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Temperature Jump Kinetic Studies of the Binding of Ligands by Myoglobin

The relaxation of a chemical system after a sudden change in an independent variable like temperature or pressure provides a means for learning about the mechanism and the rate constants of its individual steps. At Wisconsin we have employed these methods, which were pioneered by M. Eigen in Göttingen, to study serum albumin and myoglobin. The relaxation of serum albumin after a pressure jump has been studied using electrical conductivity as a means for detection.

The temperature jump method has been used to study the kinetics of binding of various ligands to sperm whale metmyoglobin. The chemical relaxation is followed spectrophotometrically at 560 mμ. The rate constants obtained yield equilibrium constants which are in agreement with those obtained directly. The dissociation rate constant for the imidazole metmyoglobin complex is independent of *p*H in the range 6-8. The variation of the association rate constant with *p*H in this range indicates that the reaction with the neutral imidazole molecule occurs more rapidly than with the protonated species.

These reactions of ferrimyoglobin are much slower than the reaction of oxygen or carbon monoxide with ferromyoglobin, perhaps because of a conformational change in the molecule. The imidazole-metmyoglobin complex is not isomorphous with metmyoglobin, according to crystallographic studies by Kendrew.

The dependence of the magnitude of the relaxation effect on imidazole concentration is in accord with theory.

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Comparison of Ferredoxin-Dependent Reactions in Isolated Chloroplasts and in Extracts of a Photosynthetic Bacterium

Ferredoxins isolated from spinach chloroplasts (I) and from the photosynthetic bacterium *Chromatium* (II) are colored (I, red; II, brown), nonheme, nonflavin, water-soluble proteins with the following properties: (i) molecular weight: I, 13,000–14,000; II, 5600–6000; (ii) Fe atoms per mole protein: I, 2; II, 3; (iii) inorganic sulfide (moles per mole protein): I, 2; II, 3. The redox potential of ferredoxin II is about 60 mv more reducing than that of ferredoxin I.

Ferredoxins I and II are each reduced photochemically by the chlorophyll-containing particles (grana) of spinach, but thus far there is no evidence for their photoreduction by the bacteriochlorophyll-containing particles of *Chromatium*. In spinach chloroplasts the photoreduction of ferredoxin is stoichiometrically linked with evolution of oxygen and formation of adenosine triphosphate (photosynthetic phosphorylation). In cell-free extracts of *Chromatium*, ferredoxin was found to catalyze two reductive reactions in which hydrogen gas serves as the hydrogen donor: reductive CO_2 fixation by way of pyruvate (the pyruvate synthase system), and the reduction of diphosphopyridine nucleotide (DPN). The pyruvate synthase system is a new primary CO_2 fixation reaction that results in the synthesis of pyruvate from CO_2, H_2, and acetyl-CoA, and leads to the synthesis of amino acids, which are known to be the main soluble products of CO_2 assimilation in *Chromatium*. With respect to DPN reduction, *Chromatium* extracts were already known to reduce DPN with hydrogen gas but only in the presence of a nonphysiological catalyst, benzyl viologen. Evidence has now been obtained that, in *Chromatium* extracts, ferredoxin II is the physiological catalyst for the reduction of DPN (and to a very limited extent of TPN) by hydrogen gas.

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Neutron Spectra from Proton Bombardment of Intermediate Nuclei

The continuous energy spectra of particles emitted from highly excited nuclei have usually been analyzed in terms of the statistical model of nuclear reactions. According to this model the shapes of the spectra are determined primarily by the level density in the residual nucleus. As a consequence, it is possible to deduce the level density of the residual nuclei from studies of particle emission from excited nuclei.

In recent experiments at this laboratory several intermediate mass nuclides have been bombarded with protons of energies between 6 and 14 Mev. The distribution in energy of the emitted neutrons was measured as a function of proton energy and emission angle using the time-of-flight method. Within the experimental accuracy the angular distributions were isotropic, as would be expected if the statistical theory is applicable. The level densities derived from the observed spectra at a given excitation energy in the residual nucleus showed, however, an apparent dependence on bombarding energy. It has not been possible to remove this dependence on bombarding energy by varying the parameters used in the analysis. In particular, the use of various theoretical estimates of the dependence of the inverse cross section on neutron energy and excitation energy were not successful. Since the level density of a nucleus should depend only on the excitation energy of that nucleus, the present experiments raise doubts about the accuracy of level densities deduced from measurements of continuous particle spectra from (p,n) or similar reactions.

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Identification by Isotopic Substitution of the EPR Signal at g = 1.94 in Non-Heme Iron Proteins

An unknown paramagnetic constituent of many materials was detected by Beinert and Sands [*Biochem. Biophys. Res. Commun.* **3**, 41 and 47 (1960)] and further characterized by Beinert and Lee [*ibid.* **5**, 40 (1961)] by its characteristic asymmetric electron paramagnetic resonance (EPR) spectrum ($g_x \approx g_y \approx 1.94$; $g_z = 2.00$), which appears on reduction. These authors suggested on the basis of chemical analyses and properties of the observed EPR spectra that a novel type of protein-bound iron complex, in a reduced state, was responsible for the signals. The complex has since been shown to have the properties of an electron carrier and to be a kinetically active component of flavoproteins [Bray, Palmer, Beinert, *J. Biol. Chem.* **239** (August 1964)] and of the electron transport system of mitochondria and *Azotobacter vinelandii*. The nature of this component, however, has remained unknown, and no iron model compound has been found that exhibits a similar EPR signal, so that the EPR signal could not be unambiguously attributed to iron. We have attempted to identify the signal by the effects of isotopic substitution. *Azotobacter vinelandii* was grown on Fe^{57} of 90 percent enrichment, an isotope of nuclear spin $\frac{1}{2}$ ($\mu = 0.09$). Since Fe^{56} has no nuclear magnetic moment, a doubling or broadening of lines should be observed on substitution of Fe^{57} for Fe^{56}. An iron protein of low flavin content (14 : 1) was isolated from a butanol extract of the *Azotobacter*. The spectrum of the Fe^{57}-protein showed broadened lines and could be reconstructed from that of the Fe^{56} protein on the assumption of a 22-gauss splitting and a final enrichment of 65 percent Fe^{57}.

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Nonlinear Viscoelastic Constitutive Equations with a Small Number of Constants

In the description of the fluid dynamics of high polymer systems, it is necessary to have realistic "constitutive equations"—equations relating the shear stress to the shear rate and other quantities. In order for a constitutive equation to be successful it must be capable of describing shear-dependent viscosity, normal stress effects, small amplitude oscillatory motions, and other phenomena. During the past few years our group has developed and studied several useful equations: (i) a three-constant Oldroyd model (*1*), (ii) a nonlinear modification of the general linear viscoelastic model (*2*), and (iii) several nonlinear modifications of the general Maxwell model (*3*).

Recently we have explored the possibility of "imbedding" the results of molecular theories of high polymers [such as those of Rouse (*4*) and Zimm (*5*)] into our nonlinear models. This procedure enables us to replace the infinite set of constants appearing in models of types ii and iii by a small number of parameters. Consequently we end up with models with a small number (two to four) of adjustable parameters which have simple dimensions and simple physical interpretations.

We believe that these relations will be useful and realistic for solving problems in polymer fluid dynamics. A limited examination of experimental data supports this viewpoint.

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Chemically Synthesized Deoxyribopolynucleotides as Templates for the DNA Polymerase of Escherichia coli

Organic synthetic work has made available a variety of short chain homopolymers of deoxyribonucleotides and deoxyribopolynucleotides containing repeating di- and tri-nucleotide sequences. Recently, one group of oligonucleotides which contain adenine and thymine in alternating sequences [$d(AT)_4$-$d(AT)_7$] have been shown to serve as templates for the DNA polymerase of *E. coli*, and the product formed in every case is of high molecular weight (in the range of millions) [A. Kornberg, L. L. Bertsch, J. F. Jackson, H. G. Khorana, *Proc. Natl. Acad. Sci. U.S.* **51**, 315 (1964)]. In further work with synthetic deoxyribopolynucleotides as templates, three reactions catalyzed by the enzyme have been discovered and are as follows: (i) The reaction mixture, dT_{11} + dA_7 + dATP + dTTP + enzyme, gives a higher molecular weight polymer consisting of long polyT and of long polyA; (ii) the reaction mixture, dT_{11} + dA_7 + dATP + enzyme, gives long deoxypolyadenylate; (iii) the reaction mixture, $d(CT)_8$ + $d(AG)_5$ + 4-deoxynucleoside triphosphates + enzyme, leads to a high molecular weight product. While the characterization of this product is as yet incomplete, it in all probability consists of one strand containing alternating C-T and the complementary strand containing alternating A-G. The characteristics of these reactions will be described. Work supported by the National Science Foundation, the National Institutes of Health, and the Life Insurance Medical Research Fund.

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Geology of the Ouachita Mountains, Oklahoma

The Stanley-Jackfork-Johns Valley-Atoka rocks of the Ouachita Mountains of Oklahoma and the Tesnus-Haymond sequence of the Marathon Mountains of Texas typify what Alpine geologists call black shale flysch, but wildflysch and calcareous flysch also are represented.

In late Mississippian and early Pennsylvanian time a minimum of 22,500 feet of alternating sandstones and shales was deposited in a rapidly subsiding, linear trough. During the cannibalistic stage of the Ouachita geosyncline the Oklahoma-Arkansas trough probably was but one part of an 1800-mile long foldbelt stretching from the Marathons to the Appalachians. Deposition of fine pelitic muds and siliceous shales was interrupted hundreds of times by deposition of quartzose sandstones. The sandstones, debouched from a shelf environment, exhibit convolute bedding, graded bedding, sole marks, and other features now commonly ascribed to turbidity current deposition.

The foldbelt is structurally complex,

with several major thrust faults striking parallel to the axis of the foldbelt and thrust toward the craton. Where observed at the surface the thrusts emerge at high angles, but some seem to flatten with depth and probably become bedding plane faults.

The facies patterns and structural characteristics compare closely with those of the Polish Carpathians, Swiss Alps, and Italian Apennines, and the characteristic sedimentary features are duplicated in intricate detail in the four mountain systems.

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Biosynthesis of Carbamyl Phosphate Synthetase in Liver Slices from Thyroxine-Treated Tadpoles

Radioactive L-leucine incorporation into carbamyl phosphate synthetase, which represents *de novo* synthesis of the enzyme, in thyroxine-treated tadpole liver slices was shown to continue after complete elimination of the radioactive material from the medium in a "pulse incorporation" experiment, suggesting formation and accumulation of precursor(s) in the reaction sequence of enzyme synthesis.

Puromycin did not inhibit to any extent the conversion of the precursor(s) to the active carbamyl phosphate synthetase, which indicates that the accumulated precursors are in the form of a peptide (or protein) which has been assembled and completed on ribosomes. The amount of the precursor(s) formed was calculated to be equivalent to a few micrograms of carbamyl phosphate synthetase per 1 gram of slices. A biphasic temperature effect and a high value for the temperature quotient suggest the involvement of conformational changes in the process of enzyme synthesis. Inhibitor experiments showed that the conversion process of precursor(s) to enzyme did not need a supply of ATP, or at least did not need a high level of ATP.

Attempts to obtain a cell-free system for the conversion of precursor(s) to active enzyme have been unsuccessful.

Available evidence excludes microsomes as the exclusive site of total biosynthesis of active carbamyl phosphate synthetase.

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Yeast Epimerase, a Protein with Enhanced Fluorescence

Yeast UDPGalactose 4-epimerase is a fluorescent protein which, upon addition of *p*-chloromercuribenzoate (*p*CMB), becomes a dark protein with the release of DPN and with preservation of catalytic activity, albeit dependent upon the addition of DPN. It has now been found that the protein, upon denaturation with alcohol, releases DPN as well as 1.4-DPNH. Hence, the fluorescent protein contains bound DPN as well as a bound reduced form of DPN. The fluorescence of the latter is more than twenty-fold enhanced as compared with that of free DPNH. Addition of sodium borohydride brings about a further enhancement of fluorescence. This additional fluorescence decays faster than the "natural" fluorescence. Full catalytic activity is restored when the additional borohydride fluorescence has disappeared and the original fluorescence level is reached. In all these studies, catalytic activity as well as variations in fluorescence (quenching, enhancement, and changes of polarization of fluorescence) are being used as additional tools in a study of conformational changes of this protein.

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Sex Chromatin Formation during the Interphase of Human Fibroblasts

The functional differentiation between homologous X chromosomes of the same somatic cell nucleus, subsumed under the general notions of "the single-active-X" or "Lyon hypothesis," persists during cultivation of human fibroblasts. Detailed information about sex chromatin formation, one aspect of the differentiation, may prove helpful in understanding it. In maximal density populations of diplo-X cells 90 to 95 percent of the cells form a distinct sex chromatin condensation but only 10 percent may do so in rapidly proliferating populations. Observation of mitoses and subsequent observations of the daughter cells reveal that distinct sex chromatin is found only in cells 16 hours or more old when the average intermitotic interval is 20 to 24 hours. Such cells incorporate little or no tritiated thymidine, indicating that typical sex chromatin is a post-replication form of the X that is condensed after nuclear DNA synthesis is almost or actually complete. If the prereplication form of the X condenses as an atypically small sex chromatin body it is not readily distinguishable from other small heteropycnotic condensations formed by autosomes. At least part of the X probably does condense, since an isochromosome formed by the long arms of two X's forms a distinctive pair of small, heteropycnotic condensations in cells at least as young as six hours old. Thus, the apparent absence of distinct sex chromatin in early cleaving embryos and its first detection in blastocysts may not accurately reflect the time at which X-chromosome differentiation begins, but provide a late estimate.

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Properties of Drosophila Cells Growing in Short- and Long-Term Cultures in vitro

We have recently reported (*Science*, in press) that embryonic cells of *Drosophila melanogaster* can be cultured in a new medium with a mean generation time of 30 hours at 30°C and no change in chromosome number through ten days of growth. When primary cultures are grown in the presence of P^{32}, label quickly appears in the acid-soluble, RNA, and phospholipid fractions, with evidence for transfer from the acid-soluble fraction to RNA. There is a lag of one hour before P^{32} appears in the DNA fraction, but it increases linearly for 24 hours thereafter. Cells grown for 24 hours in the presence of *Drosophila* DNA which was labeled with H^3-thymidine are heavily labeled. The label is found exclusively in the thymine of cellular DNA, and autoradiography reveals that it is localized in cell nuclei. The label from *Drosophila* RNA containing both H^3-cytosine and H^3-uridine is found both in RNA and DNA; in the former it is found in cytidylic and uridylic acids, in the latter in cytosine and thymine. In this case autoradiography shows label both in cytoplasm and nucleus.

Long-term cell cultures have been

maintained for 200 days by transfer to new medium every 10 days. Growth rate declines after the first few transfers, generation time becoming 48 hours, but remains constant thereafter. Cells with abnormal chromosome number appear after 160 days. Differentiation of cell types is evidenced by the appearance of large cells which divide by budding and by the formation of non-random cell aggregates. (Supported by grants from the National Institutes of Health, GM11777, and from the Wisconsin Alumni Research Foundation.)

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Study of η Decay

In an exposure of 1.21- and 1.27-Bev/c π^+ mesons in the Lawrence Radiation Laboratory 72-inch hydrogen bubble chamber, decays of η^0 mesons produced in the reaction $\pi^+P \rightarrow \pi + P + \eta^0$ have been studied. The branching ratio

$$R = N\pi^0\ \pi^0\ \pi^0 / N\pi^+\ \pi^-\ \pi^0$$

has been measured and also the shape of the spectrum in $\pi^+\pi^-\pi^0$ decay. It is not possible to reconcile the spectrum with the branching ratio under the hypothesis that the decay is dominated by the Brown and Singer $T = 0$ dipion resonance. Similarly, no fit is obtained for a matrix element varying linearly with pion energy. The branching ratio into the $\pi\pi\gamma$ decay mode, and the $\pi\pi\gamma$ spectrum will be given.

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New Evidence on Chromosome Structure and Function

Several lines of observational and experimental evidence obtained recently have increased our understanding of the fine structure of chromosomes of higher plants and animals. Feulgen microspectrophotometry of metaphase chromosomes in neuroblasts of *Drosophila melanogaster* at early and late stages of larval development reveals a twofold difference in the amount of DNA, suggesting a difference in the number of chromosomal subunits. Reconstructions in wax made from electron micrographs of serial sections of telophase chromosomes of staminate-hair cells of *Tradescantia paludosa* disclose free ends of four component chromonemata. Eight ends are apparent at prophase. Both studies support the hypothesis that the mitotic chromosome is multistranded or polynemic; that is, it contains more than two cytologically separable chromonematic subunits.

Integrated functional responses of numerous "paired" chromonematic strands in salivary-gland chromosomes of *D. melanogaster* are revealed in "pulse labeling" experiments. Incorporation of H^3-thymidine is initiated at some of the many sites along the chromosomes but is similar in the two homologues. In hybrids of *D. melanogaster* and *D. simulans* occasional differences in pattern of incorporation are detectable in "homologues," which reveal functional as well as structural differences at the chromosomal level.

With respect to RNA synthesis, a 30-second pulse with H^3-uridine produces labeling throughout the length of the chromosomes, as if all gene loci were actively engaged in synthesis. With longer treatment the label accumulates preferentially in certain loci and increases in the nucleolus. The significance of these findings will be discussed.

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Interaction of Mouse Lymphoid Tissues in vitro

It has been postulated that direct tissue interaction and cell migration may explain the role of the thymus during lymphoid embryogeny. On the other hand, recent studies have suggested that indirect, humoral activity plays an integral part in the stimulation of lymphopoiesis in the neonate and in the recovery of lymphoid cell populations after sublethal x-irradiation of adult animals. The present study was designed to test in vitro interactions between thymus, spleen, lymph node, and bone marrow.

Using a modified organ culture procedure designed to permit both lymphopoiesis and lymphoid cell maintenance, it was found that: (i) embryonic thymus has no effect on embryonic spleen differentiation, and neither normal nor irradiated adult thymus has a stimulatory effect on irradiated adult spleen fragments; (ii) in contrast, embryonic (prelymphoid) spleen stimulates lymphoid development in embryonic thymus, and adult irradiated spleen enhances lymphoid regeneration of adult irradiated thymus; (iii) when either embryonic or adult irradiated spleen is combined with normal adult isogenic bone marrow, the spleen gains lymphoid cells, in contrast to singly isolated spleen where such cells are absent (embryonic) or few (irradiated); (iv) a clear role of lymph nodes has not been observed in tissue combinations.

The mutual interactions between the different lymphoid organs will be discussed with consideration of possible mechanisms involved in the control of lymphoid differentiation in vivo.

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Sequential Expression of Enzyme Synthesis in Yeast

There is increasing evidence that the synthesis of macromolecules in cells may be ordered. In microbial cells the replication of DNA is ordered and continuous. The present report provides evidence that in synchronous cultures of yeast the synthesis of specific enzymes and of mRNA is discontinuous and that specific proteins are produced only at specific periods of the cell cycle. For example, the synthesis of β-glucosidase is stepwise and occurs over less than 20 percent of the cell cycle; the timing of enzyme synthesis is not influenced by catabolic repression or by induction. From studies of cells containing several structural genes for a given enzyme it can be shown that the appearance of enzyme is not dependent upon the function of an unstable regulatory system but rather is dependent upon the synthesis of

mRNA by an ordered transcription of cistrons. Closely linked genes are synthesized at the same period of the cell cycle, suggesting that transcription may parallel gene replication. These results suggest that the time of enzyme expression in a cell generation in yeast may be determined by the position of a gene on the chromosome.

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Detailed Final State Interaction Model for K_{e4} Decay

We assume that $K \to 2\pi e \nu$ has a Born term dominated by K^* exchange, and use a generalized Goldberger Treiman relation to determine $<K^*|A\lambda|\pi>$. We use these to determine the left-hand cut for a set of partial wave dispersion relations, which are then solved using various assumptions for the $\pi\pi$ $T = 0$ and $T = 1$ interaction. Rates, $\pi\pi$ spectra, and the π angular distribution are calculated, both for an S wave low energy $\pi\pi$ resonance (σ) and for various nonresonant scattering length terms. We also discuss modifications due to a direct $<K|A\lambda|\sigma>$ coupling.

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Exchangeable Hydrogen in Chlorophyll and the Path of Hydrogen in Photosynthesis

Nuclear magnetic resonance (NMR) experiments have shown that the δ and C-10 hydrogen atoms in chlorophyll are labile and undergo exchange readily. A combination of nuclear magnetic resonance and fully deuterated algae makes it possible to investigate the path of hydrogen in photosynthesis. Fully deuteriated algae growing in D_2O are transferred to H_2O, allowed to photosynthesize, and the chlorophyll is extracted. An NMR examination then shows that no hydrogen is introduced into chlorophyll at the δ, 7, or 8 positions from H_2O during photosynthesis. It thus appears that hydrogen exchange between chlorophyll and water does not occur to a significant extent during photosynthesis.

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Reactions of the Hydrated Electron

Free electrons trapped in their own polarization field in liquid water are called hydrated electrons and are designated by the symbol e^-_{aq}. Some of their reactions with free radicals, inorganic compounds, and organic molecules will be discussed. Hydrated electrons are generated in pure water by ionizing radiations, in solutions of negative ions by light, and in alkaline solutions by hydrogen atoms. The hydrated electron is highly reactive with many inorganic and organic compounds, carries unit negative charge, and has a standard potential of ~2.5 volts. Its reactions are readily studied in pulse radiolysis experiments by following the decay of its strong optical absorption band centered at 7200 Å. Some important reactions in irradiated water are:

$$e^-_{aq} + H_2O = H + OH^-$$
$$e^-_{aq} + OH = OH^-$$
$$e^-_{aq} + H^+ = H$$
$$e^-_{aq} + H = H_2 + OH^-$$
$$e^-_{aq} + e^-_{aq} = H_2 + 2OH^-$$

The hydrated electron reacts with most inorganic metal cations except the alkali metal and alkaline earths. And since it is a stronger reducing agent than the hydrogen atom, some unusual lower valence states such as Zn^+, Cd^+, and Pb^+ have been observed. With organic compounds, e^-_{aq} reactions are highly selective. In molecules containing strong electrophilic centers, reactions usually proceed with specific bimolecular rate constants greater than 10^{10} $M^{-1}sec^{-1}$. Over a wide range of rate constants, the reactivity of aromatic compounds toward e^-_{aq} has been found to be directly related to their reactivity toward nucleophilic reactants as expressed by Hammett's sigma function. The hydrated electron may be described as the most elementary nucleophile and by far the most reactive one.

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Tracking Migrating Fishes

Homing migrations of fishes from open water (for example, salmon) seem to require of the fish a considerable degree of directed orientation which is superimposed upon its daily random movements. Experiments in our laboratory have demonstrated that fish can be trained to use at least one of the environmental cues, the sun, as a compass for directional orientation. To accomplish this not only the azimuth position of the sun but also its altitude is utilized by trained fish. However, owing to the difficulties of tracking fish in the field, knowledge is lacking about the paths of migration and the degree of directed orientation with and without sun, at night, and under varying winds; that is, the cues to which the fish respond in the field have not been evaluated. Apparatus developed in our laboratory provides a miniature ultrasonic signaling device for underwater telemetry. An ultrasonic (70–150 kcy) tag is carried in the stomach of a freely swimming freshwater fish, the white bass, and tested in Lake Mendota. It emits a signal by which the fish's course is charted from a boat carrying receiving gear. Orienting environmental cues are observed simultaneously and correlated with orientation. Because of the high energy requirements for sound communication in water, power supply and size of the transducer are the principal limiting factors. With the present units the boat must stay within 500 meters of the marked fish and can receive the signal for about 15 hours before the power supply of the tag is exhausted. A larger unit for use in a fish such as salmon could now be constructed and can have characteristics which would increase the tracking time and permit transmission of depth at which the fish swims.

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Foundations of Mathematics

Foundational studies in mathematics used to consist in adopting some axioms or postulates as the origin of mathematical truth and deducing mathematics from them. More recently (since 1900), the structure of axiomatic-deductive theories has itself become the object of mathematical investigations. Still more recently (since 1930), a theory of mathematical processes or operations has been established, in terms of which one can measure mathematical problems against the tools proposed for solving them. The simplest operations are those which can always be completed by a preassigned

procedure, historically called an *algorithm*. Such operations we now call *computable*, meaning that they can be performed by an ideal computing machine. An ideal machine is unrestricted in the amount of its storage and scratch-work space and is not subject to errors in its functioning. With this notion made precise, one can construct a hierarchy of operations which are not computable. The first examples of uncomputable operations were *ad hoc*; but recent work has shown some long-standing problems in algebra and topology to involve such; these problems called for finding an algorithm to perform some operation, and it is now shown that no such algorithm exists. Further, there are classes of mathematical problems, of the form whether some proposition is true or false, such that no preassigned axiomatic-deductive theory can provide the answer to each question. For axiomatic-deductive theories correspond to a certain position in the hierarchy, while the class of problems may be chosen from a higher position.

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The Mechanism of Disulfide Interchange in Strong Acid Media

Sodium selenate, selenite, and meta vanadate were found to catalyze the disulfide interchange reaction (RSSR + R′SSR′ $\rightleftarrows$ 2RSSR′) between L-cystine and *N,N′*-bis-2:4-dinitrophenyl-L-cystine in concentrated HCl solutions. Catalytic coefficients have been determined and have been found to fall in the order $SeO_4^{--} > SeO_3^{--} >> VO_3^-$. This property is not shared by all of the Group VIA elements, and sodium sulfite, tellurite, arsenite, phosphite, thiosulfate, and molybdate were inactive. Like the spontaneous interchange reaction, the selenite-catalyzed reaction is very sensitive to changes in acid concentration. The data suggest an initial critical protonation of the disulfide bond followed by reversible cleavage to form a highly reactive sulfenium ion and a strongly inhibitory thiol as proposed by Benesch and Benesch. While cysteine and iodide strongly inhibit selenate catalysis, arsenite and tellurite exert no such inhibition. Cysteine inhibition of selenate catalysis is a linear function of the cysteine concentration only until the cysteine to selenate ratio is 4:1. The order of the reaction with respect to one disulfide agrees with a mechanism which postulates an initial cleavage of the disulfide bond as a requisite for interchange. The known and observed reactions between the selenium compounds and thiols suggest that they function catalytically by a removal of inhibitory thiols rather than by the generation of sulfenium ions.

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Pulse Radiolytic Studies of the Ionic Dissociation of Radicals in Water

The dissociation of OH and HO_2 to O^-_{aq} and $O_2^-{}_{aq}$ was studied, making use of differences in the reactivities of these radicals towards specific scavengers. Ferrocyanide is oxidized efficiently by OH ($k = 1.07 \times 10^{10}\ M^{-1}\ sec^{-1}$) but reacts more slowly with O^-. The value, $pK = 11.9 \pm 0.2$, was obtained for the dissociation of OH by the investigation of the effect of *p*H on the rate of ferrocyanide oxidation. Tetranitromethane was used for the discrimination between HO_2 and O_2^-; O_2^- reduces tetranitromethane much faster than HO_2 does. Our value for pK of $HO_2 = 4.45 \pm 0.25$ is in good agreement with previous results.

We have also observed an optical absorption in the region below 3000 Å in both neutral and alkaline solutions, in the presence as well as in the absence of N_2O. This absorption was obtained during the electron pulse (0.4 to 1 μsec) and is probably due to OH in the neutral and O^-_{aq} in the alkaline region. The product $G \times \epsilon$ was about (1 to 2) $\times 10^3$ molecules $(100\ ev)^{-1}\ M^{-1}\ cm^{-1}$.

At *p*H = 13 the time dependence of the optical density at 2600 Å was complex. Changes in optical density were observed even several seconds after the pulse, while the O^- is expected to decay within about 100 μsec under our conditions. This indicates that other transient species besides O^- (probably products of O^-) also absorb at 2600 Å.

The optical absorptions of O^- and OH may be used for a direct measurement of the ionic dissociation of OH.

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Argonne National Laboratory

Studies on Mammalian Thymidylate Synthetase and Its Inhibition by Fluorinated Nucleotides

5-Trifluoromethyl - 2′ - deoxyuridine (F_3TDR) has recently been synthesized in this laboratory. We have shown that it is incorporated into the DNA of and is mutagenic to bacteriophage T4, it confers radiosensitization on mammalian cells containing the analog in their DNA, it inhibits the growth of transplanted tumors, and powerfully inhibits herpes simplex keratitis in the rabbit's eye. Its 5′-monophosphate (F_3-TDRP) also inhibits thymidylate synthetase, assayed in 105,000*g* supernatant fractions from Ehrlich ascites cells by the optical method. The inhibition of the enzyme by 5-fluoro-2′-deoxyuridine-5′-monophosphate (FUDRP) has also been reinvestigated. 5, 10-Methylenetetrahydrofolate is required as a coenzyme, and kinetic studies have been carried out under a variety of conditions. Lineweaver-Burk plots of initial velocities of substrate (deoxyuridylate) and coenzyme, varied at different levels of each, have eliminated a "ping-pong" mechanism. Product inhibition studies have shown that thymidylate is a noncompetitive inhibitor of both coenzyme and substrate; thus the mechanism is ordered and not random. FUDRP, with or without preincubation, is a competitive inhibitor of substrate and an uncompetitive inhibitor of coenzyme. This means that FUDRP can only interact with the enzyme after the coenzyme has attached; thus the coenzyme attaches first to the enzyme, followed by the substrate, which was supported by dialysis experiments. F_3TDRP inhibits the substrate noncompetitively after preincubation, and dialysis experiments have shown, in contrast to FUDRP, that it is bound essentially irreversibly to the enzyme.

CHARLES HEIDELBERGER
PHILIP REYES
University of Wisconsin

Dosage Compensation as an Exemplification of Genetic Accuracy

In *Drosophila* dosage compensation has long been considered a system whereby each compensated gene has its unique group of scattered compensator genes, all in the X-chromosome. Effects of addition and subtraction of X-chromosome parts have indicated

that each compensated gene and its compensators were selected, piecemeal, for compositions such that their gene-products operate in an interlocking, balanced manner, attaining the normal degree of expression when the ratio of doses of compensated to compensators is 1 : 1, independently of their absolute doses and of the dose ratio between them and autosomal genes. In mammals, contrariwise, the same function is achieved by one overall mechanism, applying to all dosage-compensated genes. This mechanism causes all but one X-chromosome, when more than one are present, to be inactive throughout nearly all its length, thus leaving an effective dose of unity for the X-chromosome genes concerned, regardless of the number of X-chromosomes. We have restudied the situation in *Drosophila*, because of reports, based on salivary chromosome observations, that the single X-chromosome in males is as massive, relative to the autosomes, as the pair of X-chromosomes in females. For this would indicate an overall dosage-compensation mechanism essentially like that in mammals, although reverse in its operation. However, we find no evidence of any such readjustment of X-chromosome mass to compensate for its dosage. Thus, diverse X-fourth-chromosome translocations in males broaden sharply where the haplo-X passes into the diplo-IV. Dosage compensation, especially of piecemeal type, demonstrates the remarkable genetic accuracy achieved by natural selection. Conclusions concerning heterozygous disadvantage follow.

H. J. MULLER, WILLIAM D. KAPLAN
City of Hope Medical Center, Duarte, California

Denaturation of Human Carbonic Anhydrases: Cotton Effects and Ultraviolet Absorption

Native human carbonic anhydrases B and C show two or more small but well-defined Cotton effects in the region between 260 and 295 mμ. The data, obtained on a Cary 60 recording rotatory dispersion apparatus, are consistent over a wide range of concentration and absorbance of the proteins; artifacts are not involved. These effects diminish progressively in the presence of urea or guanidine hydrochloride (GCl). Enzyme B in 2*M* GCl gives a rotatory dispersion curve which is smooth, and free of Cotton effects, down to 240 mμ or below. The rotation values are also much more negative, at any given wavelength, than for the native enzyme, and enzyme C shows the same effects at considerably lower GCl concentrations. Urea denaturation leads to similar results, but only at molarities approximately 2.5 times as great as for GCl. The Cotton effects also disappear when the enzymes are denatured in acid. The native enzymes show a minimum close to 226 mμ in the rotatory dispersion curves; [R′] is of the order of −3000° for enzyme C and −3500° for B. These values, and the total character of the rotatory dispersion curves, are in accord with the view that the native proteins contain little or no α-helix. Studies of ultraviolet difference spectra in urea and guanidine hydrochloride solutions also show that enzyme C is denatured more rapidly and at lower concentrations of denaturing agent than enzyme B.

DIRCK V. MYERS, SUCHINTA MEHTA
JOHN T. EDSALL
Harvard University

Oscillatory Metabolic Function as a Challenge to Tumor Autonomy

Since marked changes have been found in the performance of the enzyme-forming systems of the newly discovered class of "minimal deviation" malignant hepatomas, we decided to determine whether such hepatomas mimicked the cyclic behavior of normal liver, referred to as circadian rhythm by Halberg, Barnum and others, and whether the response of the host liver was affected by the presence of the hepatoma. Animals bearing recently transplanted Morris Hepatoma 5123TC were placed on diets containing 0, 12, 30, 60 and 90 percent casein with glucose as the carbohydrate. Lighting was from 6 a.m. to 6 p.m. only and food dishes were withdrawn during this period. H^3-thymidine (40 μc) was injected one hour before killing, which was at 0600, 1200, 1800, and 2400, two weeks after the experiment began, using 120 normal and tumor-bearing rats. Samples were fixed for autoradiographic demonstration of DNA labeling, and aliquots were homogenized for DNA extraction and counting, and for Tdr kinase, ornithine transaminase, serine dehydrase, and glycogen determinations. In contrast to the constant rate of mitosis found by Blumenfeld in experimental epidermoid carcinomas, the 5123TC hepatoma exhibited a cyclic alteration in DNA labeling which was in general similar to normal and host liver but at a much higher level. The enzyme responses were in general unexpected and showed complex systematic patterns not reflecting the variations seen in normal liver, and in certain cases in opposite phase. Further experiments are needed to explain the cross-feedback between the hepatomas and the host animals.

Aided in part by a grant from the National Cancer Institute.

VAN R. POTTER
University of Wisconsin

The Structure of Nucleohistones in Chromosomes

It is well known that DNA in chromosomes is always combined with basic proteins (histones or protamines) and in metabolically active cells also with non-histone proteins and RNA. Biochemical studies have indicated that DNA-dependent RNA synthesis occurs mainly in the regions containing non-histone proteins, while the regions consisting essentially of DNA-histone are relatively inactive. The molecular organization of these different chromosomal regions is therefore of considerable interest. X-ray diffraction studies are still inconclusive. Electron microscopic investigations of calf thymus nucleohistone and salamander erythrocyte nuclei suggest that several DNA molecules are bound together by histones to form a multistranded bundle. Salamander erythrocyte nuclei were chosen because they consist mainly of DNA-histone and can be spread on the surface of water. Such preparations show that these nuclei contain mainly threads which are about 200 Å thick and which represent two closely associated 100-Å fibrils. Removal of histone with 0.2*N* HCl or trypsin shows that each 200-Å thread contains several thin fibrils which are digested with deoxyribonuclease and presumably represent DNA molecules. They tend to fuse after removal of histones, but partial digestion suggests that four parallel DNA strands and the histones associated with them make up a 200-Å thread. Similar threads were found

in somatic nuclei of other tissues and also in plant nuclei and seem to be a general unit of organization of DNA-histones in chromosomes. How many of these threads are present in a single chromosome has not yet been established.

HANS RIS
University of Wisconsin

Filigree Method in Numerical Analysis

In attempting to calculate numerical approximations to the solutions of differential equations, a classical procedure is to solve a closely related difference equation on a grid. When one gets to partial differential equations in several variables, such as those arising in meteorological investigations, for example, the number of grid points becomes so large as to render this method impractical, even on the largest computers.

A method is being studied whereby one can first find the solution of the difference equation at a small fraction of all the grid points, after which one can eventually take care of all grid points by handling them in successive blocks of convenient size. The initial set of grid points at which values of the solution are found are called "filigree" points because in two dimensions they are strung along the sides of squares in filigree fashion. The trick is first to isolate and solve the equations satisfied by the filigree points. Then one proceeds to solve successively for the values in the interior of each square, using the values at the filigree points along the sides of the square as boundary data.

J. BARKLEY ROSSER
University of Wisconsin

A Multiple Congenital Anomaly in Man Presumably Caused by a Minute Deletion in Chromosome 3

The great majority of all non-lethal deletions in man that have clinical consequences are undoubtedly too small to be detected microscopically. The large "cri-du-chat" deletion is a notable exception. The presence of a duplication (partial trisomy) is more readily detected and usually justifies the suspicion that in addition to the extra piece, the recipient chromosome also has a deletion. However, the possibility of an insertion not entailing a deletion can hardly be ruled out and need not be as rare an event as is often assumed. The case to be reported is unusual in that special circumstances have provided evidence for a very short deletion, which ordinarily would have gone undetected. There are two brothers afflicted by a syndrome that does not represent a known clinical entity. The patients have four phenotypically normal sibs. The parents are also normal and there seem to be no relevant anomalies in the family and no consanguinity. Whereas three of the sibs, their mother, and a number of other relatives appear to have a normal chromosome complement, the afflicted children, the remaining sib, and their father have a satellite at the end of the short arm of one chromosome 3, presumably the result of a reciprocal translocation of minute terminal segments of the short arm of an acrocentric chromosome and of No. 3. The co-occurrence of the chromosomal and the clinical anomaly is significant at the 1 percent level or better. The situation is interpreted as follows. The father and the one sib are believed to be carriers of both translocation chromosomes (of which only the satellited 3 is recognizable) whereas the two patients have only the satellited 3. The clinical picture is likely to be solely or almost solely the result of the deletion.

K. PATAU, J. M. OPITZ
W. J. DEWEY
University of Wisconsin

Gravity Observations Concerning Free Vibrations of the Earth

Free vibrations of the earth excited by the Alaskan earthquake of 27 March 1964 were recorded by two gravimeters throughout an interval of many days following this quake. The results extend and confirm with somewhat improved accuracy those obtained following the Chilean quake of 22 May 1960. The purely compressional radial mode of period 20.42 minutes was observable for more than a month after the quake. The Q value obtained for this mode is higher than the previous determinations. The first two overtones of this mode were also observed, but with lower Q values. The associated Q and frequency values are significant in revealing loci of energy loss within the earth and for testing proposed earth models.

Following this quake the spectral peak with period about 90 minutes which was noted during the Chilean quake was also observed by both instruments. However, the availability of two instruments at the same site enabled cross-correlations of the two records. The records were highly incoherent at this period, in contrast to the high degree of coherency for the free mode of period 54 minutes, and higher frequency modes. Therefore it is believed that the supposed "translational mode" of the inner core was not in fact observed.

L. B. SLICHTER
G. J. F. MACDONALD
M. CAPUTO, C. L. HAGER
University of California, Los Angeles

Behavioral and Physiological Changes Produced in Rats by Removal of the Superior Colliculi of the Brain

These observations on rats were made incidentally to an investigation of the nervous pathways from the eye to the 24-hour clock. Bilateral lesions were made in all parts of the optic system from the occipital poles to the chiasma. Of interest here are the bilateral lesions of the superior colliculi of the midbrain. These structures were rimmed with a sharp needle and then removed by suction. Postoperatively the rats were observed for many months. They appeared to be normal and healthy. They were spontaneously active, ate and drank, and showed no signs of dietary deficiency. However, when examined more closely by handling and daily records of spontaneous running activity, food and water intake, and vaginal smears, they showed a wide range of the most surprising behavioral and physiological changes:

1) Greatly reduced reaction to restraint—to being held upside-down in the palm of the experimenter's hand. Some operated rats made no effort to escape during periods as long as 10 to 32 minutes, whereas normal rats try to escape after only a few seconds. Operated recently trapped wild rats likewise showed this loss of reaction to restraint.

2) Taming—definitely noticeable in wild rats.

3) Very regular long cycles in spon-

taneous running activity—18 to 20 days in length in males; 30 to 35 days in females. (This is the first time behavioral cycles of any length have been found in male animals.)

4) Freeing of the 4- or 5-day estrous cycles from diurnal light control.

5) Marked reflex tail "rattling."

Histological study of the brain sections showed that in many instances the lesions extended to the pretectum and thalamus, possibly owing to damage to arteries. The presence of nystagmus and dilation of the pupils also indicates extension of the lesion beyond the superior colliculi. We have not yet pinpointed definite areas responsible for the behavioral and physiological effects.

CURT P. RICHTER
Johns Hopkins Medical School

On the Regulation of Chromosomal DNA Synthesis in the Polytene Chromosomes of Drosophila melanogaster

The chromosomes of *Drosophila* salivary gland nuclei exhibit a variety of labeling patterns when excised glands, incubated with tritiated DNA precursors for relatively short periods, are subjected to autoradiographic analysis. The most common pattern shows labeling, and hence DNA synthesis, restricted to unconnected chromosomal regions, the number and size of which vary from one nucleus to another. This pattern has been interpreted as indicating the existence of a large number of molecular ends in the DNA complement along the length of the chromosome. The interpretation hinges on the assumption that the large number of strands which make up the polytene chromosome are synchronized with respect to DNA synthesis.

Our analysis has now been extended by examining the labeling pattern in unpaired regions of homologous chromosomes, where the band patterns are sufficiently clear to permit unambiguous establishment of structural homology. Within these regions DNA replication appears to be absolutely synchronized, as judged by the quantitative and positional equivalence of labeled DNA after 10- to 15-minute incubations with labeled precursor. This finding reinforces the presumption that there is local synchrony within a chromosome and, when combined with the discontinuous labeling pattern observed along the chromosomes suggests, as a working hypothesis, that chromosomal DNA is subdivided into a large number of replicating units with individual, localized control. Supported by NIH research grant CA-03276 and by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

WALTER PLAUT, DAVID NASH
University of Wisconsin

Radiation-Induced Enhancement of Natural Hemolysin in Adult Rabbits

Following 500 to 600 r, the low-titered (10 to 50 50-percent units per milliliter of serum) so-called natural hemolysin in rabbits disappears from the serum in two weeks [Talmage *et al., J. Infect. Diseases* **99**, 241 (1956)], rapidly reappears, and reaches the pre-irradiation titer in a week or two. During the following three months, one or two temporary waves of enhancement occur with peak titers frequently as high or higher than 150 units. This enhancement is strikingly similar to the radiation-induced enhancement of the immune hemolysin which has been described by Hektoen [*J. Infect. Diseases* **27**, 23 (1920)] and us [*J. Infect. Diseases*, in press]. In fact, the present work strengthens the assumption that this so-called natural hemolysin, which in the absence of intentional immunization probably develops a response to the absorption of the widespread Forssman antigen in food or in infectious organisms [see Aitken, *J. Infect. Diseases* **114**, 178 (1964)], is actually identical with the nonavid part of the immune gamma-1 Forssman hemolysin that rapidly dissociates after being adsorbed on sheep red cells. Enhancement is probably the result of a greater number of inducible antibody-forming cells accompanying what Hektoen termed reparative overcompensation. Also recent work by us [*J. Infect. Diseases* **113**, 155 (1963)] suggests that the antigen, which is absorbed and gives rise to the low-titered hemolysin, never reaches the threshold amount necessary to initiate primary responses sufficient to yield anamnestic sensitivity to the antigen.

This work was supported by the U. S. Atomic Energy Commission.

W. H. TALIAFERRO
L. G. TALIAFERRO
Argonne National Laboratory

Paleoecology of the Cretaceous-Tertiary Transition in Montana

A sequence of micro-vertebrate fossils and pollen spectra in the Hell Creek and Tullock Formations of Garfield and McCone counties, Montana, permits an evaluation of terrestrial community changes from Cretaceous into Paleocene. The uppermost 80 feet of the Hell Creek Formation is characterized by reduced abundance of dinosaurs, progressive loss of most of the characteristic North American late Cretaceous mammals, introduction of new mammal species belonging to four families and one order previously restricted to the early Tertiary, and floristic changes suggesting a slightly cooler and more continental type of climate. A continuous reduction in floral diversity occurs in the stratigraphic interval from the lower Hell Creek Formation, through the Tullock Formation into the overlying Lebo Formation of middle Paleocene age. Known changes in the vertebrate communities are confined to the dinosaurs and the mammals. Three mammal zones of Cretaceous age but Paleocene aspect suggest that rates of evolution in mammals were high during this transition in community type.

Local extinction of large dinosaurs is apparently associated with transition from a subtropical type of rainforest to a temperate mixed deciduous and coniferous forest. The typically Cretaceous mammal biofacies is displaced by an invading mammal fauna of Cretaceous age but Paleocene aspect. These changes in turn are probably the result of the final retreat of the late Cretaceous epicontinental sea to the south.

The Cretaceous-Paleocene boundary is best placed at the base of the Tullock Formation.

ROBERT E. SLOAN
University of Minnesota

Recent Results on the Effects of Irradiation on Erythrocyte Chimerism in Cattle Twins

These experiments were stimulated by our observations [Stone *et al., Proc. Natl. Acad. Sci. U.S.* **51**, 1036 (1964)] that the proportions of the two antigenic cell types in chimeras may change markedly with time, and that somatic cell mating may occur between the hematopoietic tissues of the chi-

meric mixture yielding a hybrid cell type—among 25 twins which received whole-body gamma irradiation, 15 (60 percent) showed significant changes in the proportions of their cell types, whereas only 2 of the 16 (7.5 percent) unirradiated co-twins showed similar changes. The direction and magnitude of the changes were variable. In some twins the majority type increased, and in others it decreased; the magnitude of the changes ranged from 10 to 45 percent. In 3 twins, one of the two cell types disappeared almost completely. The earliest changes were observed 10 weeks and the latest 34 weeks post-irradiation. However, once a significant change had occurred, the new proportions seemed stable, suggesting that a new equilibrium had been established between the two hematopoietic tissues. Significantly, the direction of change was usually the same in both twins of a pair. Furthermore, in two twins, the proportion of the cells representing the twin's own genotype decreased, whereas in another twin, the reverse was true. Therefore, the abrogation of immunologic tolerance cannot explain these results. No evidence of somatic cell mating or changes in the transferrins or hemoglobins have been observed following irradiation.

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W. H. STONE
University of Wisconsin
R. G. CRAGLE
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Mitochondrial Responses to Ions and Hormones

A study of the nature of the effects of various ions upon the oxidation-reduction states of the mitochondrial respiratory carriers reveals that both calcium and manganous ions interact with components of the energy transfer mechanism and produce patterns of response similar to those seen when ADP is added to mitochondria in state 4. The only unusual feature of their effects is upon cytochrome a_3 where the ions induce an oxidation. The addition of magnesium to magnesium-deficient mitochondria brings about a reduction of all carriers. In the presence of parathyroid hormone, an agent known to stimulate magnesium-phosphate accumulation in mitochondria, the addition of magnesium produces a response similar to that observed with calcium in the absence of hormone and alters the typical response to calcium. Similarly, the addition of K^+ in the presence of Valinomycin, an agent known to promote K^+ uptake in mitochondria, leads to a pattern of response similar to that seen with magnesium in the presence of hormone. In the case of K^+ and Valinomycin the oxidation of cytochrome a_3 is particularly striking. These results lead to two conclusions: (i) The various ions translocated all apparently interact with the same carrier, and (ii) the mechanism of action of Valinomycin and parathyroid hormone could be to interact with this carrier, presumably a protein; and by changing its configuration, alter the specificity of the ion binding sites upon this carrier. Other alternatives are possible.

HOWARD RASMUSSEN
University of Wisconsin

Properties of Metastable Species Produced in Solid Organic Halides by Gamma Irradiation

Gamma irradiation of organic halides frozen to the glassy or polycrystalline state at 77°K produces metastable ions and free radicals which can be observed by their optical or electron spin resonance spectra. The rates of growth in concentration of these species during irradiation and of their decay during annealing or optical bleaching give evidence on their properties and interactions. Polycrystalline samples, in general, show lower radical yields, different radical species, and greater radical stability compared to samples studied in the glassy state. Caging effects, steric effects, solid state energy transfer processes, and electron trapping processes are probable sources of the observed reaction differences between the liquid, glassy, and polycrystalline states.

JOHN E. WILLARD
University of Wisconsin

Wave Form of Electrodermal Responses in Anesthetized Cats

In cats sweat glands exist only in the bare skin of the footpads of all four limbs. We have taken advantage of this fact to show the different forms of the electrodermal response (registered as a change in skin potential) which may be obtained with leads attached to two sweat gland bearing surfaces when the events occurring under the electrodes are varied in time. These are contrasted with the forms of responses obtained when one of the recording electrodes is on skin without sweat glands. It seemed worthwhile to demonstrate these simple relationships, since much has been made of differences in the form of electrodermal responses in man, whose sweat glands occur in all parts of the skin. [McLeary, *Psych. Bull.* **47**, 95 (1950)].

With a "source follower" (designed by H.L.) to eliminate mutual interference between channels of an Offner transisterized electroencephalograph, simultaneous recordings were made (i) between the pad and dorsum of each hind foot, (ii) between the pads of both hind feet, and (iii) between the dorsal surfaces of both hind feet, when both tibial nerves of cats under chloralose and urethane were stimulated with monophasic rectangular electric pulses, of which the voltage, pulse duration, and interval between pulses could be varied independently.

Results were as follows: (i) Recordings between dorsum and pad showed constant monophasic waves with the pad negative to the dorsum. (ii) The potential difference between pads was variable, and the waves were polyphasic, with the form depending on the order of stimulation of the two tibial nerves, the interval between the two stimuli, the voltages of the stimulating pulses, and the condition of the sweat glands in the two pads. (iii) Under no circumstances were potential differences recorded between the two dorsa.

These findings emphasize the necessity of taking into consideration the distribution of sweat glands in the skin in interpreting electrodermal responses in man and other primates. Supported by NIH grant B-732.

G. H. WANG
H. LUDWIG
University of Wisconsin

Complexes of Xenon Hexafluoride

Noble gas compounds are still new, and until recently studies have been limited to binary fluorides, oxides, and

oxide salts. It has now been discovered that the binary fluorides have a richer chemistry than was first anticipated, and we report here some studies on the highest fluoride-xenon hexafluoride.

Xenon hexafluoride is one of the most reactive of the hexafluorides. Chemically it is distinguished from other hexafluorides by its ability to attack dry Pyrex and quartz, suggesting that it might possess ionizing properties. Though we have not been able to measure the conductance of either xenon hexafluoride itself or of solutions in it because of electrode attack, we have been able to prepare complexes between xenon hexafluoride and fluoride donors such as alkali fluorides and fluoride acceptors such as antimony pentafluoride.

Xenon hexafluoride combines readily with the alkali metal fluorides. Two series of salts, $MXeF_7$ and M_2XeF_8, are formed depending on the conditions. The heptafluoroxenates (VI) of cesium and rubidium have been isolated as crystalline solids stable at 25°; but the potassium and sodium compounds, although probably formed in the first place, decompose on drying to octafluoroxenates (VI). Cesium and rubidium heptafluoroxenates (VI) decompose on heating according to the following reaction scheme:

$$MXeF_7 \xrightarrow[50°(Cs)]{30°(Rb)} M_2XeF_8 \xrightarrow{400°} MF$$

Potassium and sodium octafluoroxenates begin to lose xenon hexafluoride at 30°.

Xenon hexafluoride reacts with boron trifluoride, arsenic pentafluoride, and antimony pentafluoride to form complexes of composition $XeF_6 \cdot BF_3$, $XeF_6 \cdot AsF_5$, and $XeF_6 \cdot SbF_5$ and $XeF_6 \cdot 2SbF_5$, respectively. These complexes are not volatile at room temperature with the exception of $XeF_6 \cdot BF_3$, which appears to volatilize with decomposition into its components. Studies of absorption spectra (infrared and Raman) suggest an ionic contribution to the bonding.

HENRY SELIG
RAYMOND D. PEACOCK
IRVING SHEFT
Argonne National Laboratory

Recent Studies of Symbiotic Nitrogen Fixation

In several laboratories it has been suggested that the myoglobin- (Mb)-like pigments of nitrogen fixing soybean nodules donate electrons for the reduction of N_2 to NH_3. We have tested this suggestion by spectrophotometric analysis of half nodule sections with the cut surface making liquid contact with one window of a gas-tight optical cuvette.

When gas mixtures for optimum steady state rates of N_2 fixation are changed to those for zero rates, and *vice versa*, the observed Fe^{++} Mb $\rightleftharpoons$ Fe^{+++} Mb equilibrium remains constant to one part in 10^3 or less, the experimental error. A similar technique readily detects changes in the Fe^{++} Mb + O_2 $\rightleftharpoons$ Fe^{++} Mb O_2 equilibrium. We postulate that Mb serves as an O_2 carrier, whose diffusion and kinetics largely substitute for the solvent (carrier free) diffusion of O_2 to the bacteroids. Experimental and theoretical support for this hypothesis will be presented.

CONRAD S. YOCUM
University of Michigan

Symposium on Hearing

Nerve and Nucleus: A Study of Stimulus Coding in the Initial Stages of the Auditory Nervous System

The spike activity of single units in auditory nerves and cochlear nuclei was examined in anesthetized cats. The time patterns of both spontaneous activity and responses to sound were studied using a variety of selected stimulus conditions. Some interesting comparisons can be made between auditory nerve fibers and cochlear nucleus units. These include shape of "tuning curves," time patterns of spontaneous activity, responses to transient and continuous stimuli, and phenomena related to thresholds. The behavior of some units in the cochlear nucleus can hardly be distinguished from that of auditory nerve fibers. Other units in the cochlear nucleus fall into a number of different types based on responses to certain specific stimulus conditions, such as long bursts of tone, continuous tonal stimulation, or clicks at high rates. Some correlations exist between the type of unit and the location of the recording electrode within the nucleus. Apparently, information transmitted by auditory nerve fibers can be recorded in a number of ways at the level of the cochlear nucleus.

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NELSON Y-S. KIANG
RUSSELL R. PFEIFFER
Massachusetts Eye and Ear Infirmary, Boston, and *Massachusetts Institute of Technology*

Responses of Neurons in the Cochlear Nucleus to Tones and Noise of Long Duration

A study was made of the discharge characteristics of neurons of the cochlear complex in response to tones and bands of noise of 10 sec duration. All neurons studied responded to these stimuli in a sustained fashion. For some neurons, there was a monotonic function between stimulus intensity and discharge rate; in others, the relation between these two variables was nonmonotonic. Particular attention was paid to those discharge characteristics related to the distribution of interspike intervals occurring during sustained activity. In all units, and regardless of whether tones or noise were employed as stimuli, there was an increase in the variance of intervals as mean interval increased, and in many units there was a corresponding increase in the skewness of the distributions and a conspicuous shift in their modal values. Many neurons exhibited a negative correlation between the values of intervals occurring next to each other in a record. The regularity of discharge patterns varied from one neuron to another. For any individual neuron, presentation of different tonal stimuli, which produced similar mean rates of discharge, also resulted in similar discharge patterns, that is, in similar interval distributions. This was so even when it could reasonably be inferred that the various stimuli activated different combinations of excitatory and

inhibitory circuits. In some neurons, the patterns of discharge were much more irregular when bands of noise, instead of tones, were employed as stimuli. Supported by NIH grants B-896, B-2911, and MH-8354.

JAY GOLDBERG
University of Chicago
DONALD D. GREENWOOD
Duke University

Descending Activity in the Auditory System

The paper deals with electrophysiological studies on the organization of the centrifugal system which regulates admission of auditory signals into the brain. The most peripheral link in this complex system has been studied in the cat (olivo-cochlear fibers, both crossed and uncrossed) and in the pigeon (efferent cochlear fibers). These fibers can be considered homologous from functional and neurochemical standpoints. Important differences have nevertheless been disclosed between the bird and the mammal in the operational characteristics. The latter can be correlated both with behavioral features and with the fine synaptic organization of the inner ear in either group. The peripheral link receives several inputs, namely from higher levels of the brain, and it can be driven by corticofugal discharges.

JEAN E. DESMEDT
University of Brussels

Functional Characteristics of Neurons in the Medial Geniculate Bodies of Cat and Rabbit

Activities of single cells in the medial geniculate bodies of cat and rabbit were recorded with indium microelectrodes. Units were driven by tone bursts, 50 msec long, at best frequencies. Tone bursts were delivered to each ear alone or to both through a closed auditory stimulating system. Rabbits were anesthetized with urethane and cats with pentobarbital-sodium.

Analysis of 120 units, isolated in the medial geniculate bodies of rabbits, allowed classification as: (i) contralateral units driven only by the contralateral ear; (ii) ipsilateral units driven only by the ipsilateral ear; and, (iii) bilateral units driven by both ears; (iv) units driven mainly by noise, classified as miscellaneous.

The same type of analysis was made of 69 units recorded in the medial geniculate body of cats. Contralateral, ipsilateral, and bilateral, as well as miscellaneous units were found.

Percentage of units of each type for the rabbit was found to be: contralateral, 71.8; ipsilateral, 3.2; bilateral, 17.8; miscellaneous, 7.2 percent.

Percentages for the cat were: contralateral, 47.6; ipsilateral, 15.5; bilateral, 26.5; and miscellaneous, 10.4 percent.

From these findings it appears that there is a stronger ipsilateral projection to the medial geniculate body in the cat than in the rabbit.

Supported by NIH grant NB-03640.

HUGO ADRIAN
WLADIMIRO LIFSCHITZ
University of Chile, Santiago

Subjective Lateral Inhibition as a Method of Investigating Sensory Activity of the Ear, the Skin, and the Taste Nerves

Ideally, the electrophysiological recordings of sensory nerve activities should fit together with phenomena observed subjectively. Lateral inhibition is easily observable both electrophysiologically and subjectively, and it can therefore be expected to provide a useful instrument to relate these two different approaches to the same subject. Since experiments focused particularly on the localization of sensations under different stimulus conditions seem to be of interest, the different types of inhibition that may occur are discussed. Also investigated was the degree to which phenomena like directional hearing and its analogue in skin and taste sensations make it possible to define the requirements to be met by any sensory nervous system, including the auditory one.

GEORG VON BÉKÉSY
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Some Effects of Binaural Stimulation on the Activity of Single Neurons in the Inferior Colliculus of the Cat

If a neuron is activated by a tonal stimulus to one ear, stimulation of the other ear with a tone of the same frequency may result in a modification of activity which is dependent on the character of the neuron but which may also depend on the intensity and the timing of the stimuli. Thus a neuron responding to low frequency tones (some 200 to 3000 cy/sec) may be sensitive to phase differences between the binaural stimuli. With a favorable phase relationship a cell may respond hundreds or thousands of times to a given tone, while no discharge occurs when this relationship is not favorable. The spike counts are periodic functions of the phase shift, and the period is equal to the period of the stimulating frequency. Preliminary data imply that while the spike counts, as a function of phase shift, cycle with the period of the stimulating frequency, the first peak of activity is likely to occur, for a given neuron, at a constant delay regardless of the period of the stimulating tone. If a neuron is activated by stimulation of either ear alone, the activity provoked by stimulation of either ear may be augmented but it may also be partially or completely suppressed (depending on the intensity of the stimuli), if both ears are stimulated simultaneously. If a neuron is activated by stimulation of one ear only, stimulation of both may completely suppress discharges caused by stimulation of the excitatory stimulus. Parametric studies concerning the phenomena described will be presented and some implications of the findings will be discussed.

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Some Discharge Characteristics of Single Neurons in Cats' Auditory Cortex

Thirty-eight neurons, isolated in anterior ectosylvian gyrus (anterior A11) or near the dorsal tip of the posterior ectosylvian sulcus (posterior A1) in cats anesthetized with chloralose or nembutal, were chosen for detailed analysis of their responses to sound stimuli. Emphasis was on interactions resulting from binaural low-frequency tonal stimuli. The great majority of

the units responded with a single spike or a short burst of spikes to the onset of the tone regardless of stimulus duration. The variability of the latent period to the first spike and variations in spike counts for different parameters of the stimulus will be presented in some detail. Analyses of interspike intervals and distribution of spikes were made for units discharging multiple spikes.

A few neurons responded with a short burst of spikes to the termination as well as to the onset of the stimulus or only to the termination of the stimulus. Discharge characteristics of one such "off unit" will be presented. The majority of the "onset units" were driven best by stimulation of the contralateral ear. The spike counts were either a monotonic or a nonmonotonic function of stimulus intensity. The ipsilateral stimulus occasionally facilitated, but more often inhibited, the response to contralateral stimulation. Some units were apparently inhibited by low intensity and driven by high intensity monaural stimulation, as evidenced by spike counts produced when binaural stimuli were delivered. As is the case for some neurons in the inferior colliculus, a group of 16 cells was highly sensitive to small interaural differences in the phase of the stimulus at tonal frequencies ranging from 200 cy to 2.5 kcy/sec. The spike counts and latent periods here were functions of the interaural phase differences of the stimuli.

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The Contingent Negative Variation: An Electrocortical Sign of Significant Association in the Human Brain

Records from patients with chronic intracerebral electrodes reveal responses in nearly all frontal areas to stimulation in any modality. These responses can also be identified in scalp records with electronic averaging; they wane rapidly with monotonous stimuli, but are sustained when a particular stimulus is assigned conditional significance by reinforcement or association with an imperative stimulus.

In these conditions the frontal cortex develops a protracted widespread negative potential that generally sweeps back from prefrontal to motor cortex following the conditional response. This submerges the surface-negative component of the imperative response and terminates abruptly at the positive phase of the latter. At the same time the reaction time of an operant response is reduced or a conditioned response appears in a "classical" conditioning situation. This Contingent Negative Variation (CNV) reflects the probability of signal association as estimated by the subject and is independent of stimulus amplitude or energy. It is particularly large and consistent when the stimuli are purely semantic, provided that they are meaningful to the subject and that some decision or action is required.

In young children the CNV is small and variable but can be augmented by social influences such as persuasion, instruction, admonition, and competition.

Very faint or even "negative" auditory stimuli evoke large CNVs if they are followed by an exciting stimulus such as brief exposure of a provocative picture.

During partial reinforcement or extinction trials, the CNV may last 10 seconds or more, particularly in highly suggestible subjects, and may even be augmented or attenuated by hypnotic suggestion.

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Some Properties of the Slow Cortical Evoked Response in Humans

A cortical evoked response may readily be detected by average response techniques. Several laboratories, including our own, are using it, quite empirically but successfully, as an end point for "objective" audiometry to estimate hearing impairment in young children. It is difficult, however, to interpret the response in question, either in terms of neurophysiology or as a possible correlate of some psychological function such as perception. Consider the following set of properties:

(i) It does not arise from the primary auditory cortex but diffusely from cortex at the top of the head and the frontal areas.

(ii) It is slow. The first large wave (vertex-positive) has its peak at 50 msec, the next (vertex-negative) at about 100 msec, the third (vertex-positive) at 180 msec. Activity continues at least to 700 msec.

(iii) Intervals between stimuli must be about 10 seconds to obtain maximal average amplitude.

(iv) Different components of the response may vary independently.

(v) It is not specific to the auditory system but is equally well evoked by vision, touch, and electric shock. Cross-modality interactions are weak, however.

(vi) The responses are extremely variable from one stimulus to the next. Even the average response varies across subjects, across conditions, such as vigilance, indifference, habituation and drowsiness, and across successive trials.

(vii) It is a weak function of stimulus intensity, apparently a power law with an exponent about 0.12. This is much flatter than the psychophysical function for loudness.

(viii) Its threshold of detectability is near 25 db sensation level in adults but at about 2.5 db in our group of hard-of-hearing children.

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Components of the Complex Response Evoked from the Auditory Cortex of Unanesthetized Cats

Electrodes were implanted on the dura over the middle ectosylvian gyrus, the posterior lateral gyrus, and the posterior neck muscles. The two cortical leads were referred to another electrode placed in the frontal sinus, while the electromyogram was recorded between two leads in the neck muscles. The electrical activity was recorded on magnetic tape and subsequently processed on the ARC-1 computer. The ongoing electrical activity, displayed on an inkwriter, permitted homogeneous samples of evoked responses to be averaged.

The ongoing electrical activity from the cortical leads, the electrocorticogram and the electromyogram from the neck muscles, taken together, permitted the definition of three different physiological states. The averaged evoked re-

sponse to similar stimuli differed for each of these physiological states. When the electrocorticogram and electromyogram indicated that the animal was awake, three deflections appeared in the evoked response: (i) an early, surface-positive deflection at about 6 msec latency, which is unaffected by changes in state and corresponds to the classical evoked response from the anesthetized animal; (ii) a second, surface-positive response at about 32 msec latency, which remains present in light sleep but is absent in deep sleep or after anesthesia; and (iii) a late, surface-negative deflection at about 55 to 60 msec latency which is absent for all states other than the awake.

Since variation in the response waveform due to state was ascertained, it was possible also to study the effects of changes in stimulus conditions. The addition of background noise showed that the early component was reduced by noise intensities lower than that which completely masked the response.

Taken together, the variation in waveform due to state and that due to stimulus indicate that the different components are independently manipulable. The conclusion is reached that more than one subsystem contributes, in parallel, to the evoked response complex in the unanesthetized, awake cat.

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Activity of Neural Units in the Auditory Area of the Cerebellum of Decerebrate Cats

Indium microelectrodes of 2 to 4μ diameter were used to study spontaneous and evoked activity of single cells in the auditory area of the cerebellum in cats acutely decerebrated under short-acting Viadril (Pfizer) anesthesia. Continuous white noise and tones, or short bursts of the same, were delivered to one or both ears. Evoked slow waves also were recorded. Electrode tracts were located histologically.

Many cerebellar units are "spontaneously" active at relatively high rates of discharge (100 per sec). Some are not affected by auditory stimuli; others are. Especially striking is the inhibition produced by tone bursts which may be fairly sharply frequency-specific.

Some units, thought initially to be spontaneous, were found to be under drive from tonal stimuli repeated at one-second intervals; firing gradually ceased when tones were turned off and recruited when repetitive stimulation resumed. There was, however, no clear drive to single stimuli. When the discharge rate was well developed, single tones bursts caused both initial acceleration and later inhibition. No units were found which responded in a sustained manner with sharp on and off of discharges.

Most units studied were clearly related to evoked responses. One or more discharges might occur, often on the negative components of evoked responses, sometimes on the positive wave. Trains of six to eight discharges were observed throughout the period of the evoked response. Sometimes there were two bursts, an early one during the evoked response and a later one after an intervening silent period.

Although unit discharges and evoked responses both showed some frequency specificity, no clear cut tonotopic localization in the cerebellum was defined.

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