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Fatty Acids in Sedimentary Rocks

Palmitic and stearic acids have been isolated from a variety of sedimentary rocks ranging in age from Recent to the Cambrian Alun Shale. Crude extracts obtained by conventional procedures were separated by gas-liquid chromatography. The identity of the individual acids or esters was confirmed by physical methods.

PHILIP H. ABELSON,
THOMAS C. HOERING, PATRICK L. PARKER
Geophysical Laboratory,
Carnegie Institution of Washington

Photosynthetic Phosphorylation at Temperatures Above and Below 0°C

The key event in photosynthesis, the conversion of radiant energy into physiologically useful chemical energy, is now known to be closely related to the formation of adenosine triphosphate (ATP) and reduced pyridine nucleotide by the reactions of photosynthetic phosphorylation (cyclic and noncyclic photophosphorylation). Photosynthetic phosphorylation can be investigated in subcellular particles (chloroplasts) when CO_2 assimilation is excluded and the chemical events resulting from light absorption by chlorophyll are limited to the synthesis of pyrophosphate bonds of ATP and the reduction of pyridine nucleotide.

We have investigated photosynthetic phosphorylation over a temperature range from −10° to 15°C under experimental conditions which select either shorter or longer chemical pathways for ATP formation. [The presently envisaged reaction mechanisms are discussed in *Proc. Natl. Acad. Sci. U.S.* **47**, 1314 (1961).] In accordance with the idea that the shortest chemical pathway should be least sensitive to temperature, cyclic photophosphorylation catalyzed by phenazine methosulfate (PMS) gave, in isolated chloroplasts, markedly higher yields of ATP at temperatures below 0°C than (i) cyclic photophosphorylation catalyzed by flavin mononucleotide or vitamin K_3 (menadione), or (ii) noncyclic photophosphorylation with pyridine nucleotide or ferricyanide. By reducing the electron flux (low light intensity), ATP formation catalyzed by PMS became independent of temperature in the range −10° to 15°C.

The observed temperature effects provide further support for (i) the proposed mechanisms for cyclic and noncyclic photophosphorylation, and (ii) the view that photosynthetic phosphorylation by chloroplasts is autonomous and distinct from the more temperature-sensitive oxidative phosphorylation in mitochondria [see *Science* **122**, 9 (1955)].

DANIEL I. ARNON, DAVID O. HALL
University of California, Berkeley

Significance of Dark Reversion of Phytochrome in Flowering of Short-Day Plants

Flowering of soybean and chrysanthemum, two short-day plants, is promoted by subjecting them to several daily dark periods of about 16 hours and is inhibited by illuminating them near the middle of each long dark period with low-intensity (50 foot candles, for example) unfiltered incandescent-filament light. Ten minutes of such light is more than adequate to inhibit flowering of soybean, but 2 or more hours are required to prevent flowering of chrysanthemum.

The light, however, need not be continuous during such a period to inhibit the flowering of chrysanthemum. In fact, it is an effective inhibitor if introduced in a succession of cycles each consisting of a few minutes of light and many minutes of darkness throughout the period of 2 or more hours. By this procedure flowering of chrysanthemum is inhibited by less than 10 percent of the light that would be required if the illumination were continuous.

The effectiveness of intermittent lighting on chrysanthemum is explained as being dependent on certain characteristics of phytochrome, the photoreceptive pigment that controls flowering. The pigment exists in two forms that are interconverted by red and far-red light. The far-red form is believed to be the biologically active one, but it is unstable and reverts slowly in darkness to the inactive red-absorbing form. In chrysanthemum, dark reversion of active phytochrome reduces the phytochrome to an ineffective level before it completes its flower-inhibiting function. Experiments involving different durations of darkness between successive periods of light give a measure of the time required for active phytochrome to revert to an inactive level. For chrysanthemum the time is about 1 hour. Poinsettia resembles chrysanthemum in that a single brief irradiance with incandescent-filament light does not inhibit flowering. Cocklebur, however, resembles soybean in that its flowering is readily inhibited by brief illumination with incandescent-filament light.

H. A. BORTHWICK
H. M. CATHEY
U.S. Department of Agriculture, Beltsville, Maryland

Wave Distortion for Magnetic Moment Effects in Nucleon-Nucleon Scattering

Magnetic moment effects in nucleon-nucleon scattering are not negligible [A. Garren, *Phys. Rev.* **96**, 1709 (1954); G. Breit, *ibid.* **99**, 1581 (1955); M. E. Ebel and M. H. Hull, Jr., *ibid.* **99**, 1596 (1955); A. Garren, *ibid.* **101**, 419 (1956)], especially in view of progress in data analysis. Their calculation is interfered with by wave function distortion of uncertain magnitude even at reasonably small scattering angles [G. Breit, *Phys. Rev.* **106**, 314 (1957)]. On the basis of approximate knowledge of nucleon-nucleon interactions, the distortion effect is estimated as appreciable. The difficulty is largely removed by treating magnetic moment effects for the one pion exchange (OPE) phase parameters separately from those of the low orbital angular momentum (L) group. Knowledge of magnetic effects for the latter is not needed in usual analysis since they may be lumped with those of mesonic origin.

Quantitative understanding of mesonic interactions for low L is in a too rudimentary stage to make reliable corrections for magnetic moment effects possible. Since these mesonic interactions are large and not exactly calculable, the magnetic moment effects are as yet not needed for fundamental purposes except perhaps for tests of charge independence. For the OPE group an approximate calculation is possible. Formulas and their bearing on experimental data will be presented.

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G. BREIT, H. M. RUPPEL
Yale University

Intermediates in Biological Nitrogen Fixation

The importance of ammonia as an intermediate in biological nitrogen fixation is supported by several lines of evidence: nitrogen-fixing organisms prefer ammonia to other sources of nitrogen; when they have been using N_2 as a sole source of nitrogen they will utilize ammonia immediately when it is supplied to them without delay for adaptation; the pattern of assimilation of N^{15} from N_2 into amino acids and the kinetics of the process are as predicted for ammonia as an intermediate; when N_2^{15} is supplied to cultures of *Clostridium pasteurianum* growing under

specific conditions and to cell-free preparations from the organism, the cultures produce and accumulate free ammonia with very high concentrations of N^{15}. Although the importance of ammonia in nitrogen fixation is generally accepted, the intermediates with a reduction level between N_2 and ammonia have not been established. Recent experiments with cell-free preparations from *C. pasteurianum* have not supported the occurrence of free or bound hydrazine as an intermediate in nitrogen fixation. The suggestion that dihydropyridazinone-5-carboxylic acid is an intermediate could not be confirmed. Hydroxylamine often has been suggested as an intermediate in nitrogen fixation, but cell-free preparations fixing $N_2{}^{15}$ vigorously showed no accumulation of N^{15} into free or bound hydroxylamine. The difficulty in demonstrating free or readily released intermediates of nitrogen fixation suggests that products between N_2 and ammonia may remain tightly bound to the enzyme complex during stepwise reduction.

R. H. BURRIS
University of Wisconsin

Localized Fluorometry of Oxidation-Reduction States of Intracellular Pyridine Nucleotide in Brain and Kidney Cortex of the Anesthetized Rat

Blood oxygen levels, as measured continuously by an oximeter [G. A. Millikan, *Proc. Roy. Soc. London* **B123**, 218 (1937)] or by a platinum microelectrode [P. W. Davies and D. W. Bronk, *Federation Proc.* **16**, 689 (1957)], may give too high an approximation of cellular oxygen concentrations available to the respiratory chain of mitochondria and may respond insensitively or sluggishly to changes in metabolism caused by physiological function.

Spectrophotometry and fluorometry of cell suspensions and excised tissues led us to suggest that measurements of the fluorescence emission of the reduced pyridine nucleotide component of mitochondria would be applicable to a thick layer of tissue and would be relatively insensitive to the state of oxygenation of hemoglobin [B. Chance and F. Jobsis, *Nature* **184**, 195 (1959)]. We can now report continuous recordings of changes of intracellular oxidation-reduction levels of various tissues and organs *in situ* in the anesthetized animal. The peak of fluorescence emission of the aerobic kidney cortex is, on a relative energy basis, at 472 mμ, as compared to 466 mμ for its isolated mitochondria, and the intensity approximately doubles in anoxia. Chemical studies identify this increase of fluorescence chiefly with the reduction of pyridine nucleotide, with some contribution from the cytoplasmic material.

By means of a modified microfluorometer [B. Chance and V. Legallis, *Rev. Sci. Instr.* **30**, 732 (1959)], a 20-micron area of brain or kidney cortex can be selected, and continuous observations can be made over a period of a few hours in the anesthetized animal. Increased pyridine nucleotide reduction in the brain cortex is not observed until the inspired oxygen concentration falls to 8 percent, and half-maximal effect is obtained with a 4-percent concentration. On the assumption that properties of isolated mitochondria are similar to properties of mitochondria in vivo, average intracellular tensions of 1.3 millimeters and 0.1 millimeter, respectively, have been computed. By using two microfluorometers it has been possible to compare the responses of pyridine nucleotide of the brain and of pyridine nucleotide of the kidney cortex of the same animal to a variety of pharmocologically active substances. For example, we note in vivo the increased reduction of mitochondrial pyridine nucleotide caused by the oxybarbiturates but find that the sensitivity of the kidney is higher than that of the brain cortex. Localized circulation control by vasoconstrictors such as norepinephrine is also observed. The degree to which adenosine diphosphate and phosphate activate metabolism has been studied in a preliminary fashion, and the kidney cortex appears to be fully activated [State 3, B. Chance, G. R. Williams, *Nature* **176**, 250 (1955)].

B. CHANCE, P. COHEN,
F. JOBSIS, B. SCHOENER
Johnson Foundation,
University of Pennsylvania

Magnetic Resonance Studies of Metal-Enzyme-Substrate Interactions

In enzymatic reactions involving metal ions, the study of possible ternary complexes of metal, enzyme, and substrate has been hampered by the lack of an observable parameter characteristic of such complexes. It has now been found that the interactions of the various components of an enzymatic reaction involving paramagnetic ions can be measured by the effect of the resulting complexes on the longitudinal and transverse relaxation times of the nuclear spin of the protons of water in the system. The experimental and theoretical aspects of the marked decrease in the longitudinal relaxation time of water protons, T_1, due to paramagnetic ions such as the manganous aquo-cation has been studied by many investigators; the magnitude of the effect is of the order of 10,000 for a 1*M* solution of manganous ions. The efficacy of the manganous ion in affecting the relaxation rate of the water protons depends sensitively on the immediate environment of the ion, and for this reason the measurement of T_1 of the water protons in enzyme systems has proved most useful in probing the active site of enzymes which are activated by manganese. It has been found that the metal-enzyme complexes for some enzymes, or the metal-enzyme-substrate complexes for other enzymes, enhance the effect of free manganous ion by a factor of the order of 10.

The existence of ternary complexes of manganese, substrate, and enzyme has been demonstrated for several enzymes such as creatine, phosphokinase, 3-phosphoglycerate phosphokinase, pyruvate phosphokinase, and enolase. The formation of ternary complexes is specific for the substrates of these enzymes. In the case of enolase it has also been shown to require the presence of active enzyme, since the ternary complex disappeared in 4*M* urea. Furthermore, for the ternary complex of metal-substrate creatine phosphokinase, measurement of both the enhancement of the proton relaxation rate and the electron spin resonance spectrum has led to the conclusion that the metal ion is bound only to the nucleotide and not to the enzyme in the ternary complex. The determination of metal-substrate and metal-enzyme dissociation constants by this technique will also be discussed. From the encouraging results thus far obtained, it would appear that magnetic resonance techniques show promise of being a powerful tool in elucidating the interactions of enzyme and substrate at the active site of the enzyme.

MILDRED COHN
Johnson Foundation,
University of Pennsylvania

Neuronal Extension and Glial Supply

Our knowledge concerning the functional significance of glia cells is still insufficient. The present study represents an attempt to correlate the number of glia cells with known morphological features (length of axon) of neurons. Clark's column in the spinal cord provided an opportunity to compare the glial supply of nerve cells which are functionally identical but have different length axons. The number of perineuronal glia cells in Clark's column shows a linear relationship to the length of the axons. Additional counts of the glia index (number of glia cells per nerve cell) indicate less glial supply in nuclei with very short connections than in nuclei with long connections.

Glia cells, particularly oligodendroglia, are considered "auxiliary metabolic units" attached to nerve cells wherever the morphological extension of the neuron renders the maintenance of metabolism difficult. Increased functional demands on a nerve cell apparently are not met with hypertrophy but with the attachment of glia cells to the nerve cell.

REINHARD L. FRIEDE
WIECHER H. VAN HOUTEN
University of Michigan

Structure of Crystal Surfaces

The diffraction of slow electrons can be used to determine the arrangement of the surface atoms of a crystal. This is well known [C. J. Davisson and L. H. Germer, *Phys. Rev.* **30**, 705 (1927); L. H. Germer, *Z. Physik* **54**, 408 (1929)], but the original method is so tedious that it has attracted few investigators (H. E. Farnsworth and his co-workers). In a newer method the diffracted electrons are accelerated to a fluorescent screen and can be photographed. Work in this field now gives promise of becoming extensive enough to build up a great body of knowledge regarding the two-dimensional structure of

crystal surfaces, potentially surpassing in extent knowledge of three-dimensional structures. On most, but not all, clean surfaces studied, the arrangement of surface atoms is the same as the arrangement in similar planes within the crystal, but for nickel crystals the surface plane is displaced outward by about 0.1 angstrom. Foreign atoms or molecules often take up positions on a clean surface structurally related to the arrangements of the atoms of the crystal, without significantly disturbing the latter. This is true of various atoms and molecules adsorbed upon (111) and (100) planes of nickel crystals. But upon the next less dense (110) plane, adsorption of carbon, oxygen, hydrogen, or deuterium causes immediate rearrangement of surface nickel atoms so that, in each case, the resulting superficial plane contains just half the normal number of metal atoms. The three completely different structures resulting from adsorption of carbon, oxygen, and hydrogen (or deuterium) will be described.

L. H. GERMER
Cornell University
A. U. MACRAE
Bell Telephone Laboratories

Neural Discriminations Achieved by Bats in Echolocation

Bats rely on echolocation when intercepting flying insects, when drinking by skimming water surfaces on the wing, and when catching aquatic insects or fish at the surface. Their performance is not impaired by total darkness or blinding. Interception maneuvers will be demonstrated in motion pictures by Webster and Cahlander. One to five echoes of the pulsed orientation sounds (duration 0.5 millisecond to 10 milliseconds) enable them to distinguish the significant echoes from many others returned by much larger objects at nearly the same distances. Neurophysiological recordings by Grinnell have demonstrated the following properties of single units or of synchronously discharging populations of brain cells: (i) response to the second as well as the first of two pulses of sound timed to simulate the emitted pulse and its echo, even when the second pulse is 20 to 25 decibels fainter than the first and follows it by only 1 or 2 milliseconds (corresponding to a range of 17 to 34 centimeters); (ii) differential sensitivity to sounds arriving from different directions (thresholds of certain units change by as much as 9 decibels per degree); (iii) responses to a narrow band of frequencies and to these only at low intensities (such cells would discriminate against a loud emitted pulse in favor of its echo); and (iv) sensitivity to a signal when signal and noise arrive from different directions; a signal that would otherwise be masked may have only a slightly higher threshold than in the quiet. These findings help explain the auditory discriminations required by echolocation, including the resistance of bats to jamming.

D. R. GRIFFIN, A. D. GRINNELL, J. J. G. MCCUE, D. CAHLANDER, F. A. WEBSTER
Harvard University and *Massachusetts Institute of Technology*

Some Primary Abilities in the Areas of Nonverbal Divergent Production

Divergent production means generating a variety of items of information from a given item of information. Abilities of fluency, flexibility, and elaboration, which belong logically in this general category, are believed to be uniquely important for creative thinking in general.

Prior to the study on which this report is based, six different divergent-production abilities dealing with semantic (verbal meaning) information had been recognized. Although the structure-of-intellect model predicts the existence of six parallel abilities for dealing with purely symbolic information and also six for dealing with figural (concrete, perceived) information, only two of each category had been previously investigated and isolated in factor-analytic studies.

Twenty-one tests were designed especially to determine whether seven of the remaining eight predicted factors in these two categories could be distinguished. The experimental subjects were 238 Navy pilot trainees in one sample and 205 boys and girls in a ninth-grade class in another.

Five of the seven factors investigated were found in the analysis of the ninth-grade results, and four of the seven in the adult-male results. On some tests the functioning was somewhat different in the two samples, but four of the new factors appear to be common to the two groups.

It is believed that the divergent-production abilities in the symbolic area are important for creative mathematicians and cryptographers and that the divergent-production abilities in the figural area are important for creative visual artists and inventors.

J. P. GUILFORD, PHILIP R. MERRIFIELD, ARTHUR GERSHON
University of Southern California

Observations on the Physiological Thermostat in Homoiotherms

In an effort to determine the nature of the physiological thermostat in the homoiotherm, a series of experiments have been performed that have involved heating and cooling of small volumes of hypothalamic tissue in the unanesthetized dog. These experiments showed that (i) thermoregulatory responses could be evoked by changes in hypothalamic temperature as small as 0.2° to 0.3°C, and (ii) the most sensitive tissue was localized in the volume just rostral and ventral to the anterior commissure within 2 to 3 millimeters of the midline.

In a second series of experiments, microelectrodes were placed in the sensitive preoptic region of urethanized cats to record the electrical activity of single cells during periods of local heating and cooling. These experiments showed the following results, after recording from about 500 units: (i) about one cell out of five rapidly increased its discharge rate with increased temperature ($Q_{10} = 5$–10); (ii) a majority of the cells showed minimal temperature sensitivity ($Q_{10} = 1$–2); (iii) no cells were found which increased discharge rate with cooling; and (iv) temperature-sensitive cells were not found in the supra-optic nucleus or the posterior hypothalamus.

The responses of the temperature-sensitive and -insensitive neurons, taken together, are sufficient to provide the temperature set point and thermal load error information for the physiologic thermostat.

J. D. HARDY, H. T. HAMMEL, T. NAKAYAMA
Yale University

Enzymes of DNA Synthesis in Nuclei of Sea Urchin Embryos

The system of enzymatic deoxyribonucleic acid (DNA) synthesis discovered by Kornberg and his associates has been found in tissues of higher organisms. The enzyme would be expected to be a component of the nucleus, where the DNA synthesis presumably takes place. A favorable material should be isolated nuclei from a cell population in which all of the cells were engaged in DNA synthesis at the time of isolation. This condition can be met by isolating nuclei from sea urchin embryos at early stages of development. In the work under discussion, the cleavage and blastula stages of eggs of *Strongylocentrotus purpuratus* were used, and the isolation procedure was one developed by Hinegardner, involving osmotic lysis of the cells in 0.002*M* $MgCl_2$, followed by separation of nuclei on a sucrose gradient.

The assay method was that utilized by Kornberg and his collaborators, involving measurement of the incorporation of a P^{32} label in one of the deoxynucleoside triphosphates into PCA-insoluble material (this work was made possible through the aid of Arthur Kornberg).

The following observations may be reported. (i) The isolated nuclei do synthesize DNA by the polymerase system. (ii) The product can be shown to be DNA by the fact that the incorporated counts band with the total DNA of the system in a cesium chloride gradient. (iii) Some of the DNA in the isolated nuclei can serve as primer; the nuclei carry out measurable synthesis without the addition of DNA. (iv) The addition of dissolved DNA in an amount equal to or even less than the amount already present in the nuclei enhances the synthesis several-fold, suggesting that much of the DNA in the nucleus is not effective in priming enzyme that is present there. (v) Net synthesis is limited; a doubling has not yet been achieved. (vi) The nuclei also contain kinases. Synthesis can be obtained with 3-deoxynucleoside monophosphates plus adenosine triphosphate in the presence of a labeled deoxynucleoside triphosphate. (vii) Nuclei isolated from later stages of development, at which the rate of cell division is much lower than that during early cleavage, are less active in the same assay system.

On the basis of the findings so far it is possible to invoke both the state of the nuclear DNA and the enzyme content of the nucleus in devices for the control of DNA synthesis. This research was supported by the U.S. Public Health Service (NIH grant RG-6025).

RALPH HINEGARDNER, DANIEL MAZIA
University of California, Berkeley

Electronic and Steric Factors in Induction of Cancer by Hydrocarbons

Under special conditions which are easily satisfied, a single administration of any of a large number of polycyclic aromatic hydrocarbons induces cancer selectively, invariably, and very rapidly. The effects are similar to a sublethal dose of radiation. Despite divergent molecular structure, the chemical compounds have in common the property of inducing cancers of certain types. Substituents with potency in converting an otherwise ineffective parent molecule to a powerful carcinogen are $-CH_3$, $-NH_2$, and additional ring structure in a critical location. Albert Szent-Györgyi discovered that the carcinogenicity of aromatic molecules is correlated with their ability to form charge transfer complexes. But charge transfer per se is not enough to cause cancer. Steric factor is involved. There is a striking structural resemblance between three types of molecules: polycyclic aromatic hydrocarbons, steroid hormones, and base pairs of nucleic acids. To induce cancer, hydrocarbons must resemble base pairs of nucleic acids in solid geometry and must be powerful electron donors.

CHARLES HUGGINS
NIEN-CHU YANG
University of Chicago

The Kinetics of Unimolecular Dissociation

The most direct experimental test of the theories of the kinetics of dissociation of excited polyatomic molecules would be to study the kinetics of dissociation of isolated molecules containing precisely known amounts of internal excitation energy. Such an experiment is not as yet possible, but it can be approached.

One method by means of which molecules can be given precisely known amounts of excitation is photoexcitation. Unfortunately, the dissociation of a single excited molecule cannot be detected in a simple, straightforward manner. However, if, in addition to excitation of the molecule, the photon also ionizes that molecule, the dissociation leads to a fragment ion which can be identified and studied quantitatively by means of a mass spectrometer.

A complication arising from the ionization process is that the molecule ion no longer contains a known amount of excitation energy. The range and probabilities of various excitations can, however, be determined, and hence the data can be interpreted in a simple manner.

With the above object in mind, a series of investigations of simple organic molecules [B. Steiner, C. F. Giese, M. G. Inghram, *J. Chem. Phys.* **34**, 189 (1961)] and free radicals have been carried out by means of a combination of an ultraviolet vacuum monochromator and a mass spectrometer. The results and comparisons of these results with theory will be presented.

MARK G. INGHRAM
University of Chicago

High-Pressure Chemistry

Bridgeman and his students and colleagues did a number of incidental experiments on the chemical effect of high pressures, which were of great significance. Following the earliest of these, Conant and his students made much more thorough studies, and subsequent workers have investigated with extreme care the effects of pressures up to about 10,000 atmospheres.

Our researchers are concerned with the range above 10,000 atmospheres, where we suspect there is a great increase in the relative effects of pressure on chemical reaction rates. We observe from the work of Anders and Jameson and their coworkers that diamonds can be formed in milliseconds under conditions of several hundred thousand atmospheres. Theoretically, it seems likely that a general theorem applies: the retarding effect of repulsive energy barriers will essentially be eliminated by the compressions involved at these pressures. Therefore, it is our expectation that in the vicinity of 100,000 atmospheres possible reactions occur instantaneously, limited only by the rates with which the electrons can rearrange to form the new bond structure.

Experimental evidence for these effects will be presented. Specifically, the effects of very high pressures on several general organic materials, applications to the cooking of food, and detailed consideration of the breakdown and transformations observed in plastics will be discussed.

W. F. LIBBY
University of California, Los Angeles

Excitatory and Inhibitory Mechanisms in Visceral Pain and Analgesia

Visceral pain is felt in the parts of the body below the skin surface which normally are not sensed or projected into consciousness. Unlike cutaneous pain, visceral pain is not evoked by physical forms of injury. The only receptors present in viscera which might serve to signal injury or any other change are found in the vicinity of capillaries as the unmyelinated free branching terminals of C- and A-gamma-delta *paravascular* nerves (R. K. S. Lim, C. N. Liu, F. Guzman, C. Braun, *J. Comp. Neurol.*, in press). Being sensory, these survive sympathectomy and thus differ from the vasomotor perivascular plexus in the adventitia of arterial vessels, which disappear after removal of the sympathetic chain. The paucity of sensory terminals in the viscera, as compared with the skin, may account for the inadequacy of physical stimuli to cause pain, since chemical stimulation by intra-arterial injection readily evokes pain from cutaneous and visceral sites [C. Braun, F. Guzman, E. W. Horton, R. K. S. Lim, G. D. Potter, *J. Physiol.* **155**, 13 (1961)]. With the vocalization reflex to nociception as an indicator of pain, histamine, acetylcholine, and 5-hydroxytryptamine (in 25- to 500-μg doses) and bradykinin and substance-P (in 1- to 2-μg doses) have been found to cause vocalization in dogs on intra-arterial injection into somatic, abdominal, cardiac, and cranial vessels. Dorsal root ganglionectomy abolishes this response (F. Guzman, C. Braun, R. K. S. Lim, *Arch. intern. pharmacodynamie*, in press; G. D. Potter, F. Guzman, R. K. S. Lim, *Nature*, in press). According to Keele [C. A. Keele, in *Polypeptides Which Affect Smooth Muscles and Blood Vessels*, M. Schachter, Ed. (Pergamon, New York, 1960)], bradykinin or similar polypeptide may be formed after injury, promoting inflammation and pain. Vocalization in response to chemical stimulation can be blocked by analgesic drugs. In viviperfusion experiments of the innervated spleen it was found that morphine blocks vocalization in the central nervous system only, while acetylsalicylic acid blocks it both centrally and peripherally. Psychic inhibition of vocalization may also occur.

ROBERT K. S. LIM
Miles Laboratories, Elkhart, Indiana

Races and Incipient Species in Drosophila paulistorum

A remarkable case of transition between race and species has been found by Dobzhansky and Spassky in *Drosophila paulistorum*, one of the six sibling species of the *willistoni* species group. Five of the six incipient species proved to be reproductively isolated, by lack of sexual attraction and by sterility of the male hybrids, to an extent sufficient for some of them to coexist sympatrically in at least three different localities. However, a sixth race, the Transitional one, living in western Colombia, can interbreed freely with most of the others. Collections of further material from northeastern and central Brazil disclosed the existence in this same region of another Transitional race, which forms a genetic bridge especially between the Amazonian race (living from Panama to Pará) and the Andean–South Brazilian race (living from Colombia, Peru, and Bolivia to southern and central Brazil). A population sample from Belem (in the state of Pará, Brazil) is particularly interesting. Some of the strains established from the Belem material cross to and yield fertile hybrids with the Andean–South Brazilian group, but some do not cross to other strains from the same locality. Populations from Maranguape (Ceará, Brazil) and Salvador (Bahia, Brazil) cross and give fertile hybrids with both Amazonian and Andean–South Brazilian strains, but not with certain strains from Belem.

This research was supported in part by the Conselho Nacional de Pesquisas.

C. MALOGOLOWKIN
Columbia University and University of Brazil

Effects of Chemostimulation of Brain and of Bacterial Endotoxins on Eating and Drinking

Previous work by Sebastian P. Grossman in our laboratory showed that, in the lateral hypothalamic "feeding area," minute

crystals of sympathomimetic substances, adrenalin or noradrenalin, stimulate eating in the satiated rat, while parasympathomimetic substances, acetylcholine or carbachol, stimulate drinking. Furthermore, intraperitoneal injection of the sympathetic blocking agent ethoxybutamoxane differentially blocks the effect of noradrenaline (and also normal hunger), while the parasympathetic blocking agent atropine sulfate differentially blocks the effect of carbachol (and also normal thirst).

The present study extends this work by showing that drinking can be elicited by carbachol elsewhere—for example, in the preoptic area and the ventromedial nucleus of the hypothalamus. A dose-response study in the "feeding area" shows some drinking in response to 3×10^{-4} mole of carbachol, maximum drinking in response to between 24 and 72×10^{-4} mole, and convulsions with higher doses. For eliciting eating, the optimal dose of noradrenaline is considerably higher.

Dubos and Schaedler have inhibited drinking in mice by systemic administration of endotoxins from bacteria such as *Escherichia coli*. We replicated this finding in rats but found inhibition of eating also (not alleviated by tube feeding of water). Both effects began after 30 minutes and lasted 24 hours. Protection against a second dose lasted 10 days, but when delayed until the 20th day, a second dose produced an almost immediate severe effect. Small injections in the hypothalamic "feeding area" produced no obvious effect. The techniques used in this study may provide a way of analyzing mechanisms involved in the behavioral phenomena of feeling sick.

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NEAL E. MILLER
KAY S. GOTTESMAN
JOHN E. HOLMES
Yale University

Active Sites of Enzyme Precursors

Several proteolytic enzymes occur in vivo in the form of inactive precursors which require limited enzymatic hydrolysis in order to become active catalysts. Previous studies of two of such activation reactions—of trypsinogen and chymotrypsinogen—have suggested that in each case the splitting of a single peptide bond suffices to produce activation, the resulting change in tertiary configuration of the protein molecule presumably giving rise to the formation of a single enzymatically active site.

Recent studies of the activation of another enzyme precursor, pancreatic procarboxypeptidase A, to be described in this presentation, reveal a more complex molecular configuration of this zymogen. This protein, upon tryptic activation, gives rise to two different enzymatic activities, an endopeptidase, resembling in specificity the pancreatic chymotrypsins, and an exopeptidase, carboxypeptidase A. These two activities are associated with different portions of the parent zymogen molecule and with two different enzymatic centers. Procarboxypeptidase A, although seemingly a single, homogeneous protein, in fact occurs as a molecular aggregate of two and three subunits, respectively, which can be disaggregated prior to activation by appropriate means. After activation, the active center of the endopeptidase can be chemically labeled by phosphorylation with diisopropylphosphofluoridate, which causes irreversible inactivation. Carboxypeptidase A, in contrast, is insensitive to diisopropylphosphofluoridate. The active center of carboxypeptidase includes the groupings involved in the binding of zinc, the intrinsic metal essential for enzymatic activity. Since one of the ligand groups is a sulfhydryl (Vallee *et al.*) contributed by one of the two "half cystines" of the apoprotein, this group can be labeled, under appropriate circumstances, by alkylating agents and differentiated from the other, less reactive, "half cystine" sulfur.

HANS NEURATH
University of Washington

Metabolism of Inorganic Nitrogen and Its Compounds in Microorganisms

Cell-free extracts prepared from *Azotobacter vinelandii* OP by disrupting the cells (suspended in the medium in which they were grown) with an ultrasonic probe (Kcy/sec) incorporate appreciable amounts of nitrogen gas. Two independent methods used to check the enzyme activity included use of the stable isotope N_2^{15} and the radioactive tracer N_2^{13}. The main activity is associated with the cell particles collected between 25,000 and 144,000*g*, but ultrasonic treatment of these particles releases part of the nitrogen-fixing system into the supernatant fluid. The particles can be partially solubilized by a variety of treatments. The relation between hydrogenase and nitrogenase in these extracts will be discussed. A cobalt requirement of about 0.05 μg/lit. has been established for *Azotobacter* when it is fixing nitrogen. Vitamin B_{12} is, however, more effective than cobalt at equivalent concentrations.

Colbalt is also required by a range of bacteria and yeast when they are utilizing nitrate nitrogen. The growth requirement (in micrograms of Co per liter) in *Rhizobium spp.* is 0.1 to 2, and in *Azotobacter* and *Escherichia coli* it is lower, 0.01 to 0.1.

Cobalt or vitamin B_{12} is required for the induction of nitrate reductase in bacteria. The enzyme in *Azotobacter* is associated with ribosomal particles in cells exposed to nitrate for 3 hours. At a later stage of growth the enzyme is released from the ribosomes and is then present in the supernatant fluid left after centrifuging at 144,000*g*. The ribosomal particles have been further resolved by means of a sucrose density gradient technique, and the site of induction of nitrate reductase has been established.

D. J. D. NICHOLAS
P. W. WILSON
University of Wisconsin

Galactose Incorporation into Cell-Wall Lipopolysaccharide in Mutant Strain of Salmonella typhimurium

Mutants of *Salmonella typhimurium* deficient in UDP-galactose-4-epimerase produce an abnormal cell-wall lipopolysaccharide which is distinguished from the wild type by the absence of galactose, mannose, rhamnose, and abequose. The only neutral sugars detected in the mutant lipopolysaccharide are glucose and an aldoheptose, chromatographically similar to D-*glycero*-L-mannoheptose. Cell-free extracts of the epimeraseless mutant catalyze the transfer of galactose from UDP-galactose-C^{14} into cell-wall lipopolysaccharide. The enzymic activity is localized in the particulate fraction sedimenting at 12,000*g*. This fraction is also rich in lipopolysaccharide, which appears to be the acceptor for galactose. The reaction is measured by the determination of radioactivity precipitable by trichloroacetic acid, with UDP-galactose-C^{14} as the substrate. Mg^{++} or Mn^{++} are required, and no incorporation is observed when UDP-galactose is replaced by galactose-C^{14} or galactose-C^{14}-1-P. The incorporated radioactivity is nondialyzable, and it is not released by treatment with RNAse, DNAse, or trypsin. The radioactivity product has been isolated by phenol extraction and acetone precipitation. The product purified in this manner is free of nucleic acid and protein but contains all of the lipopolysaccharide of the cell. Mild acid degradation by the method of Westphal and Lüderitz [*Angew. Chem.* **66**, 407 (1954)] indicates that galactose is incorporated into both the lipid and the polysaccharide fractions of the lipopolysaccharide.

M. J. OSBORN, SAMUEL M. ROSEN,
L. ROTHFIELD, B. L. HORECKER
New York University School of Medicine

Age of Salt Marsh Peat in Relation to Recent Changes in Sea Level

Radiocarbon age determination on samples of peat collected in salt marshes at Barnstable, Massachusetts, show that the peat has grown upward at a rate of approximately 3.3×10^{-3} feet per year since 2100 years ago. Prior to 2100 years before the present (B.P.) and back beyond 3700 B.P., the rate of vertical accretion was about 10×10^{-3} feet per year. Since the surface of the high marsh, where peat is formed, is at approximately the level of mean high water, it is assumed that these rates indicate approximately the change in sea level relative to the land. Comparison with data from southwestern Louisiana [H. R. Gould and E. McFarlan, Jr., *Trans. Gulf Coast Assoc. Geol. Soc.* **9**, 261 (1959)], a region considered to have been stable during the recent period, suggests that the change in relative elevation of sea and land at Cape Cod has been due primarily to subsidence of the land rather than to eustatic rise in sea level.

ALFRED C. REDFIELD
Woods Hole Oceanographic Institution
MEYER RUBIN
U.S. Geological Survey

The Currents in the Ionic Centrifuge

The book by Ferraro and Plumpton [V. C. A. Ferraro and C. Plumpton, *Magneto Fluid Mechanics* (Oxford Univ. Press, New York, 1961)] gives the valuable equations 7.112 and 7.1113 (chap. 7, p. 138). On their right-hand sides are the hitherto neglected forces of reaction of the ions and electrons mutually passing through each other. These hitherto neglected expressions involve, besides the local characteristics of the plasma, an additional τ whose value depends, by an integration, upon the values of more distant characteristics of the plasma.

When the plasma abuts upon a highly conducting metallic surface such as the cylinder in the ionic centrifuge [J. Slepian, *Proc. Natl. Acad. Sci. U.S.* **47**, 313 (1961)], there will be a zero net component of current density parallel to any circle of the cylinder. This may be seen from the equation

$$\mathbf{j} = \frac{\rho_1\rho_2}{\rho_0\tau}\left(\frac{Z_1}{m_1} + \frac{1}{m_2}\right)\left(\overline{\mathbf{V}}_1 - \overline{\mathbf{V}}_2\right)e \qquad \text{F\&L (7.108)}$$

But $\overline{\mathbf{V}}_{1\theta}$ and $\overline{\mathbf{V}}_{2\theta}$ must each be zero, as otherwise the cylinder must give up some positive ions and some electrons to the discharge. Hence $\mathbf{j}_\theta = 0$.

Now, applying this to the first, and much experimented upon, discharge described for the ionic centrifuge [J. Slepian, *Proc. Natl. Acad. Sci. U.S.* **47**, 313 (1961)], we get, letting $\overline{\mathbf{V}}_{1r} - \overline{\mathbf{V}}_{2r} = 0$, the total ion current at the cylinder, just one-half the initial ion current, with a voltage of $(e/8\pi\ c^2 m_1)B^2r^2$ electromotive units.

Applying this to the second discharge [J. Slepian, *Proc. Natl. Acad. Sci. U.S.* **47**, 313 (1961)], not yet tried out experimentally, we get for the cylinder voltage a value close to zero. The cylinder ion current is nearly the whole initial ion current. The discharge is at low voltage initially, rises to a very high value in crossing the magnetic field, and falls to a low voltage again when it is caught upon the cylinder.

JOSEPH SLEPIAN
Pittsburgh, Pennsylvania

Sensation Measurement by Inverse Judgments

The simple procedure of asking observers to estimate the apparent intensity of various stimuli has repeatedly shown that the subjective magnitude grows in direct proportion to the stimulus magnitude raised to a power. For each sensory system the input-output function has a characteristic exponent, ranging from 0.33 for the brightness of light to 3.5 for electric shock to the fingers.

A strong test of this psychophysical law can be made by asking observers to judge an inverse or reciprocal aspect—softness instead of loudness, for example. Judgments of softness should generate another power function whose exponent is the negative of the exponent for loudness.

Five pairs of inverse aspects have been scaled: lightness and darkness of gray papers, loudness and softness of sounds, roughness and smoothness of emery cloths stroked by the finger, longness and shortness of lines, and largeness and smallness of squares projected on a screen.

Direct estimations of the apparent magnitudes of stimuli presented in irregular order produce reasonably good power functions, both for an attribute and for its inverse. Somewhat cleaner sets of reciprocal power functions have been obtained, however, with the method of magnitude production, in which the observer adjusts the stimulus to produce apparent magnitudes named by the experimenter.

The reciprocality between tactual roughness and smoothness was also tested by cross-modality matching. One group of observers adjusted a loudness to match apparent roughness, another group, to match apparent smoothness. Two reciprocal power functions were generated. The exponents correspond to the ratios between the exponent for loudness (0.6) and the exponents for roughness-smoothness ($\pm$ 1.5).

S. S. STEVENS
Harvard University

The History of Lake Petenxil, Departamento de El Petén, Guatemala

Pollen analyses of two cores, collected by G. L. Cowgill and U. M. Cowgill in Lake Petenxil, adjacent to various Maya sites, indicate three major vegetation periods: (G_1) anterior to about 3000 B.P., with *Quercus* and species of *Moraceae* dominant in the arboreal pollen, and with much grass but very little composite pollen; (G_2) from some time subsequent to 3000 B.P. to about 1300 B.P., with *Quercus* dominant, moderate *Pinus*, low *Moraceae*, and very abundant composite as well as grass pollen; (G_3), with *Terminalia* and *Moraceae* spp. the dominant tree pollen and all herbaceous pollen poorly represented. The inorganic material of the sediment is mainly metahalloysite. During G_1, sedimentation was slower than it was later on, and the resulting sediment was more organic. Exchangeable calcium and potassium show marked variations; the maxima of the former appear to be correlated with maxima in the *Quercus-Gramineae* pollen association. *Zea mays* pollen occurs very sparingly at the bottom, at a level probably about 4000 years old. It is maximal in G_2 around 1600 B.P., and it declined rapidly, with a slight secondary maximum 600 years later. The pollen of G_3 is concordant with the modern vegetation; G_2 clearly indicates intense agricultural activity; the interpretation of G_1 is somewhat problematic. (This study is supported by the Henry L. and Grace Doherty Charitable Foundation and by National Science Foundation grants Nos. 8916, 15606, and 17831.)

MATSUO TSUKADA, URSULA M. COWGILL, G. E. HUTCHINSON
Osborn Zoological Laboratory, Yale University

Synthesis of Lactose by Particulate Enzyme Preparations from Guinea Pig and Bovine Mammary Glands

Gander *et al.* [*Arch. Biochem. Biophys.* **69**, 85 (1957)] reported the biosynthesis of lactose 1-phosphate from UDP-D-glucose and α-D-glucose 1-phosphate (G-1-P) by enzyme fractions from bovine mammary tissue and proposed that lactose is formed according to the reactions:

$$\text{UDP-D-glucose} \xrightarrow{\text{galactowaldenase}} \text{UDP-D-glucose} + \text{pyrophosphate}$$

$$\text{UDP-D-galactose} + \text{G-1-P} \xrightarrow{\text{galactosyl transferase}} \text{lactose 1-phosphate} + \text{UDP}$$

$$\text{lactose 1-phosphate} \xrightarrow{\text{phosphomonoesterase}} \text{lactose} + \text{phosphate}$$

According to these authors, UDPG served as the source of both the galactose and the glucose moieties of lactose.

Experiments on the incorporation of isotopic substrates such as acetate 1-C^{14} into lactose in vivo have shown that the D-glucose and D-galactose moieties have different labeling patterns and are therefore not in close equilibrium with each other [Shambye, Wood, and Kleiber, *J. Biol. Chem.* **226**, 1101 (1957)], as Gander *et al.* claimed that they were. Furthermore, attempts to synthesize lactose 1-phosphate by the method of Gander and his co-workers in other laboratories were not successful.

In view of these discrepancies, a reinvestigation of the enzymes and substrates involved in the biosynthesis of lactose has been undertaken. With particulate preparations from lactating guinea pig or cow mammary glands as the enzyme source and a mixture of uridine diphosphate galactose-C^{14} and D-glucose as substrates, lactose was synthesized according to the reaction:

$$\text{UDP-D-galactose} + \text{D-glucose} \xrightarrow{\text{galactosyl transferase}} \text{lactose} + \text{UDP}$$

There is also an active epimerase present in the preparations that interconverts uridine diphosphate D-glucose to UDP-D-galactose. Lactose synthesis can thus be obtained with UDP-D-glucose-C^{14} and D-glucose as substrates.

No evidence could be obtained, with either particulate or cell-free preparations, for the formation of lactose or lactose 1-phosphate when glucose 1-phosphate was used as an acceptor for D-galactose from the sugar nucleotide.

Furthermore, when unlabeled lactose 1-phosphate was added to the reaction mixture containing UDP-D-galactose-C^{14} and D-glucose, practically no radioactive lactose 1-phosphate could be detected. All the radioactivity resided in the synthesized lactose, indicating that the lactose could not have been formed via the intermediate lactose 1-phosphate.

WINIFRED M. WATKINS
Lister Institute, London
W. Z. HASSID
University of California, Berkeley

The Nerve Fiber as a System in Continuous Flow: Microcinematographic and Electronmicroscopic Demonstrations

The discovery (Weiss, 1944) of the permanent "damming up" of the substance of nerve fibers in front of chronic constrictions and of the downward advance of the bulge upon deconstriction has led to the concept that neurons are not static fixtures but streams of substance moving continuously from central production sites in the nucleated cell body into the periphery at rates of the order of 1 millimeter per day, carrying both replacements for major macromolecular components of the axon and "neurosecretions" destined for peripheral discharge. Later data on enzyme and isotope gradients in nerve added circumstantial evidence to this concept, yet the mechanism of convection had remained obscure.

Time-lapse motion pictures, under the phase-contrast microscope, of myelinated sensory nerve fibers of young mice and chicks, explanted with their central ganglia into nutrient solution, have now revealed true peristaltic waves in the surface of nerve fibers and the actual centrifugal movement thereby produced of the viscous axonal column at the predicted rate.

Moreover, electronmicroscopic studies of the "damming" phenomenon in chronically constricted nerves, besides confirming the earlier microscopic findings, have shown that, proximally to a partial block, (i) the larger "neurofibrils," which are definitely tubular, become greatly distended, to vesicles, and (ii) mitochondria accumulate in dense piles, in which many then disintegrate.

These facts demonstrate (i) that there is a constant movement of fluid distally inside the tubules, and (ii) that mitochondria likewise travel distally in the axonal stream.

These new findings have brought not only more direct confirmation of the axonal flow but methods for its more detailed study.

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PAUL WEISS
A. CECIL TAYLOR
P. AIYAPPAN PILLAI
Rockefeller Institute

Individuality as Exhibited by Inbred Animals; Its Implications for Human Behavior

A high degree of individuality of various sorts—anatomical, biochemical, and physiological—exists even in inbred animals, which are often regarded as uniform. The individuality observed in rabbits, rats, mice, hamsters, and baby chicks is discussed. Many of the items under discussion, which include not only excretion and blood patterns but also choices of food and beverage and inclination to exercise (many of which have been observed in our laboratories), have not been studied from the genetic standpoint and are probably out of reach of classical genetics because they may involve complex gene-cytoplasm interactions, possibly of the same character as those involved in the well-recognized phenomenon of differentiation.

Regardless of possible genetic interpretations, however, the facts are that each individual mammal and fowl—even a member of an inbred strain—has a distinctly individual set of behavioral leanings. Inevitably this must be even more true of members of the human family, who have much more diverse ancestry than inbred animals have.

This dimension of human behavior is admittedly difficult to deal with, but it must be taken into account in any realistic appraisal of human behavior. The so-called "behavioral sciences," which most often merely bow to biology and then pass on their way, must sooner or later face the facts of inborn individuality.

ROGER J. WILLIAMS, RICHARD B. PELTON, FRANK L. SIEGEL
University of Texas

Alterations in Learning Ability Caused by Changes in Cerebral Serotonin or Catechol Amines

The discovery that schizophrenia may possibly arise from a disturbance of serotonin metabolism in the brain (Woolley and Shaw, 1954) has prompted us to explore whether other mental diseases also may be causally related to this hormone. Inherited idiocies such as phenylketonuria and galactosemia might arise from such an abnormality imposed at birth. To test this, the induction of the disease (including the mental failure) in animals would be helpful. A simple but quantitative maze-learning assay was devised. With this easy and accurate test, the effect on learning ability of changes in the serotonin content of the brain has been studied. Increases in serotonin caused specifically in the brain by administration of 5-hydroxytryptophan (HTP) plus the antiserotonin BAS (Woolley *et al.*, 1957) resulted in complete failure to learn the maze. Less specific increases caused by administration of iproniazid (which also increases catechol amines and other compounds) likewise reduced learning ability, but less than HTP plus BAS. Decreases in serotonin and catechol amines caused by feeding large amounts of DL-phenylalanine plus L-tyrosine (Huang *et al.*, 1961) increased learning ability. Thus, learning ability greater than normal was induced in adult mice. By contrast, when a deficiency in serotonin plus catechol amines was induced in newborn mice, their learning ability when they were mature was found to be greatly reduced. Just as in the human disease (phenylketonuria), the time of life at which the deficiency was induced was crucial to the mental failure.

D. W. WOOLLEY
Rockefeller Institute

A Further Mathematical Aid in Optimizing Engineering Designs

Last year I showed [C. Zener, *Proc. Natl. Acad. Sci. U.S.* **47**, 537 (1961)] that one may attack directly the problem of obtaining the minimum of a certain class of polynomials. This class embraces all polynomials whose number of terms exceeds by unity the number of variables. For this class, the minimum was obtained as an explicit analytic function of the constant coefficients.

During the past year Duffin has provided considerable additional insight into the problem of minimizing polynomials. First (R. J. Duffin, in preparation), he showed that the problem of minimizing a polynomial in n variables and $n + \sigma$ terms may be reduced to the problem of maximizing a certain function in a $\sigma - 1$ dimensional space. Second (R. J. Duffin, *Operations Research*, in press), he showed that the problem of minimizing polynomials may be approached without ever using the concept of calculus.

In the present paper I extend Duffin's previous analysis by applying a perturbation procedure to polynomials whose number of terms exceeds by more than unity the number of variables.

C. ZENER
Westinghouse Research Laboratories