Commission and the National Institutes of Health.

H. FERNANDEZ-MORAN
University of Chicago

RNA in Learning and Memory

Many minutes must elapse after an animal has had an experience before an enduring memory is formed in its brain. This "fixation time," probably due to continuing physiological activity of neurons, is measured by cutting off such activity at varied intervals after starting it and testing for retention of the altered performance. A maintained nondynamic change must be structural, and much attention has been given recently to macromolecules, especially RNA. We have found a drug that interferes with RNA synthesis to prolong fixation time and, perhaps more encouraging, one that enhances RNA to shorten fixation time. An asymmetrical cerebellar lesion in the rat leads to a postural asymmetry of the hind legs; this disappears after a high spinal section, made before fixation has occurred, but persists if the interval between the appearance of asymmetry and cord section is long enough. In normal animals this fixation time is 45 minutes; in animals treated with 8-azaguanine it is 70 minutes; and in those given a malononitrile dimer (U-9189, Upjohn) it is under 30 minutes. The U-9189 also speeds learning of an avoidance situation by intact rats.

R. W. GERARD
T. J. CHAMBERLAIN
G. H. ROTHSCHILD
University of Michigan

Studies on Two Components of Carbonic Anhydrase from Human Erythrocytes

We have studied the kinetic properties of the two major carbonic anhydrase isoenzymes in human erythrocytes [Nyman, *Biochim. Biophys. Acta* **52**, 1 (1961); Rickli and Edsall, *J. Biol. Chem.* **237**, PC258 (1962)]. Separation of carbonic anhydrase from hemoglobin has been achieved by a very gentle procedure involving chromatography of hemolyzate directly on Sephadex G-75. This yields essentially the same components obtained by more drastic procedures. Blood from several individuals contained the two components in nearly the same proportions in all cases; no evidence for genetic differences is yet apparent.

Initial reaction rates were measured spectrophotometrically in a stopped flow apparatus at 25°C, in 0.025*M* phosphate buffer. The two enzymes, tentatively denoted as CAI and CAII, yielded the following values for the kinetic constants (V_m denotes maximum velocity, E_o is molar enzyme concentration, and K_M the Michaelis constant).

*p*H	V_m/E_o (10^3 sec^{-1})	K_M (m*M*)
	CAI	
6.3	5.6	6
7.0	17	2.5
7.5	23	1
	CAII	
6.3	270	17
7.0	560	11
7.5	640	9

For the dehydration reaction at *p*H 7.0, CAI gave V_m/E_o = 20,000 sec^{-1} and K_M = 33m*M*; CAII gave 310,000 sec^{-1} and 66 m*M*. When cobalt replaces the zinc atom normally present in the enzyme, the maximum velocities are reduced by a factor of nearly 2, for both components I and II.

Nearly five times as much I as II is present in the erythrocyte, so that both components contribute significantly to the total catalysis of carbon dioxide transport in the blood.

BARBARA R. GIBBONS
JOHN T. EDSALL
Harvard University

Visual Discriminations of a Subject with Acquired Unilateral Tritanopia

The subject of this experiment exhibits a retinopathy of the macula. Consequently he can now perceive a definitely outlined, darkened area near the center of his affected eye.

Results of the Farnsworth D-15 test showed that many lines for the subject's color matches ran in the direction of the tritanopic axis. Acuity in the defective eye was reduced. A drawing of the field of the lesion was possible because the subject could measure its characteristics by "projecting" it against a light surface.

Determinations were made of wavelengths in the normal eye that matched spectral colors seen in the colorblind eye. Neutral point determinations were also made against a white light of 5500°K. In general, it was found that, up to a neutral point at about 570 mμ, the subject matched all of the lights in the defective eye by a color seen in the normal eye as a blue of about 475 mμ. Above the neutral point, the subject matched wavelengths in the defective eye by any narrow band in the normal eye lying in the range from about 620 mμ to the long-wave end of the visible spectrum.

Luminosity data for the normal eye are in accord with the usual luminosity

function. Determinations in the defective eye were made with some difficulty; because of the retinal injury, threshold values are high. However, they seem to show that luminosity loss occurs in the 550 to 650 $m\mu$ region of the spectrum.

Observations on color matching with the Farnsworth continuous matching test show that the "missing fundamental color process" occupies a spectral position near the violet end of the spectrum.

C. H. GRAHAM, YUN HSIA
Columbia University
F. F. STEPHAN
Princeton University

Isolation and Characterization of the Unit of Electron Transfer in Heart Mitochondria

Two developments led to the isolation of the elementary unit of electron transfer: (i) the discovery by Fernandez-Moran of a repeating structural unit (the elementary particle) in mitochondrial membranes and (ii) the isolation by Green *et al.* of structural protein (devoid of oxidation-reduction groups) accounting for 60 to 70 percent of the total mitochondrial protein. A particle has been isolated and reconstituted that uniquely contains all of the fixed components of electron transfer; the enzymic activity increases concomitantly with the purification of the particle. The rates of oxidation of DPNH and succinate are 2.5 times greater than those of sonicated mitochondria; the concentration of fixed components is increased by a factor of 2.2. The molecular weight of such a particle was calculated to be 2.1×10^6. The purification can be carried a stage further, to a particle which is 3.2 times more concentrated with respect to the fixed components than the mitochondrion and which has a molecular weight of 1.4×10^6. In this highly purified particle the enzymic activity does not parallel the increased concentration of components. The isolated or reconstituted particles (adjudged to be homogeneous by established criteria) appear to be a physical and functional aggregate of the four complexes known collectively to constitute the electron transfer system. Four lines of evidence show that the theoretical molecular weight of the elementary particle of the mitochondrion is no greater than 1.4×10^6. The available biochemical and biophysical evidence obtained by correlative studies supports the view that the isolated elementary particle is essentially identical with the repeating unit observed by Fernandez-Moran in the intact mitochondrion. Work supported by the U.S. Atomic Energy Commission and the National Institutes of Health.

D. E. GREEN, P. V. BLAIR, T. ODA
University of Wisconsin

Radio Stars with Large Red Shift

Five objects with extremely small angular diameters have been identified by radio astronomers. Accurate positions obtained at the Caltech Radio Observatory, when compared with Palomar photographs, had shown apparently stellar objects near the radio positions. Spectra obtained by Schmidt and Greenstein had revealed that no identifiable lines could be found; that no two objects had any lines in common; and that weak emission lines were present in at least three of the five objects. This extraordinary lack of success in spectroscopic identification had lead to the suggestion that these were stars of very low luminosity within our own galaxy and were possibly the remnants of supernova explosions. However, the discovery by Schmidt in 3C 273 of a series of regularly spaced emission lines, which resembled the Balmer series, became the key to recognition of these objects as unusual and very distant galaxies.

The study of 3C 48, a 16th magnitude object of nearly stellar appearance, has shown that the weak emission lines present are in fact forbidden lines of neon and oxygen. The strongest line is identifiable as the permitted resonance doublet of ionized magnesium, redshifted from $\lambda 2798$ to $\lambda 3832$. Another far ultraviolet line, $\lambda 2975$, the auroral transition in [Ne V], is also identifiable.

The galaxies are at great distances; the redshift of 3C 273 is 0.158 and of 3C 48, 0.3675. The object 3C 48 has an apparent velocity of +110,000 km/sec, which makes it the second most distant galaxy known (about 4 billion light years). Its luminosity is between 50 and 150 times that of our own galaxy, and is concentrated within less than 500-parsec diameter. Such objects are almost certainly the results of a giant explosion and represent the early stages in the development of the large radio sources.

An important consequence of the observed strength of the emission lines in such luminous objects is that far ultraviolet lines should be observable with the Palomar reflector to very large redshifts, $d\lambda/\lambda_0$ between one and two, that is, very close to the boundary of the observable universe.

JESSE L. GREENSTEIN
Mount Wilson and Palomar Observatories

Relationship between RNA Synthesis and Loop Structure in Lampbrush Chromosomes

Ribonucleic acid synthesis in lampbrush chromosomes occurs on loops which project from the main axis of the chromosome. These loops contain DNA in addition to RNA.

The synthesis of RNA in the chromosome is a DNA-dependent process, as judged by its sensitivity to inhibition by actinomycin D. Nucleolar RNA synthesis is also blocked by this antibiotic. The addition of actinomycin D to isolated chromosomes, or to intact oocytes, not only inhibits RNA synthesis, but also leads to a disappearance of the chromosomal loops. A modified actinomycin C_3 (which does not block RNA synthesis) does not cause loop retraction.

Other agents which inhibit nuclear RNA synthesis, such as the arginine-rich histones, also cause the loops to disappear. Inhibitors of protein synthesis, such as puromycin, do not have this effect.

The results suggest that the morphology of an active chromosomal site is not only closely related to its capacity to synthesize RNA, but also is dependent upon it.

MITSUO IZAWA, V. G. ALLFREY
A. E. MIRSKY
Rockefeller Institute

Charge Exchange Attraction between Primary Ions and Un-ionized Molecules in Radiation Chemistry

When a molecule is ionized in the vicinity of a similar molecule, a short-range attractive force of considerable strength may exist between them, in addition to the polarization and Van der Waals interactions. This is due to the possibility of electron exchange between the ion as long as it has not

relaxed to redistribute its charge and thus destroy the identity in geometry of the ion and neutral molecule. As soon as the ion relaxes the charge exchange interaction is no longer possible because of the electronic Franck-Condon principle, which forbids electrons to move if nuclei must move also. In the case of hydrocarbons, this constitutes a type of three-electron bond somewhat analogous to that in He_2^+ which has a strength of 2.5 ev except that four atoms, two C and two H, are bound. As the two hydrocarbons draw together, the H atoms may form a normal H_2 bond, leaving a one-electron bond between the C atoms analogous to that in H_2^+ which has a strength of 2.6 ev. Thus, the Weiss reaction, which is the direct bonding between a hydrocarbon ion and a neutral hydrocarbon eliminating H_2, can occur. Analogous reactions probably occur in many other systems. This research was supported by the Directorate of Chemical Sciences, Air Force Office of Scientific Research, under contract No. AF 49(638)-901.

LARRY KEVAN
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Horizontal Convection in the Earth's Mantle: A Mechanism for Strike-Slip Faulting

Local horizontal shear can be introduced into the earth's interior by horizontal gradients of temperature in regions of the interior that can be considered as horizontally stratified fluids. There is considerable evidence of lateral temperature gradients at shallow depths in the mantle associated with differences in oceanic-continental structure. At about 200 km some evidence is to be found for a layer of low strength. Such evidence includes: (i) a low-velocity layer, (ii) low energy release in earthquakes, and (iii) anomalous absorption of compression waves. If we assume a fluid layer with horizontal temperature gradients exists at this depth, then circulation in a horizontal plane can occur. By drag on the material above the 200-km horizon, horizontal dislocations can be induced in the crust, especially in regions of low strength, such as faults. The energy balance and the velocity distributions will be described. The process envisions small-scale circulation near continental margins as the driving mechanism for strike-slip earthquakes; the latter will occur in locations where faults are found above the circulation.

LEON KNOPOFF
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Continuous Conduction of Action Potentials by Peripheral Myelinated Nerve Fibers

An analysis has been made of the flow of action currents during conduction by single, undissected fibers of nerve trunks with intact sheath or desheathed.

1) The nerve (diameter 0.4 to 0.5 mm) is placed in an extended two-dimensional conducting medium (glucose Ringer's) and a microelectrode (diameter 30 μ) traveling in 0.5-mm steps measures the potential in the field of action currents.

2) Two rectangular, grounded silver plates are placed on the conducting medium so that the nerve lies at the axis of a 2-mm wide rectangular slot. The potential difference between points at the surface of the nerve and ground measures directly the density of the membrane action current.

The perfectly consistent and reproducible results are direct and incontrovertible evidence that conduction of action potentials is a continuous process. All points of the 2.5- to 3-mm-long internodal segments become successively active and at each point the action current flows through the membrane in three successive directions, outward, inward, and outward. Numerous assemblages of serial recordings have been obtained with more than 20 undissected, single fibers. None of them shows the slightest sign of discontinuities of conduction at the nodes of Ranvier. Work supported in part by grant B-2650 from the U.S. Public Health Service.

R. LORENTE DE NO
V. HONRUBIA
Rockefeller Institute

Chimpanzee Behavior: Removal of Foreign Body from Companion's Eye

The social activity of grooming by anthropoids has been much observed by students of comparative behavior. Chimpanzees that are friendly often exhibit mutually excited interest in grooming. These tendencies are not usually accredited to motives of "mutual aid." The operator, or groomer, appears to select his objectives and perform action patterns for his own pleasure, although he may treat or condition the groomed animal's skin in a way favorable to its health.

The present study reports a chance incident observed in Pan and Wendy, a pair of 30-plus-year-old chimpanzees, long-time companions who lived in an outdoor enclosure at the Yerkes Laboratories of Primate Biology at Orange Park, Florida. The date of this observed event was 21 February 1954. Pan was engaged in vigorous show-off activity before two men who stood some distance from the enclosure. He may well have kicked up some sand during his performance. At any rate, Wendy came whimpering up behind him, demanding his attention. He turned to her quickly. She sat down on the ground. He immediately crouched in front of her, looking in her eye at close range. With his two forefingers he manipulated the lids of her left eye so as to reveal the pink mucous membrane that evidently carried a foreign body which he removed. Relief came promptly and both chimpanzees remained quietly near each other for several seconds. Impromptu color photographs recorded the progress of this event which seems to be of a more advanced social order than grooming.

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Field Theory of Weak Interactions

A new approach to the study of higher order weak interaction effects, developed together with G. Feinberg, will be reported. We find that such effects may be observable even at low frequencies. The cause of this can be traced precisely to the unrenormalizability of weak interaction field theory in the sense of conventional perturbation expansions. These expansions, useless for our purposes, are circumvented by a new technique of summing leading singular parts of infinite sets of perturbation graphs. As a first model we treat charged vector meson theory and start with pure leptonic processes. The Bethe-Salpeter equation (for uncrossed graphs) is soluble by a new

iteration scheme. In leading order we reproduce the conventional zero energy results, provided g^2 is replaced by $3\ g^2/4$ (g = meson lepton coupling). However, there exists energy-dependent deviations from the conventional second-order results. These are in principle observable in μ-decay.

The applicability of the theory to semileptonic and nonleptonic phenomena depends on properties of the baryon and meson currents and on the effects of the strong interactions. Preliminary considerations along these lines are given. A main result is that $\Delta S = -\Delta Q$ leptonic transitions are of the same order as $\Delta S = \Delta Q$ processes in a theory which does not introduce the former from the outset ($\Delta T = 1/2$ interaction). At the same time, effective neutral lepton currents remain negligible in a theory which does not contain them *ab initio*.

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Immediate Cause of Phototropic Curvature in the Maize Seedling

For some time the cause of phototropic curvature has been controversial because of conflicting claims of: (i) photolytic auxin destruction on the lighted side of the seedling; (ii) lateral movement, either of auxin or of an auxin precursor, across the plant from lighted to shaded side; and (iii) effects of light on transport and on auxin sensitivity of the tissue. The phenomenon has therefore been reexamined by use of purified carboxyl-labeled C^{14}-indole acetic acid (IAA).

When the labeled acid was applied externally to the tips of intact maize coleoptiles, illuminated from one side with light dosages that caused the "first positive" curvature, the amount of acid emerging on the shaded side was about 75 percent of the total. If the tips were halved longitudinally and analyzed, the shaded half of the tissue contained 65 percent of the total. With long light exposures such as cause "second positive" curvatures, the amount emerging on the shaded side was 66 percent of the total. From subapical sections given similar high light exposures the shaded side yielded 54 percent of the total, and sections of oat coleoptiles gave the same figure. All of the radioactivity applied to such sections was recoverable after illumination. Correspondingly, bioassay showed no decrease in the endogenous auxin formed by the coleoptile tips after long light exposures. It is concluded that photolytic destruction of auxin is not the cause of phototropic curvature. Instead, both in the first and second positive responses, C^{14}-indoleacetic acid (whether exogenous or endogenous) is itself transported laterally from the lighted to the shaded side.

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Turnover of Nuclear Proteins in Amoeba

The turnover of total nuclear protein was followed in *Amoeba proteus* during growth and cell division by combining labeling with tritiated amino acids with a micrurgical technique. Amoebae were grown in a medium containing a mixture of seven tritiated amino acids (including lysine, arginine, and histidine) for a full-cell cycle. The cells were subsequently cultured on nonradioactive nutrients, and approximately every 36 hours about 50 percent of the cytoplasm was removed with a microneedle and then discarded. The nucleated fragments continued to synthesize new cytoplasm but the timing of the operations prevented cells from reaching mitosis. The operations resulted in gradual replacement of the original radioactive cytoplasm without direct disturbance to the nucleus (15 operations would give about a 10,000-fold dilution of original cytoplasm). Under these conditions the behavior of the radioactive proteins could be observed autoradiographically, and the following points were demonstrated. The nucleus contains a large fraction of protein which has a high affinity for the nucleus but at least part of this is constantly moving between the nucleus and cytoplasm. Such migration has been clearly shown by L. Goldstein [*Exptl. Cell Res.* **15**, 635 (1958)]. All of the nucleus-specific protein identified by labeling is released from the nucleus during mitosis and dispersed throughout the cytoplasm. The chromosomes and spindle do not contain a higher concentration of radioactive protein than the cytoplasm. The nucleus-specific protein rapidly returns to the two daughter nuclei during late telophase and early interphase. A series of 30 successive operations results in the disappearance of all detectable radioactive protein from the nucleus. Therefore, all major protein components of the nucleus, including histones, are turning over. No nuclear protein could be identified which retained label under the same conditions that result in complete retention of radioactivity in DNA labeled with tritiated thymidine.

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Proposed Important Mental Tools for Scientific Thinking at the High School Level

Some experimentation during the Ohio "Project: Honoring the Science Scholar" suggests that the basic logical structure of a quantitative law of nature and its limits can be focused in the minds of students through puzzles whose solutions are based on unifying principles. For example, the Koenigsberg bridge problem has a law: It is *always true* that a land area with an odd number of bridges is a path terminus *no matter what* the number of bridges or land areas *provided that* no boats are used. Individuals of high school to post-doctoral level usually solve such problems by following the course (described for example in Hadamard, *Psychology of Inventions in the Mathematical Field*) of acquiring *familiarity* with the phenomena, obtaining a *hunch* about the answer or law involved, and finally achieving *progress* by detailed analysis. Preliminary teaching experiments indicate that the logical structure of laws and methods of finding them can be learned and fixed in mind with aid of the italicized phrases, and that these concepts are probably the most important mental tools for scientific thinking that can be developed in high school. It is proposed that the next most important mental tool is facility in translating concept of quantitative reality into algebraic symbol and back again. An additional viewpoint of importance is that the practice of science is an art; and personal predilection rather than quantitative thinking usually determines whether a problem calls for paper and pencil, experimental apparatus, reference to publications, or inquiry to a well-informed source.

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The Ionic Centrifuge Can Give Fusion Nuclear Power

The ionic centrifuge is a vacuum vessel enclosed by two circular end plates and by a straight surrounding cylinder made up of closely spaced slats. The gas which is introduced at the center by means of centrally placed tubes streams across an intervening parallel magnetic field, is ionized thereby, and discharges to the end plates and to the slats of the surrounding cylinder.

First I will describe the distribution of the ion current we obtained over the end plates and the slats of the floating cylinder (*1*). On increasing the negative voltage applied to the end plates, the floating negative voltage of the cylinder did not remain approximately zero, but it rose, remaining nearly equal to that on the end plates, until its voltage equaled $-[e/8\pi c^2 m)\ (B^2 r^2$ e.m.u.)] after which it remained constant. The ion current which it received remained constant at one half the total, with the initial current varying from 1 to 100 amperes, and with a wide variation of *B*. One textbook (*2, 3*) agreed with the ion current distribution observed. If the gas used was capable of nuclear fusion, and the cylinder was kept at zero voltage, nuclear fusion might have been observed.

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Comparative Structures of Cytochromes c

The amino acid sequence of horse heart cytochrome *c* has been described [*Nature* **192**, 1121 (1961)]. Recently, the structures of this protein from human [*J. Biol. Chem.* **237**, PC 3575 (1962)], dog, and monkey hearts have been determined in this laboratory; structures from chicken and pig hearts have been determined by E. Margoliash of Abbott Laboratories (private communication). All of these cytochromes are *N*-acetylated and contain 104 amino acid residues. In the six species there are 17 loci at which substitutions have been found. Some of these substitutions are conservative in that an amino acid is replaced by one with a similar side chain, whereas others are radical in that residues with different side chains are substituted. These latter substitutions must be at loci which play little or no role in determining the functional properties or native conformation of the cytochrome *c*. Conversely, there are several regions of the sequences of these cytochromes at which no changes have been found; these could represent areas which may be critical for the functional integrity of the protein. As expected, more closely related species possess proteins which are similar. Those of human and monkey differ in only two residues, those of horse and pig differ in only three residues. Such comparative structural studies of a protein should permit further understanding of the evolution of the single gene which presumably determines the structure of cytochrome *c*.

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Characteristics of the Process of Aging in Algal Cells

Metabolic activity during the life cycle of cells does not remain constant but undergoes changes whose direction, rate, extent, timing, and dependence on external conditions vary with the progress in cell development. Rates of respiration and photosynthesis in synchronized suspensions of algal cells generally decline toward the end of the life cycle. Quantum efficiency of photosynthesis and saturating light intensity are lower during the later developmental stages.

The overall capacity for organic synthesis also varies in the course of cell development. In younger cells synthesis of organic matter takes place at an appreciable rate even in a nitrogen-free suspending fluid. In cells of later developmental stages the synthetic activity, both in nitrogen-free and nitrogen-containing media, rapidly declines toward the time of cell division. The dependence of the level of synthetic activity on the age of cells can be demonstrated also on cells separated from a nonsynchronized cell suspension by fractional centrifugation. The level of metabolic activity and its decline in the process of cell development is a basic characteristic of aging on the cellular level.

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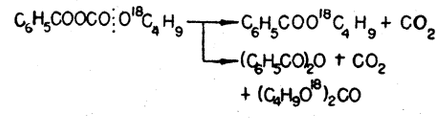
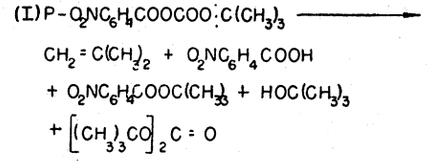
Studies on Mixed Carboxylic-Carbonic Anhydrides

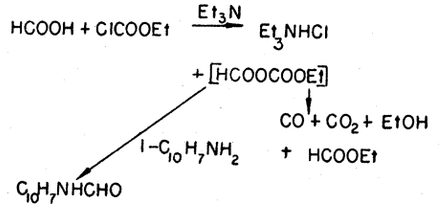
It has been shown [Michejda, Tarbell, Saunders, *J. Am. Chem. Soc.* **84**, 4113 (1962)] that benzoic *sec*-butylcarbonic anhydride decomposes entirely by acyl oxygen cleavage:



It has now been possible to prepare *p*-nitrobenzoic *tert*-butylcarbonic anhydride as a stable crystalline material and to study its decomposition at 100°C; it undergoes mainly alkyl oxygen cleavage to yield isobutene, along with ester, acid, *tert*-butyl alcohol, and di-*tert*-butyl carbonate.



Evidence has been obtained for the formation of mixed formic-carbonic anhydrides; the reaction mixture acts as a formylating agent under conditions where none of the stable components of the mixture do. The mixed anhydride decomposes at 0°C to yield CO, CO_2, ethanol, and ethyl formate.



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Isotopic Abundance Variations in Meteorites

Reynolds showed that meteoritic xenon has an isotopic composition different from that of terrestrial xenon. This suggested the possibility that other elements might show similar variations. It is found to be true in some other cases, that is, barium (Umemoto), molybdenum (Murthy), silver (Murthy), and lithium and boron (Shima). Molybdenum samples of different isotopic composition are found within the Aroos

meteorite. Some other elements have been investigated but no differences between the meteoritic and terrestrial samples beyond observational error have been found. We suggest that (i) meteoritic matter differs from the terrestrial and (ii) a still different sample of molybdenum has been injected in the Aroos meteorite. Some iron meteorites contain samples of both primitive lead and radiogenic lead without an adequate amount of uranium and thorium to have produced the radiogenic lead. Also, chondrites contain more radiogenic lead than could have been produced by the uranium and thorium present in them. These facts also indicate that lead has been transferred from some other source into these objects. Finally, bismuth appears to have been introduced into the dark part of the Pantar meteorite, according to Reed's observations. All these transfers of non-volatile elements indicate high temperature volatilization and injection under high pressure. Yet the Widmanstätten figures of the irons and diffusion borders of the metal particles in chondrites indicate that low temperatures have obtained. We suggest a quick injection of material in a collisional process and favor a comet head collision as one most likely. It is well known that the inert gases of meteorites differ from those of the earth, but attention is directed here to the much more abundant and nonvolatile elements.

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Control of Pupal Diapause by the Direct Action of Light on the Insect Brain

Pupae of the Tussah silkworm, *Antheraea pernyi*, overwinter within their silken cocoons in a state of arrested development termed "diapause." We find that the termination of diapause, as signaled by the resumption of development, is markedly influenced by the photoperiod to which the cocoons are exposed. Continuous light, or continuous darkness, or 16 hours of illumination per day favor the termination of diapause. By contrast, the dormancy persists at room temperature as long as the cocoons are exposed to 8 hours of illumination per day.

In experiments performed with Charles Walcott the cocoon was found to serve as a light-integrating sphere. Light transmitted or reflected within the cocoon ultimately enters the pupa by way of a zone of transparent cuticle overlaying the insect's brain.

By surgical methods we have been able to show that light acts directly on the pupal brain; consequently, the effects of photoperiod are not disturbed after all nerve connections between the brain and the rest of the insect are severed.

The photoperiod acts within the brain to control the neurosecretory cells which secrete the "brain hormone" prerequisite for the resumption of development. By suppressing this secretion, an 8-hour day enforces a continuation of pupal diapause.

This effect totally disappears if the exposure is delayed until the brain has already secreted a threshold titer of hormone; this occurs on the first or second day of the 19 days required for adult development at 25°C. Studies supported in part by research grants from the National Institutes of Health and the National Science Foundation.

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Do Stellar Chromospheres Evolve?

High-dispersion spectrograms of integrated sunlight show weak double emission components at the centers of the broad H and K absorption lines of Ca II; it is known that these emission features originate in the chromosphere.

Spectrographic observations have been made of many main-sequence stars (similar to the sun in luminosity) in the solar neighborhood and in the Pleiades, Hyades, Praesepe, and Coma clusters. They show that the H-K emissions average very much stronger in the cluster stars than for those in the local field, and that they are strongest in the Pleiades. It is known also that the ages of the Pleiades, Hyades-Praesepe-Coma, and the sun are approximately 5×10^7, 5×10^8, and 5×10^9 years, respectively.

The most likely interpretation of the observations, therefore, is that the strength of the H-K reversals in a main-sequence star, and hence the general degree of its chromospheric activity, is a decreasing function of its age. This idea is consistent with all the observations described here as well as with others which are relevant to the problem.

A strong correlation is known to exist over the solar surface between local magnetic field strength and H-K emission intensity. Thus, a decrease in the chromospheric activity of a star may well be due to a decrease in its average surface magnetic field strength.

If stellar chromospheric activity decreases with time, this has presumably been true of the sun also. Hence the flux of ionizing radiation falling upon the earth and other planets may have been considerably greater in earlier epochs than at present.

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Glycoside Formation with a Nonacylated Glycosyl Halide

Mercaptolysis of methyl 3-amino-3-deoxy-α-D-mannopyranoside hydrochloride (I) has been shown (Wolfrom, Horton, Garg, *J. Org. Chem.* in press) to yield an approximately equal mixture of the anomers of ethyl 3-amino-3-deoxy-1-thio-D-mannopyranoside hydrochloride. Replacement of the ethylthio group by chlorine was effected by chlorination under mild conditions and the resultant amorphous 3-amino-3-deoxy-D-mannopyranosyl chloride (hydrochloride) (II) was readily convertible to I with methanol. The yield of II was good.

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